PAPER • **OPEN ACCESS**

The effect of sodium bisulfite immersion to the potential of *Candi* banana peel ethanol extract as radical scavenger and UV protection

To cite this article: R A Laeliocattleya and J Wijaya 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **230** 012024

The effect of sodium bisulfite immersion to the potential of *Candi* banana peel ethanol extract as radical scavenger and UV protection

R A Laeliocattleya and J Wijaya

Department of Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: deeochalina@gmail.com

Abstract. Banana peel consists of bioactive compounds like phenolics, flavonoids, tannin, carotenoids and alkaloids, preventing and reducing free radical and impeding UV-ray radiation. Banana peel immersed with sodium bisulfite solution prevents browning reaction by enzymatic and non-enzymatic reaction. This research aims to study the effect of sodium bisulfite immersion to the content of bioactive compounds, antioxidant activity and Sun Protection Factor (SPF) of *Candi* banana peel. Stages conducted in this research include: sample preparation (non-immersion (E1) and immersion (E2) of samples with sodium bisulfite) as well as an extraction of *Candi* banana peels with ethanol solvent by ultrasonic. Afterwards, each extract is determined by the content of bioactive compounds (phenolic, flavonoid, tannin and β-carotene), antioxidant activity (IC_{50}) and SPF value. The result of this research shows that the contents of phenolic, flavonoid, tannin and β-carotene in ethanol extract of non-immersion (E1) *Candi* banana peel are 2613.60 mg/kg; 59.34 mg/kg; 6895 mg/kg and 103.97 mg/kg respectively. Moreover, the antioxidant activity (IC_{50}) is 2573.25 ppm and the SPF value is 7.34. Furthermore, the contents of phenolic, flavonoid, tannin and β-carotene in ethanol extract of *Candi* banana peel with immersion (E2) are 4303.40 mg/kg; 125.27 mg/kg; 4881 mg/kg and 158.36 mg/kg respectively. The antioxidant activity (IC_{50}) is 3210.28 ppm and the SPF value is 10.67. The research result shows that the contents of phenolic, flavonoid, β-carotene and the SPF value are higher in *Candi* banana peel with immersion than that of non-immersion. Meanwhile, both treatments show inversed result of tannin content and antioxidant activity.

1. Introduction

The depletion of ozone layer as a protector of sunlight radiation due to an increase in free radicals on the earth causes the possibility of increasing various diseases and health disorders. The exposure to abundant sun radiation with high intensity can cause skin hyperpigmentation which causes the skin becoming dull and scaly and unfortunately can even increase the risk of skin cancer. This effect is mainly caused by UV A (320-400 nm) and UV B (290-320 nm). For this reason, a skin protection is needed; one of them is by applying sunscreen.

In sunscreen preparations, besides photoprotective compounds, antioxidant and moisturizing compounds are also vitally needed. Based on the research Svobodova et al. [1], it is suggested that one of the active ingredients in sunscreen is phenolic compounds. One of the phenolic compounds believed to play a role as active ingredients of sunscreen is a derivative of cinnamic acid and

flavonoids. UV protection effect is caused by the presence of a benzene ring which is able to absorb UV wavelengths.

Fruits, which one of them is bananas, contain bioactive components which are high in antioxidants. Malang Regency, East Java, is one of the most banana-producing regions which provides *Candi* banana (*Musa paradisiaca*). Bananas can be processed into various products; one of the products is in the form of banana peels which accounts for around 38% of all fruit weight [2]. Yellow ripe banana peels are rich in flavonoids, phenols, and β-carotene which are a source of natural antioxidants possessing a property called as photoprotective activity against UV light [3].

Sodium bisulfite is often used in the food industry to prevent enzymatic and non-enzymatic browning. Sulphite solution can be used when soaking banana peels. In enzymatic browning, sulphite will reduce disulfide bonds in enzymes to prevent enzyme catalyzation the oxidation of phenol compounds causing browning [4]. Polyphenol Oxidase (PPO) activity can be prevented by replacing the substrate on the active side of the enzyme. The bioactive content of the *Candi* banana peel extract can be identified by extraction. The alternative methods are required since the conventional extraction method presents weaknesses requiring a longer time to conduct. Ultrasonic wave extraction requires faster time and can be operated at a moderate temperature which is suitable for heat sensitive compounds.

2. Materials and Methods

2.1. Materials

The materials used include: samples in the form of *Candi* banana peels, 96% ethanol, petroleum ether, alumina oxide (Al_2O_3), sodium bisulfite, Folin Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), NaOH, sodium bisulfite ($NaHSO_3$), gallic acid, quercetin, β-carotene, standard tannic acid, methanol, DPPH, ethanol pa and nitrogen gas.

2.2. Methods

2.2.1. Sample preparation

The sample was a *Candi* banana with a maturity level of 6-7. *Candi* bananas were washed with flowing water. The end of the base is cut, the skin was peeled, and the rind was cut into small pieces. Then the sample was soaked with 100 ppm sodium bisulfite solution for 15 minutes (E2). As for the E1 sample, the immersion stage is not carried out with sodium bisulfite. Banana peel was drained and dried by using a cabinet dryer at 45°C for 11 hours. The sample was blended until achieving certain degree of smoothness and was sieved by using an 80 mesh sieve to obtain the *Candi* banana peel flour.

2.2.2. Sample extraction

Candi banana peel flour (E1 and E2) weighed for 15 grams was dissolved with ethanol solvents (material: ethanol ratio = 1: 8 gr/ml). Extraction was conducted by using an ultrasonic bath at a frequency of 39 kHz, for 30 minutes. Extraction of the sample was filtered with fine filter paper. Each filtrate obtained was evaporated by using a rotary vacuum evaporator at 40°C and 40 rpm. Each extract was sprayed with nitrogen gas to remove the remaining solvents to obtain the extracts of E1 and E2.

2.2.3. Total phenolic compounds (TPC) determination

Determination of phenol content was carried out based on several following measurements [5]: the absorbance was measured by using a UV-Vis spectrophotometer at λ 759 nm; the total phenol content was expressed as the equivalent of mg of Gallic acid per kg of sample extract (mg GAE / kg extract) as presented by the following formula:

$$\text{TPC (Total Phenolic Compounds)} = \frac{C \; x \; V \; x \; FP}{W} \quad (1)$$

Where: C = phenolic concentration (x value) in ppm (mg/L), V = volume of extract used (ml), FP = dilution factor, W = sample weight (kg)

2.2.4. Total flavonoid compounds (TFC) determination

Determination of flavonoid content was based on following modifications [6]: absorbance measurements were conducted by using UV-Vis spectrophotometer at λ 355 nm; the total flavonoid content was expressed as mg quercetin per kg sample extract (mg QE / kg extract) by the following formula:

$$\text{TFC (Total Flavonoid Compounds)} = \frac{C \; x \; V \; x \; FP}{W} \quad (2)$$

Where: C = flavonoid concentration (x value) in ppm (mg/L), V = volume of extract used (ml), FP = dilution factor, W = sample weight (kg)

2.2.5. Total tannin compounds (TTC) determination

Determination of tannin content was conducted by using a spectrophotometer method based on several measurements [7]: the absorbance was measured using a UV-Vis spectrophotometer at λ 765 nm; the total tannin content was expressed as mg equivalent of tannic acid per kg of sample extract (mg TE / kg extract) by the following formula:

$$\text{TTC (Total Tannin Compounds)} = \frac{C \; x \; V \; x \; FP}{W} \quad (3)$$

Where: C = tannin concentration (x value) in ppm (mg/L), V = volume of extract used (ml), FP = dilution factor, W = sample weight (kg)

2.2.6. Total β-carotene determination

The total β-carotene was determined by the column chromatography method as described by several measurements with some modifications [8]. Firstly, sample of 1 g was added with 8 mL of petroleum ether: acetone (1:1). The flask was covered with alumina foil and was shaken for 10 minutes and was filtered by using filter paper. The filtrate obtained was stored at room temperature. The residue was added with 8 ml of petroleum ether: acetone (1:1) covered with alumina foil and was shaken for 10 minutes. This treatment was repeated twice. Then, the filtrate was diluted with petroleum ether: acetone (1:1) until the total volume of 25 ml; afterwards the filtrate was transferred to separator funnel and was added with 10 mL of aquadest. The separator funnel was shaken and incubated to the mixture which was separated into 2 phases. The lower part (water: acetone phase) was discarded and the upper part (ether phase) was transferred to a tube. Then, 0.5 g of Na_2SO_4 was added to each of 10 mL of ether phase's volume. Then, the mixture was loaded into the column of chromatography. Petroleum ether: acetone (10:1) solvent was added to the column until the mixture turned into crystal clear. Then the absorbance of the mixture was measured by using UV-Vis Spectrophotometer at 450 nm. Total β-carotene was defined as weight of β-carotene (mg) equivalent per kg of sample. The formula for calculation is:

$$\text{Total β-carotene} = \frac{\text{C} \times \text{V} \times \text{FP}}{\text{W}} \quad (4)$$

Where: C = β-carotene concentration (mg/L), V = mixture volume (L), FP = dilution factor, W = sample weight (kg)

2.2.7. IC_{50} value of antioxidant activity determination

Extraction samples, E1 and E2 were dissolved in methanol solvent and concentration series of 30, 60, 90; 120 ppm and 150 ppm. Each sample was taken at 4 ml, then was added with 1 ml of 0.2 mM DPPH ethanol, and the absorbance was measured by using a spectrophotometer [9]. Calculation of antioxidant activity is presented by the following formula:

$$\text{Antioxidant Activity (\%)} = \frac{Abs\ of\ blank - Abs\ of\ sample}{Abs\ of\ blank} \text{ x } 100\% \quad (5)$$

2.2.8. Sun Protection Factor (SPF) value determination

The extracted sample was weighed at 0.1 gram and was dissolved in 10 ml ethanol analysis pro. Testing the sample was conducted by measuring its absorbance using a UV-Vis spectrophotometer at λ 290-320 nm with a measurement interval of 5 nm. Testing the SPF value was performed by using constant set [10].

The sample SPF value is calculated using formula below:

$$SPF = CF \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda) \quad (6)$$

Where: CF = Correction factor = 10, EE = Spectrum eritremal effect at wavelength (λ), I = Spectrum of solar intensity at wavelength (λ), Abs = Absorbance of sunscreen products at wavelength (λ).

3. Results and Discussion

3.1. Bioactive compounds content (TPC, TFC, TTC and total β-carotene)

Table 1 shows the TPC value of *Candi* banana peel extract with sodium bisulfite immersion which is higher than a without soaking process. Immersion with sodium bisulfite can prevent browning reactions in the flour extraction process of *Candi* banana. According to Pasaribu [11], the prevention of browning reaction is to hinder the phenolase activity itself; therefore, soaking with bisulfite solution is effective in preventing the brown colour of fruits and vegetables. Phenolase is enzyme that catalyse the oxidation of phenolic compounds. By preventing the phenolase activity, so TPC value of *Candi* banana peel extract with sodium bisulfite immersion is higher than that of non-immersion.

Table 1. Bioactive Compounds Content of *Candi* Banana in a non-immersion extract (E1) and an immersion extract (E2) with sodium bisulfite.

Bioactive Compounds Content	Extracts	
	Non-Immersion (E1)	Immersion with Natrium Bisulfite (E2)
TPC (mg/kg)	2613.16 ± 101.12^{a}	4303.40 ± 153.17^{b}
TFC (mg/kg)	59.34 ± 3.89^{a}	125.27 ± 9.36^{b}
TTC (mg/kg)	6895 ± 209.07^{a}	4881 ± 155.80^{b}
Total β-carotene (mg/kg)	103.97 ± 7.63^{a}	158.36 ± 12.19^{b}

The TFC value of Candi Banana in immersion with sodium bisulfite is higher than that of non-immersion. Flavonoids are polyphenol group compounds which are widely distributed in plants in the form of glycosides which bind to a sugar; therefore, flavonoids are grouped as polar compounds. The prevention of this browning reaction is to hinder the phenolase activity itself. The two inhibitors which are widely used include sulphite and vitamin C. Sodium bisulfite can bind to Cu which is a cofactor to activate enzymes [11]. Sodium bisulfite can be a browning inhibitor on flour composed of saccharide monomers, because sodium bisulfite decreases the α-amylase phase which is thought to be the cause of browning flour.

The TTC of *Candi* banana peel extract has decreased, which is conducted without soaking with bisulfite at 6895 mg/kg extract, but is performed with bisulfite immersion to 4881 mg/kg extract. The decrease in tannin is thought to be due to tannin damage in the hydrolysis process during the extraction and the ongoing heating. Tannins can be hydrolysed to glucose and tannic acid [12]. Sulphite compounds contained in sodium bisulfite cannot absolutely stop the browning reaction but can only slow the browning reaction. This is due to the addition of bisulfite solution as an anti-browning compound by forming a disulfide bond with the PPO enzyme to inhibit oxygen binding. As a result, the formation of oxygen bonding with disulfide causes enzyme activity to decrease.

The content of β-carotene in *Candi* banana peel extract without bisulfite immersion is 103.97 mg/kg extract, while the content with bisulfite immersion increases to 158.36 mg/kg extract. β-carotene is a class of carotenoids which are hydrophobic, lipophilic, insoluble in water, and soluble in solvents such as acetone, alcohol, and chloroform [13]. Carotenoids are considered as fat-soluble pigments which are widely distributed in nature which have many benefits to their colouring power [14]. This pigment can absorb ultraviolet (UV) light and show colour in the visible light spectrum. The structure of chromophore in carotenoids which have conjugated double bonds is responsible for the absorption of light [15].

3.2. IC_{50} value

Table 2 shows the IC_{50} value of banana peel extract without bisulfite immersion is 2573 ppm, whereas the banana peel extract soaked with bisulfite shows a larger IC_{50} value, which is 3210 ppm. The IC_{50} (inhibition concentration) value is defined as the concentration of the sample to inhibit oxidation by 50% or the concentration of the test sample to capture 50% radical DPPH. According to Blois [16], the greater the IC_{50} value, it indicates the weaker antioxidant ability. The antioxidant activity of *Candi* banana peel with sodium bisulfite immersion is lower than that of non-immersion. This is allegedly caused by a decrease in tannin content in banana peel soaked with bisulfite affecting the antioxidant activity of banana peel extract. Tannins can be hydrolysed to glucose and tannic acid [12]. The test results of both sample extracts show that IC_{50} values are more than (>) 200 ppm in both types of extracts; therefore, the *Candi* banana peel extract has a small ability to counteract free radicals.

Table 2. IC_{50} value of *Candi* Banana in a non-immersion extract (E1) and an immersion extract (E2) with sodium bisulfite.

Extract	IC_{50} (ppm)
Non-Immersion (E1)	2573.25 ± 91.71^{a}
Immersion with Natrium Bisulfite (E2)	3210.28 ± 123.35^{b}

3.3. SPF value

Table 3 shows SPF value in banana peel extract soaked with bisulfite shows a greater value of 10.67 which can potentially be a sunscreen with a maximum level of protection. Contrastingly, banana peel extract which is not soaked with bisulfite shows a smaller SPF value of 7.34 which can potentially be a sunscreen with an extra level of protection [17]. Phenol compounds have conjugated double bonds in the benzene ring. If it exposed to UV light, the resonance will occurs in the form of electron transfer. Flavonoids have photoprotective properties because of the presence of a chromophore group which is considered as a conjugated aromatic system having the ability to absorb light rays in the UV wavelength range in both UVA and UVB [18]. The β-carotene pigment can absorb light in the spectrum of ultraviolet (UV) light and in the visible light because it also has cromophore group [15].

Table 3. SPF value of *Candi* Banana in a non-immersion extract (E1) and an immersion extract (E2) with sodium bisulfite.

Extract	SPF Value
Non-Immersion (E1)	7.34 ± 0.93^{a}
Immersion with Natrium Bisulfite (E2)	10.67 ± 1.24^{b}

4. Conclusion

The contents of phenolic, flavonoid, β-carotene and the SPF value are higher in *Candi* banana peel with sodium bisulfite immersion than that of non-immersion. Meanwhile, both treatments show inversed result of tannin content and antioxidant activity. *Candi* banana peel with sodium bisulfite

immersion has an ability as radical scavenger. Besides, it also has an ability as UV protection in maximum level.

References

[1] Svobodova A, Psotova J, Walterova D 2003 Natural phenolics in the prevention of UV-induced skin damage *Biomed. Pap.* **147** 137-145.
[2] Gonzalez-Montelongo R, Lobo M G, Gonzalez M 2010 Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds *Food Chem.* **119** 1030–1039.
[3] Atun S, Arianingrum R, Handayani S, Rudyansah, Garson M M 2007. Identification and antioxidant activity test of some compounds from methanol extract peel of banana (*Musa paradisiaca linn*.) *Indo. J. Chem* **7** 1 83-87.
[4] Wood E J, Archer M C, Palmer J K 1989 An experiment in enzyme characterization: banana polyphenoloxidase Practical Biochemistry for Colleges Pergamon Press Cambridge UK.
[5] Vuong Q V, Goldsmith C D, Dang T T, Nguyen V T, Bhuyan D J, Sadeqzadeh E, Scarlett C J, Bowyer M C 2014 Optimization of ultrasound-assisted extraction conditions for phenolic content and antioxidant capacity from *Euphorbia tirucalli* using response surface methodology *Antioxidants* **3** 604-617.
[6] Atanassova M, Georgieva S, Ivancheva K 2011 Total phenolic and total flavonoid contents, antioxidant activity and biological contaminants in medicinal herbs *J. Univ. Chem. Technol. Metal.* **46** 1 81-88.
[7] Pratik D T, Prakash D P, Chandrashekhar K C 2016 Quantitative estimation of tannins from *Pueraria tuberosa* by uv spectrophotometry *Int. J. Pharm. Sci. Rev. Res*. **37** 1 210-212.
[8] Cagampang B G and Rodrigues F M 1980 Methods of analysis for screening crops of approximate qualities analytical services laboratory Institute of Plant Breeding UPLB Philippines.
[9] Molyneux P 2004 The use of of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity *Songklanakarin J. Sci. Technol* **26** 211–219.
[10] Sayre R M, Agin P P, Levee G J, Marlowe E 1979 Comparison of in vivo and in vitro testing of sunscreening formulas *Photochem. Photobiol.* **29** 559-566.
[11] Pasaribu P, Waluyo T K, Hastuti N, Pari G, Sahara E 2016 Effect of addition of sodium bisulfite and multilevel ethanol washing on the quality of Porang flour *(Amorphophallus muelleri blume) Jurnal Penelitian Hasil Hutan* **34** 3 241-248. [In Indonesian]
[12] Sukardi, Mulyarto A R, Safera W 2007 Optimization of extraction time on tannin content in guava leaf extract powder (*Psidii folium*) and its production costs *Jurnal Teknologi Pertanian* **8** 2 88-94. [In Indonesian]
[13] Thane C and Reddy S 1997 Processing of fruit and vegetables: effect on carotenoids *Nutr. Food Sci.* **97** 2 58–65.
[14] Niizu P Y 2003 Fontes de caroten´ oides importantes para a sa´ ude humana [Mastering thesis (Food Science)] Faculdade deEngen-haria de Alimentos Universidade Estadual de Campinas (UNI-CAMP) Campinas Brasilia Brazil.
[15] Mezzomo N, Ferreira S R S 2016 Carotenoids functionality, sources, and processing by supercritical technology: a review *J. Chem.* **2016** 1-16.
[16] Blois M S 1958 Antioxidant determinations by the use of a stable free radical *Nature* **181** 1999-1200.
[17] Wilkinson J B 1982 Harry's Cosmeticolgy 7th edition Penerbit George Godwin London UK.
[18] Nichols J A, Katiyar S K 2010 Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms *Arch. Dermatol. Res.* **302** 71–83.