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Original Article

VITAMIN C LEVELS AND ANTIOXIDANT ACTIVITY OF PINEAPPLE WET CANDIED BASED ON THE LEVEL OF PINEAPPLES RIPENESS *(ANANAS COMOSUS* VAR. QUEEN*)* AS A FUNCTIONAL FOOD PRODUCT

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ABSTRACT

Objective: The purpose of this study to find out the levels of vitamin C and antioxidant activity of pineapple wet candied based on the level of ripeness of pineapple as a functional food product.

Methods: This type of research was true experimental with posttest-only control research design. The determine vitamin C level method using spectrophotometry UV-Vis and the antioxidant activity testing using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical inhibition method. Statistical test using One Way Anova test continued Post Hoc test.

Results: The results of the determination of vitamin C levels in pineapple wet candied with full green, half yellow and full yellow ripeness levels, respectively, were 4.22; 17.24; 0.75 mg/gram. The results of the antioxidant activity test (IC50) on wet candied pineapple with ripeness levels of full green, half yellow and full yellow, respectively, were 61.86; 49.98; and 72.11 mg/l, Based on the level of antioxidant intensity of each ripeness, the levels were strong, very strong and strong. The results of the One Way Anova test on the level of ripeness of pineapple fruit in wet candied for vitamin C levels and antioxidant activity both of them had a significant difference with P value = 0.000 (P<0.05), which indicates that there were a difference in antioxidant activity levels of wet candied pineapple based on the ripeness level of pineapple. The results of the Pos Hoc test showed a P value = 0.000 (P<0.05) with the highest antioxidant activity value in half-yellow pineapple wet candied.

Conclusion: The conclusion of this study is there are differences in vitamin C levels and antioxidant activity in pineapple wet candied based on the level of ripeness as a functional food product. Wet candied with half yellow pineapple contains the highest vitamin C and the highest antioxidant activity.

Keywords: Functional foods, Fruit ripeness level, Antioxidants, Vitamin C, Pineapple wet candied

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INTRODUCTION

Indonesia is a region with a tropical climate and a location in the equator. Indonesia allows the growth of various kinds of lush plants such as fruits and vegetables. Fruits contain various vitamins needed by the body, one of which is vitamin C. Vitamin C acts as an antioxidant and is effective in overcoming free radicals that damage cells or tissues, including protecting the lens from oxidative damage caused by radiation [1].

Vitamin C is easily soluble in water; therefore when undergoing the process of slicing, washing and boiling foods containing vitamin C will experience a decrease in levels. The content of vitamin C in fruit and food will be damaged due to the oxidation process by the outside air, especially when heated. Storage is carried out at low temperatures (in the refrigerator) and cooking that does not cause color changes in foods containing vitamin C [2]. Vitamin C has anti-infective properties by helping to heal diseased or damaged body parts [3]. A person who is deficient in vitamin C is likely to reduce the antioxidant power in his body making it easy for stress, cancer sores, gingivitis and bone pain [4]. Vitamin C is useful for preventing cancer sores, maintaining healthy teeth and gums, and protecting from other diseases caused by vitamin C deficiency [5].

Pineapple is one type of fruit that is in great demand by the public. Pineapple is elliptical in shape, the skin of the fruit is scaly. The recommended vitamin C requirement is 30-60 mg per day, while the average vitamin C adequacy for a family is (53.7±2.2) mg [6]. Pineapple was known as a fruit rich of the enzyme bromelain, vitamin C, beta carotene and carotenoids. Vitamin C, beta carotene and carotenoids are known as free radical scavenging antioxidants, so consuming pineapple regularly can protect the body from various diseases, including cancer, coronary heart disease and premature aging [7]. Pineapple fruit in fresh condition only has a shelf life of 1-7 d at a temperature of±22 °C [8]. The amount of pineapple production is very abundant at harvest time, but it is not proportional to the level of consumption so that the selling price in the market is very cheap. Pineapple fruit is not utilized at the time of harvest, it is necessary to make efforts to extend the shelf life, increase the economic value and diversify the product so that it can be accepted by consumers [9].

The level of vitamin C in fruit is strongly influenced by the variety, environment, place of growth, the use of various types of fertilizers and the level of fruit maturity [10]. Classification of the level of ripeness of pineapples based on different skin colors from green to yellow to produce pineapples in 3 (three) categories, namely raw, ripe, and very ripe. Green pineapple fruit is included in the raw category, yellowish-green is included in the ripe category and full yellow is included in the very ripe category [11].

The results of the research by Syahrumsyah *et al.* (2010) showed that vitamin C levels in full green pineapple were 2.24%, half yellow pineapple were 1.92% and full yellow pineapple fruit was 1.75%. Raw fruits contain higher levels of vitamin C than ripe fruits. Vitamin C levels in fruit will increase until the fruit is ripe, and will decrease when the level of maturity has been exceeded. This decrease is due to the level of vitamin C in ripe fruit, which will turn into glucose [12]. Ripe fruit will increase water content, total dissolved solids, color, aroma, fruit texture, starch and sugar, while vitamin C content generally decreases [13].

Pineapple fruit that undergoes processing can increase shelf life and a wider marketing reach. This processing can be an added value to the income of pineapple farmers [14]. Pineapple fruit can be processed into various products such as pineapple jam, lunkhead, syrup, jelly, pineapple chips and Wet candied fruit. Processing pineapples into various kinds of products is one of the efforts so that pineapples are not wasted and rotten during the main harvest [10].

Wet candied is a preserved food with sugar and one form of processed food that is much liked by the public. The taste is sweet

mixed with a distinctive taste. The fruit used for sweets is fruit with an old maturity level with a hard fruit texture. Wet candied can be divided into two types, namely Wet candied and dried candied fruit [15]. The purpose of giving sugar is to preserve sweets, give a sweet taste, and prevent the growth of microorganisms (fungi).

One of the components of functional food that have a physiological function for the body is an antioxidant [16]. Antioxidants are substances that are able to neutralize or reduce the negative effects of free radicals. Free radicals are molecules that have an unpaired electron group in an outer circle. Types of antioxidants that can catch the free radicals include ascorbates, tocopherols, flavonoids, and the presence of lycopene [17]. Measurement of antioxidant activity with The DPPH method (2,2-diphenyl-1-picrylhydrazyl) is a simple method and using a small number of samples in a short time [18].

Based on the background, researchers are interested to find out the effect of pineapple ripeness on functional food of pineapple wet candied on vitamin C levels and antioxidant activity.

MATERIALS AND METHODS

Plant material

The material used for the study was the pineapple fruits with three category of ripes (Full green, half yellow and full yellow fruits). The pineapple fruit that used in this study was confirmed as the queen pineapple, which had the characteristics of sharp thorny leaves, oval fruit resembling a cone. Plant determinations result was carried out in the Biology laboratory, Faculty of Mathematics and Natural Sciences, Sebelas Maret University (UNS). The determination results of the sample was 1b, 2b, 3b, 4b, 7b, 9b, 10a, Class: 57: Plants with rosette leaves 92B, 100A, 101B, 102A, Family 23: *Bromeliaceae*, Genus 1. *Ananas*, Species: *Ananas comosus* (L) Merr. (pineapple) Variety: queen [19].

Chemical and reagent

Destilated water Kimia ARD®, Mg powder Merck®, HCl Merck®, methanol p. a, merck®, $AlCl_3$ 10% Merck®, acetic acid 5% Merck®, DPPH (*2,2-difenil-1-pikrilhidrazil*) Sigma®, wet candied pineapple fruit full green, full yellow, and half yellow, ascorbic acid powder merck®, KMnO4 merck®, and filter paper ashless grade Cytiva®

The preparation of samples

The pineapple wet candied-making preparation is the full green, half yellow, and full yellow Pineapple fruit was peeling off the skin. Pineapple was cut into pieces as desired with uniform size and the center of the pineapple is removed then each pineapple flesh is weighed as much as 500 grams and washed with clean water. Pineapple flesh is soaked in 1% lime water for 1 hour, then washed with clean water and drained. Cinnamon and cloves are added to a 50% sugar solution and brought to a boil; after boiling the pineapple flesh slices are added to the sugar solution and brought to a boil again. Pineapples is soaked in a sugar solution for 1 night, the longer the soaking in the sugar solution the better the sweetness. The wet candied pineapple is then put into a plastic standing pouch and stored in the refrigerator.

The procedure of preparation of samples solution were pineapple wet candied which has full green, half yellow, and full yellow, each weighed as much as 200 g, washed thoroughly and put into a juicer to separate the juice and pulp. Pineapple wet candied juice was filtered using filter paper to separate the residue and the filtrate. The filtrate was taken as much as 0.5 ml and then put into a 10 ml volumetric flask and then aqua dest was added to the mark. The filtrate obtained is ready to be used as a sample solution for qualitative and quantitative tests of vitamin C [3].

Identification of vitamin C with $KMnO_4$

Reagent A 5 ml sample is added with 5 drops of $KMnO_4$ reagent, if the sample is positive for vitamin C it can fade the purple color of $KMnO_4$ and over time, the solution will turn brown [20].

Determination of vitamin C levels

The vitamin C levels was determination with the Spectrophotometry method. First step is the preparation of 100 ppm ascorbic acid standard solution. Ascorbic acid was weighed as much as 5 mg and dissolved in aquadest up to 50 ml in a volumetric flask. The second step was the determination of ascorbic acid maximum wavelength, which scaning 10 ppm ascorbic acid in the range of 250-290 nm. The maximum wavelength was used for scaning the ascorbic acid Calibration Curve. The standard curve of ascorbic acid was obtained by diluting the standard solution prepared and then taken as much as 0.4; 0.6; 0.8; 1 and 1.2 ml then put into a 10 ml volumetric flask and add aquadest to the limit mark to get a concentration of 4; 6; 8; 10 and 12 ppm. Each concentration was measured for absorbance at the maximum wavelength that had been obtained, then a calibration curve and linear equation were made for quantitative tests of samples containing vitamin C. The equation was used for the quantification of vitamin C levels in pineapple wet candied. The sample solution was put into a cuvette, the cuvette was inserted into a UV-Vis spectrophotometer and its absorbance was measured at the maximum wavelength. The absorbance value obtained is entered into the calibration curve equation y = bx+a so that the x value or vitamin C concentration in the sample can be calculated. The concentration obtained is then entered in the following formula (Equation 1) [21]:

$$\% \text{ vitamin C} = \frac{\text{consentration of vitamin C in sample}}{\text{consentration sample}} \times 100\ \% \ldots\ldots \text{(Eq.1)}$$

Determination of vitamin C levels was replicated 3 times [22].

Antioxidant activity test DPPH method

The antioxidant activity test of pineapple wet candied using the DPPH method [23]. The wet candied pineapple test solution was made in various concentrations of 8 g/ml, 16 g/ml, 32 g/ml, 64 g/ml, and 128 g/ml. Each concentration was pipetted 1 ml into a test tube, added 1 ml of DPPH solution and then added with 2 ml of methanol. Shaken until homogeneous, then incubated at 37 °C for 30 min, the absorbance was measured at a wavelength of 507 nm (maximum wavelength of DPPH). As a comparison, vitamin C was used. Calculation of DPPH radical immersion using the Equation 2:

$$\% \text{ Inhibition} = \frac{\text{Control absorbantion–sampel absorbantion}}{\text{Control absorbantion}} \text{x}100\% \ldots\ldots \text{(Eq.2)}$$

RESULTS AND DISCUSSION

The characteristic of pineapple wet candied

Making wet candied from the three types of ripe pineapple fruit produces almost the same organoleptic characteristics. organoleptic test results are presented in the following table 1 and fig. 1.

Identification of vitamin C with $KMnO_4$

The results of the identification of pineapple wet candied fruit of full green, half yellow, and full yellow pineapple wet candied showed positive results, indicated by the change purple color of $KMnO_4$ turning brown (table 2).

Fig. 1: Pineapple wet candied making result

Table 1: Characteristic of vitamin C in pineapple wet candied

Ripeness levels	Flavours	Colors	aroma	Texture
Full green	sweet, slightly sour	light yellow	Pineapple aroma	crunchy
Half yellow	sweet, slightly sour	light yellow	Pineapple aroma	crunchy
Full Yellow	sweet, slightly sour	yellow	Pineapple aroma	crunchy

Table 2: Identification of vitamin C in pineapple Wet candied with $KMNO_4$

Subject	Indication test	Result	Interpretation
Ascorbic acid	The purple color of KMnO4	The purple color of KMnO4 disappears and becomes brown	+
Destilated water	disappears and becomes	The purple color of KMnO4 was stay	-
Full green pineapple wet candied	brown	The purple color of KMnO4 disappears and becomes brown	+
Half yellow pineapple wet candied		The purple color of KMnO4 disappears and becomes brown	+
Full yellow pineapple wet candied		The purple color of KMnO4 disappears and becomes brown	+

Note: (+) = contains vitamin C, (–) = does not contain vitamin C

Identification of vitamin C with $KMnO_4$ reagent occurred reduction and oxidation (redox) reactions. Vitamin C acts as a reducing agent, which is oxidized, while $KMnO_4$ acts as an oxidizing agent, which is reduced. Vitamin C can reduce permanganate ions because permanganate can be reduced in an acidic environment to manganese ions, while vitamin C is oxidized by permanganate ions because it has the potential to release H+ions into dehydroascorbic acid. Ion permanganate accepts electron ions that escape from vitamin C so the purple color of KMnO4 disappears [24]. The addition of potassium permanganate reagent to pineapple wet candied results in a changing color of KMnO4 from purple to brown which indicates the formation of manganese ions (Mn^{2+}). The reaction mechanism of vitamin C compounds with KMnO4 reagent can be seen bellow:

$$5C_6H_8O_6+2MnO_4^-+6H^+ \rightarrow 5C_6H_8O_7+2\ Mn^{2+}+3\ H_2O$$

Determination of vitamin C levels

Determination of vitamin C levels in pineapple wet candied was carried out using the UV-Vis spectrophotometry method because ascorbic acid is a compound that have chromophore and auxochrome groups in its structure so that it can be detected by UV detectors and provides absorption at UV wavelengths, in addition to UV spectrophotometry methods. UV-Vis has the advantages of being fast easy, has a low detection limit and has a high level of accuracy and precision [25]. In this research, the maximum wavelength is searched; the maximum wavelength is the wavelength that gives the largest absorption and is the same in each concentration. The maximum wavelength of a compound can be different if it is determined under different conditions and tools, so before determining the concentration, the maximum wavelength of ascorbic acid is determined. Determination of the maximum wavelength in this study was carried out by measuring the absorbance value of 10 ppm ascorbic acid solution in the wavelength range of 250-290 nm. The results of the absorbance measurement of 10 ppm ascorbic acid obtained the highest absorbance value at a wavelength of 266 nm with an absorbance value of 0.676.

Vitamin C levels in samples of pineapple wet candied can be determined using a calibration curve by measuring the absorbance of the sample and then calculated using a linear regression equation. The regression equation (fig. 2) generated from the calibration curve is y = 0.0588x+0.088 and the correlation coefficient (R^2) is 0.9985.

Based on the research results, it is known that the calibration curve obtained is linear because the value (R^2) is close to 1, the correlation coefficient is used to determine the linearity of an analytical method. The correlation coefficient value close to 1 indicates the relationship between absorbance and concentration has a linear correlation where all points lie on a straight line [26]. Determination of vitamin C levels in candied pineapple fruit (table 3) was carried out using the UV-Vis spectrophotometry method, whose absorbance was measured at a wavelength of 266 nm.

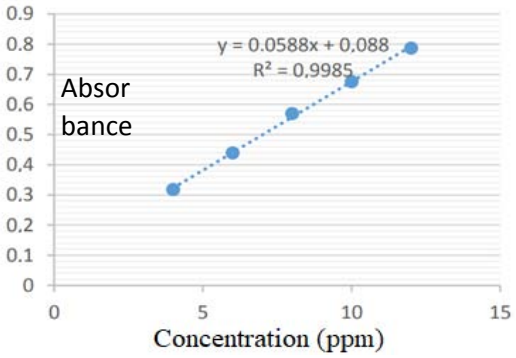

Fig. 2: The calibration curve of vitamin C

Table 3: Calculation results of vitamin C levels in wet candied pineapple

Subject	Levels average±SD (mg/gram)
Full green pineapple wet candied	4.22±0.171
Half yellow pineapple wet candied	17.24±0.189
Full yellow pineapple wet candied	0.75±0.047

Note: The data were presented in mean+SD, n=3

The results of the calculation of the average vitamin C content of wet candied pineapple±SD value obtained indicate that the method used has a high precision value. The precision criterion is determined based on the value of the resulting standard deviation is small and close to zero, the smaller the SD value indicates, the more precise the resulting data [27]. According to Putri and Setiawati (2015) the recommended ADI (Nutritional Adequacy Rate) of vitamin C is 30-60 mg per day, while in±2000 mg of half yellow pineapple wet candied it has been able to suitable the ADI, this is due to the processing process.

Based on the results of this study, it can be seen that half yellow pineapple wet candied has the highest average of vitamin C content, compared the candied full green, and full yellow pineapple candied. Based on the results of this study, it can be seen that vitamin C levels are influenced by the level of maturity, vitamin C levels in fruit will increase until the fruit is half ripe, and will decrease when the level of maturity overs, this is because vitamin C levels in overripe fruit will turns into glucose. Ripe fruit will increase water content, total dissolved solids, color, aroma, fruit texture, starch and sugar, while vitamin C content generally decreases [13].

Antioxidant activity test DPPH method

The antioxidant activity test of wet candied fruit with variations in the level of fruit maturity was carried out using the DPPH method

principle (table 4). The principle of measuring antioxidant activity using the DPPH reagent method is a change in the intensity of the purple color of DPPH, which is comparable with the concentration of the DPPH solution. DPPH free radicals which have unpaired electrons will give a purple color. Color will change turns yellow when the electrons are paired. The change in intensity of this purple color occurs due to the reduction of free radicals produced by the reaction DPPH molecule with a hydrogen atom. The hydrogen atom released by sample compound molecules to form 2,2-diphenyl-1-picrylhydrazine and causes DPPH color decay from purple to yellow. This color change will give a change in absorbance at length the maximum waveform of DPPH when measured using UV-Vis spectrophotometry [28].

Table 4: IC_{50} value of antioxidant activity test

The levels of ripeness	Average±SD of IC_{50} (mg/l), n=3
Full green	61,86±0,75
Half yellow	49,98±0,85
Full yellow	72,11±0,69
Vitamin C	12,09±0,033

Note: The data were presented in mean+SD, n=3

Based on the antioxidant activity test on candied pineapple with various levels of ripeness of full green, half yellow, and full yellow, respectively, the average IC_{50} value was 61,86; 49,98 and 72,11 mg/l (fig. 3).

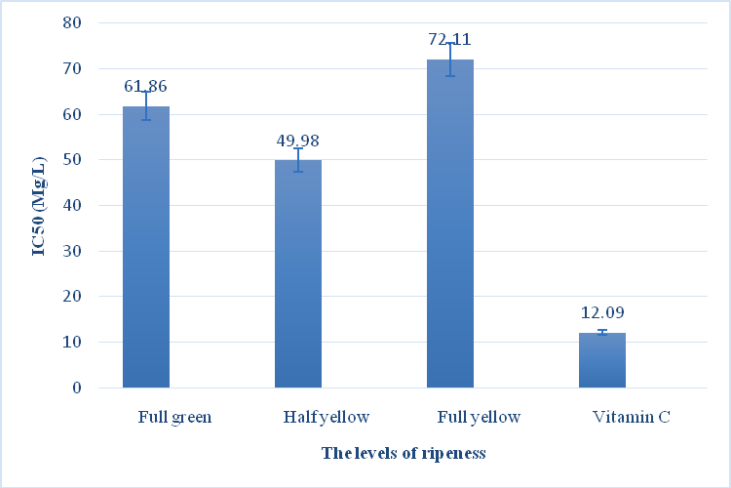

Fig. 3: Antioxidant activity test result, Note: The data were presented in mean+SD, n=3

Based on the IC_{50} value, it can be categorized as the potential antioxidant activity of pineapple wet candied with various levels of ripeness full green, half yellow and full yellow, the potential value respectively strong, very strong, and strong. The result of antioxidant activity can be influenced by the vitamin C content of each pineapple wet candied. The antioxidant activity of the three ripe pineapples on wet candied fruit was significantly different p value<0,05 (asym. sig=0,00. Based on the statistical test results, the antioxidant activity of vitamin C was significantly different (p value<0,05).

Vitamin C was a very strong antioxidant compound because it had a small IC_{50} value, which is 12,09 g/ml (IC_{50} value is less than 50). Vitamin C have four hydroxyl groups. Vitamin C can react directly with the hydroxyl anion by donating one electron to form a semihydroascorbate compound which is not reactive and then undergoes a disproportionation reaction to form an unstable dehydroascorbate. Dehydroascorbate will be degraded to form oxalic acid and threonic acid. Vitamin C is a polar compound, the higher the concentration of vitamin C, the more abundant hydroxyl groups and the intensity of antioxidant activity increases [29].

CONCLUSION

The conclusion of this study is there are differences in vitamin C levels and antioxidant activity in pineapple wet candied based on the level of ripeness as a functional food product. Wet candied with half yellow pineapple contains the highest vitamin C and the highest antioxidant activity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Rezvani HT, Moradi P, Soltani F. The effect of nitrogen fixation and phosphorus solvent bacteria on growth physiology and vitamin c content of Capsicun annum L. Iran J Plant Physiol. 2013;6:673-82.
2. Biswas AK, Sahoo J, Chatli MK. A simple UV-vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. LWT- Food Sci Technol. 2011;44(8):1809-13. doi: 10.1016/j.lwt.2011.03.017.
3. Rahman N, Ofika M, Said I. Analysis of vitamin C levels of gadung mango (Mangifera sp) and golek mango (Mangifera indica L.) based on maturity level using iodimetric method. Chem Acad J 2015;4:33-7.
4. Gahler S, Otto K, Bohm V. Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. J Agricul Food ChemJ Agric Food Chem. 2003;51(27):7962-8. doi: 10.1021/jf034743q, PMID 14690380.
5. Handrian RG, Meiriani H. Increasing levels of vitamin C in lowland tomato (Lycopersicum escelentum Mill.) with GA hormone. Agrotechn Onl J 2013;2:333-9.
6. Putri MP, Setiawati YH. Analysis of vitamin C levels in fresh pineapple (Ananas comosus L.) and canned pineapple fruit with UV–vis spectrophotometry method. Wiyata J 2015;2:34-8.
7. Lingga L. Hypertension free without drugs. Jakarta: Agro Media Library; 2012.
8. Lies MS. Making various processed pineapple. Jakarta: Puspa Swara; 2001.

9. Syahrumsyah H, Murdianto W, Pramanti N. Effect of addition of carboxymethyl cellulose (CMC) and pineapple (Ananas comosus (L.) Merr.) maturity level on pineapple jam quality. J Agricul Tech. 2010;6:33-40.
10. Suyanti. Guidelines for processing 20 types of Fruit. Jakarta: Self-Help Spreader; 2010.
11. Lustini A, Saparudin PA. Classification of pineapple maturity levels using red–green–blue and hue–saturation–intensity color spaces. Digit J Inf Tech. 2019;2:1-8.
12. Oktoviana Y, Aminah S, Sakung J. Effect of storage time and sodium benzoate concentration on vitamin C levels of red chili (Capsicum annum L). Chem Acad J 2012;1:193-9.
13. Julianti E. Effect of maturity level and storage temperature on the quality of Dutch eggplant (Cyphomandra betacea). Indonesian Hortic J 2011;2:14-20.
14. Afrianti LH. 33 Kinds of fruits for health. Bandung: Alphabeta; 2010.
15. Muaris H, Fruit C. Gramedia Pustaka Utama; 2003.
16. Puspita NS, Tamrin RA. Kajian Pembuatan minuman Fungsional dari daun Sirsak (Annona muricat Linn.) dengan penambahan Bubuk Jahe (Zingiber officinale). J Sains Tekno Pangan. 2019;4:2086-95.
17. Andriani Y, Ekstrak Betaglukan UAA. Dari saccharomyces cerevisiae. J Gradien 2007;3:226-30.
18. Hasnaeni H, Aminah A. Uji aktivitas antioksidan dan profil fitokimia ekstrak kayu beta-beta. Lunasia Amara Blanco JFG 2019;5(1):101-7. doi: 10.22487/j24428744.2019.v5.i1.12404.
19. Backer CA, Brink RCB. Flora of java. Taxon. 1966;15(2). doi: 10.1002/j.1996-8175.1966.tb00230.x.
20. Shaha RK, Rahman S, Asrul A. Bioactive compounds in chilli peppers (Capsicum annuum L.) at various ripening (green, yellow and red) stages. Annals Biol Res. 2013;4:27-34.
21. Klimczak I, Małecka M, Szlachta M, Gliszczynska swigło A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J Food Compos Anal. 2007;20(3-4):313-22. doi: 10.1016/j.jfca.2006.02.012.
22. Karinda M, Fatimawali, Citraningtyas CG. Comparison of results of determination of vitamin C levels of dodol mango using UV-vis spectrophotometry and iodometry methods. J Pharmacol. 2013;2:86-9.
23. Brand Williams W, Cuvelier EMME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995;28(1):25-30. doi: 10.1016/S0023-6438(95)80008-5.
24. Chandra B Zulharmita, Putri WD. Determination of vitamin C and B1 levels in red dragon fruit (Hylocereus lemairel (Hook.) britton and rose) using UV–vis spectrophotometric method. J Hygea Pharm. 2019;11:62-74.
25. Ngibad K, Herawati D. Comparison of measurement of vitamin C levels using UV-vis spectrophotometry at UV and visible wavelengths. Borneo J Medical Lab Technol. 2019;1:77-81.
26. Selpiana E, Destiarti L, Nurlina. Comparison of Pb(II) determination methods in kapuas river by UV–vis spectrophotometry separate calibration method and standard addition. J Equatorial Chem. 2016;5:17-23.
27. Padmaningrum RT, Marwati S. Validation of cyclamate analysis methods by spectrophotometry and turbidimetry. J Basic Sci. 2015;4:23-9.
28. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26:211-21.
29. Lung JK, Destiani DP. Uji aktivitas antioksidan vitamin A, C, E dengan metode DPPH. Farmaka. 2017;15:53-62.