DETERMINATION OF VITAMIN C CONTENT IN BELL PEPPER (*Capsicum annuum L.*) WITH DIFFERENT PROTIC POLAR SOLVENT BY UV-VIS SPECTROSCOPY

Fawait Afnani [1*], Jamilah Hamidi Yanti[1], Wiwit Sri Werdi Pratiwi[2]
[1] Department of Diploma 3 Pharmacy, Yannas Husada Pharmacy Academy, Jl. Letnan Singosastro No.3, Kraton, Bangkalan 69115, East Java, Indonesia
[2]Department of Marine Science, University of Trunojoyo Madura, Jl. Raya Telang 2, Kamal, Bangkalan 69162, East Java, Indonesia
*Email: fawaitafnani@gmail.com

Received 15 April 2023
Accepted 31 October 2023

Abstract

Bell pepper can be an antioxidant and has many health benefits because of the high content of vitamin C. Vitamin C contents in yellow and orange bell peppers were extracted using different protic polar solvents and analyzed using the UV-Vis Spectrophotometry method. In this research, the bell peppers were extracted using the maceration technique for two days in 90% concentration of solvents (methanol and ethanol, respectively). Subsequently, the maximum wavelength was determined, and then 100 ppm ascorbic acid was used as a standard solution to analyze vitamin C content. Linearity based on a calibration curve is used to obtain the correlation coefficient of concentration between the standard solution and vitamin C levels in the sample. The result of UV-Vis spectroscopy analysis of this sample shows 373.5 nm of λ_{max}. The linearity is shown in the equation y = 0.0006 x + 0.019. The vitamin C content in all samples had significant differences based on randomized complete block design (RCBD) test result with $\alpha = 0.05$. The vitamin C level in the yellow pepper sample with an ethanol solvent (340 mg/100 g) was higher than that of the orange pepper sample with ethanol (251 mg/100 g). Meanwhile, the vitamin C content in the sample of yellow peppers with methanol solvent (562.5 mg/100 g) was smaller than that of orange peppers with methanol solvent (757.5 mg/100 g). These contents indicated a different result in the level sources of vitamin C, even if the maceration process used a solvent with a higher dielectric constant.

Keywords*: bell pepper, protic polar solvent, UV-Vis spectroscopy, vitamin C*

Introduction

Vegetables and fruits have an important role in improving nutrition (Nadeem *et al*.,2011). One of the vegetable commodities that contains nutrients is the bell pepper. Bell pepper contains relatively high amounts of important nutrients such as vitamins (vitamin C, Vitamin A, and vitamin E), potassium, carotenoids, fibers, fats, proteins, capsaicinoids, polyphenols, flavonoids, volatile oil, and carbohydrates in varying concentrations (Nadeem et al., 2011; Duracasu, 2007; Pathirana, 2013). The nutritional content of pepper depends upon the genotype, and some pepper varieties have been reported to have high levels of vitamin C, vitamin A, and folate(Hoffman et al., 1983). These nutritional values can enrich many health benefits, especially the good source of vitamin C as an immunity. Vitamin C has played an important role as an antioxidant with anti-inflammatory and immune system enhancement, protection against cancer, collagen and carnitine synthesis, cholesterol metabolism, and formation of neurotransmitters. Lack of vitamin C in the human body causes mouth ulcers, muscle pain, weight loss, and lethargy. (Abiri and Vafa, 2020; Rosmainar Lilis et al., 2018).

Vitamin C is better known as ascorbic acid. Different levels of vitamin C depend on maturity stages, varieties, storage, and growth conditions (Abiri and Vafa, 2020; Lee, 2000). Several maturities indicated by colors may be due to differences in containing levels of vitamin C. Determination of vitamin C levels of bell pepper in various colors that depending on maturity stages shown that green, yellow, orange, and red bell pepper was obtained 16.52 mg/100 mg; 159.61 mg/100 mg; 121.38 mg/100 mg; 81.19 mg/100 mg, respectively (Nerdy,2018). Therefore, the result revealed that bell peppers with yellow and orange colors have high vitamin C content. Nevertheless, this vitamin C content depended on extraction to uptake vitamin C, also known as ascorbic acid, in bell pepper.

The process of taking an ascorbic acid compound needs a method that must be adapted from its characterization. Vitamin C is sensitive to heat and drying, so maceration is used to extract vitamin C (Nadeem *et al*.,2011). One factor that influenced the extraction rate was the type of solvent (Utami *et al*., 2020). Vitamin C compounds are polar, so a polar solvent is needed (Verdiana *et al*., 2018). Protic polar solvents (methanol and ethanol) were used to obtain different levels of vitamin C in yellow and orange bell peppers, especially for yellow bell peppers using ethanol solvent, whereas another one is dissolved by methanol. According to the result, yellow bell peppers contained higher vitamin C than orange bell peppers (Nerdy, 2018). In addition to the dielectric constant at 20 °C, methanol has a value of 32.6 and ethanol 22.4 (Mirbagheri *et al*., 2017).

There are several methods to determine vitamin C levels. Spectroscopy and iodometry methods are basic in the instrument and non-instrument analysis category. UV-VIS Spectroscopy is mostly used to determine ascorbic acid because it absorbs the UV rays at 200-400 nm. Meanwhile, the iodometric method is a titration method by using several oxidizing agents such as KIO_3 (potassium iodate), potassium permanganate ($KMNO_4$), and potassium dichromate ($K_2Cr_2O_7$) to form dehydroascorbic acid and iodide ions. The excess I_2 in the solution can be titrated with sodium thiosulphate solution. As reported, the spectroscopy method was more efficient in determining levels of vitamin C (Karinda *et al*., 2013; Ullah *et al*., 2012). Bell pepper with different colors (yellow indicated more mature than orange) will determine levels of vitamin C with protic polar solvent by spectroscopy method and has not been reported previously. This research aims to show the difference in vitamin C levels in yellow and orange bell peppers with protic polar solvents.

Research Methods

Materials

Chemicals used are bell pepper (yellow and orange), Vitamin C (Merck), distilled water (Brataco), ethanol 90% (Merck), and Methanol 90% (Merck).

Instrumentation

The apparatus and instruments used are analytical balance (Shimadzu), volumetric flask 25 mL, 50 mL, 100 mL (Iwaki), beaker glass 10mL, 50 mL, 100 mL (Pyrex), test tube (Pyrex), Buchner funnel, volume pipette 5 mL, 10 mL (Pyrex), measuring pipette 5 mL, 10 mL (Iwaki), Erlenmeyer 25 mL, 50 mL (Iwaki), Whatman No.2 filter paper, set of distillation equipment, blender (Philips), cutting blades, oven, and UV-Vis (Thermo Electron Sci Inst UV-VIS Spectrophotometer 335908P-000).

Procedure

1) Sample preparation of bell pepper extract
   Fresh-peeled fruits of bell peppers (yellow and orange) were washed, blended until smooth, and carefully weighed 100 g, respectively. Separate become 2 parts of each sample, and then the samples were macerated with 150 mL of ethanol 90% and 150 mL of

methanol 90% for 2 days in a closed beaker glass and at room temperature. The macerate was filtered by Whatman filter paper to get the filtrate and then concentrated by a distillation apparatus at 50℃ to yield the slurry-dense extract.

2) Preparation of vitamin C stock solution 100 ppm
   50 mg of vitamin C was weighed, put into a 500 mL volumetric flask, added double distilled water to the marking line, shaken until dissolved, and homogenized the mixture.
3) Determination of the maximum wavelength of vitamin C solution
   5 mL vitamin C stock solution 100 ppm was pipetted into a volumetric flask 50 mL, added double distilled water to the marking line, and then homogenized it. The solution was measured at a wavelength range of 250–400 nm. The maximum wavelength has been obtained to indicate the highest absorbance value (Ahmad *et al*., 2015).
4) Determination of the calibration Curve of vitamin C solution
   The stock solution of vitamin C 100 ppm was pipetted into a volumetric flask 50 mL (4, 6, 8, 10, 12 ppm), double distilled water was added to the marking line and then homogenized. The measurement was done at 373.5 nm to obtain an absorbance value. The plotting of absorbances vs concentrations is needed to make a linear calibration curve (Kamaruddin *et al*., 2021).
5) Determination of vitamin C levels in yellow and orange bell pepper
   1 g of extracted-dense sample was weighed, put into a 100 mL volumetric flask, added double distilled water to the marking line, and then mixed well up to homogenize. 2 mL of sample filtrate was pipetted, put into a volumetric flask 50 mL, and then added double distilled water to the marking line. The sample was measured at maximum wavelength absorption up to three times respectively. The interpolation of sample absorbance was regressed into a linear calibration curve of vitamin C.

Results and Discussion

Vitamin C is almost found in all plants, such as fresh fruits and vegetables (Grosso *et al*., 2013). The availability of vitamin C in bell pepper may play a role as a good source of replacement for reducing nutrient deficiencies, a nutritional supplement, and many medicines for influenza and colds because of high global consumption in several countries (Philips *et al*., 2006; Wahyuni *et al*., 2013; Lane *et al*., 2014). Thus, vitamin C is extracted using the maceration method. This method was selected to prevent the decomposition of the compound, particularly vitamin C, when compared with other methods that used heating, such as Soxhlet and reflux (Wahyulianingsih *et al*., 2016). The methanol solvent in process maceration generated a higher yield in yellow or orange bell peppers (Table 1). A study has reported that the yield of lemon (*Citrus limon (Linn) Burm. F*) using methanol solvent obtained higher than ethanol and acetone solvents (Mardawati *et al*., 2018; Verdiana *et al*., 2018). According to alcohol compounds was polar, the polarity is on the –OH group. Thus, the longer the carbon chain, the lower the polarity (Hasanah *et al*., 2019).

Table 1. Results of yield extraction bell pepper (orange and yellow) using ethanol and methanol solvents

Solvent	The yield of Extracted Bell Pepper (%)	
	Orange	**Yellow**
Ethanol	2.91	2.86
Methanol	4.44	3.94

Furthermore, the maximum wavelength measurement results using UV-VIS spectroscopy analysis of vitamin C solution showed 373.5 nm of λ_{max}. this absorption will provide the maximum sensitivity for each unit of the highest concentration (Pricilia, 2015). According to absorption maximum wavelength at around 373.5 nm and absorbance value, based on these results, the correlation coefficient at about 0.9842 shows the occurrence of linearity between the variables x and y (Figure 1).

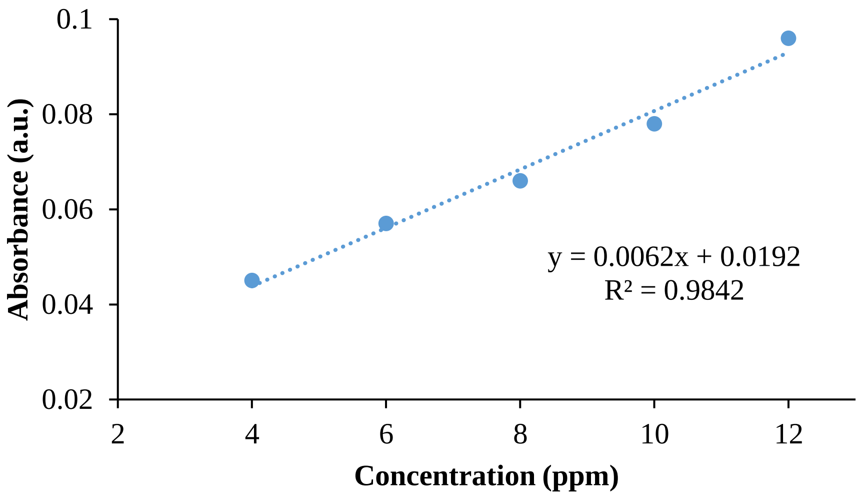

Figure 1. Calibration curve of vitamin C solution

The linear equation is used for determining vitamin C levels by putting in the absorbance value that obtained its absorption. Calculations have been done to obtain data of vitamin C levels in yellow and orange colors of bell pepper. The conversion of measurement unit was used to measure vitamin C levels in milligram per 100 grams. The data of measurement result can be shown in (Table 2).

The vitamin C content in all samples had significant differences based on randomized complete block design (RCBD) test result with α= 0.05. Vitamin C levels in yellow bell pepper with ethanol contained lower than in orange bell pepper with methanol. The obtained levels were different from the result obtained on previous research, that vitamin C levels of yellow bell pepper was higher than orange one, despite using a different solvent (Nerdy, 2018). Whereas vitamin C levels of yellow bell pepper in methanol contained higher than orange bell pepper in ethanol. Overall, between solvents that were used for extraction shown methanol obtained result higher than ethanol. The graph of vitamin C levels in yellow and orange colors of bell pepper can be shown in (Figure 2).

Table 2. The data of vitamin C levels in yellow and orange colors of bell pepper using UV-Vis spectroscopy at wavelength 373.5 nm

Data		**Solvent**					
		Ethanol			**Methanol**		
Number		**1**	**2**	**3**	**1**	**2**	**3**
Absorbance (A)	Yellow Bell Pepper	0.027	0.0269	0.0276	0.0327	0.0323	0.0326
		0.0271 ± 0.016[a]			0.0326 ± 0,019[c]		
	Orange Bell Pepper	0.0228	0.0261	0.0238	0.0372	0.0372	0.0372
		0.0238 ± 0.014[b]			0.0372 ± 0.000[d]		

*Based on the Randomized Complete Block Design (RCBD) test result with α= 0.05, the superscript letters show a significant difference (P<0.05)

Two main factors led to the different result of this research: the dielectric constant of methanol is higher than ethanol so that when the sample was being concentrated will increase reaction rate due to electrical energy and the impurities of solvent with the presence of polar and non-polar compounds that contained in bell pepper (Mirbagheri *et al*., 2017)(Dirar *et al*., 2019).

Other reasons can also be significant differences in vitamin C levels due to several factors such as varieties, growth, harvesting and post-harvest conditions, and maturity stages (Mateos *et al*., 2013). Also, due to the loss of some vitamins during the storage stages, temperature, exposure, color changes, acid levels, and storage time (Muhammad, 2016; Tosun, 2008). Decreased levels of vitamin C have to be prevented by being kept closed to avoid the oxidation process (Bieniasz *et al*., 2017.). However, interactions and other bioactive compounds of vitamin C were too complex, so extracting them need protic polar solvent(Contini *et al*., 2008; Naczk *et al*., 2006; Razali *et al*.,2012).

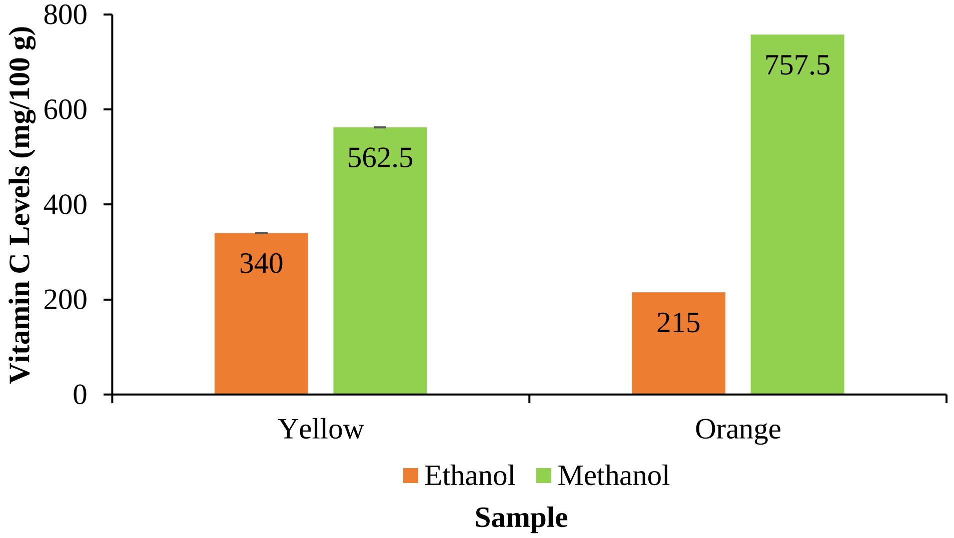

Figure 2. The graph of vitamin C levels in yellow and orange colors of bell pepper

Conclusions

The present study showed that vitamin C content in yellow and orange bell peppers with protic polar solvent by spectroscopy method had significant differences based on randomized complete block design (RCBD) test result with α = 0.05. In the same ethanol solvent, the vitamin C content in yellow bell pepper was 340 mg/100 gram, higher than in orange bell pepper at 215 mg/100 gram. Nevertheless, in the methanol solvent, there is a difference in vitamin C content in yellow bell pepper of 562.5 mg/100 gram, which was higher than in orange bell pepper of 757.5 mg/100 gram. The study suggested that solvents with different dielectric constants influenced the vitamin C content in bell peppers.

Author Contribution

All authors have read and agreed to the published version of the article.

Conflict of Interest

The authors have declared that no conflict of interest is associated with this work.

References

Abiri, B., and Vafa, M., 2020. Vitamin C and Cancer: The Role of Vitamin C in Disease Progression and Quality of Life in Cancer Patients, 1–11.

Ahmad, A., 2015. Penetapan kadar fenolik dan flavonoid total ekstrak metanol buah dan daun patikala (Etlingera elatior (Jack) RM SM). *Scholarhub.Ui.Ac.Id*, *2*(1).

Bieniasz, M., Dziedzic, E., 2017. The effect of storage and processing on vitamin C content in Japanese quince fruit. *Search.Proquest.Com*.

Contini, M., Baccelloni, S., Massantini, R., 2008. Extraction of natural antioxidants from hazelnut (*Corylus avellana L.*) shell and skin wastes by long maceration at room temperature. *Elsevier*.

Dirar, A., Alsaadi, D., Wada, M., 2019. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *Elsevier*.

Durucasu, I., 2007. Effects of grilling on luteolin (3’, 4’, 5, 7-tetrahydroxyflavone) content in sweet green bell pepper (Capsicum annuum). *Europepmc.Org*.

Grosso, G., Bei, R., 2013. Effects of vitamin C on health: a review of evidence. *Tadbirsalamat.Com*.

Hasanah, N., Putriani, E., 2019. Pengaruh Jenis Solubility Promotor dan Waktu Reaksi pada Sintesis α-Terpineol dari Minyak Terpentin menggunakan Katalis Zeolit Alam Lampung Teraktivasi. *Journal.Unnes.Ac.Id*, *8*(2).

Hoffman, P. G., Lego, M. C., and Galetto, W. G., 1983. Separation and Quantitation of Red Pepper Major Heat Principles by Reverse-Phase High-Pressure Liquid Chromatography. *Journal of Agricultural and Food Chemistry*, *31*(6), 1326–1330.

Kamaruddin, H., 2021. Determination of Polyphenol Content in Sawo Fruit (Manilkara zapota) Based on Geographical Location. *Jtpc.Farmasi.Unmul.Ac.Id*, *5*(3).

Karinda, M., Fatimawali, F., 2013. Perbandingan Hasil Penetapan Kadar Vitamin C Mangga Dodol Dengan Menggunakan Metode Spektrofotometri Uv-Vis Dan Iodometri. *Ejournal.Unsrat.Ac.Id*, *2*(01).

Nerdy., 2018. Determination of vitamin C in various colours of bell pepper (Capsicum annuum L.) by Titration Method. *Pdfs.Semanticscholar.Org*, *14*(1), 164–177.

Lane, D., 2014. The active role of vitamin C in mammalian iron metabolism: much more than just enhanced iron absorption. *Elsevier*.

Lee, S., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Elsevier*.

Mardawati, E., Filianty, F., 2008. Kajian aktivitas antioksidan ekstrak kulit manggis (*Garcinia mangostana L*) dalam rangka pemanfaatan limbah kulit manggis di Kecamatan Puspahiang Kabupaten. *Jurnal.Unpad.Ac.Id*.

Mateos, R., Jiménez, A., Román, P., 2013. Antioxidant systems from pepper (*Capsicum annuum L.*): involvement in the response to temperature changes in ripe fruits. *Mdpi.Com*.

Muhammad Auwal., 2016. Effect of different storage condition on pH and vitamin C content in some selected fruit juices (pineapple, pawpaw and watermelon). *Journalbank.Org*.

Mirbagheri, M., Mohebbi-kalhori, D., and Jirofti, N., 2017. Evaluation of Mechanical Properties and Medical Applications of Polycaprolactone Small Diameter Artificial Blood Vessels. *International Journal of Basic Science in Medicine*, *2*(1), 58–70.

Moneruzzaman, K., Hossain, A., 2008. Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato.

Naczk, M., 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Elsevier*.

Nadeem, M., Anjum, F., Khan, M., 2011. Antioxidant potential of bell pepper (Capsicum annum L.)-A review. *Academia.Edu*.

Pathirana, R., 2013. Peppers: Vegetable and Spice Capsicums , 2nd edition, by Paul W. Bosland and Eric J. Votava . *New Zealand Journal of Crop and Horticultural Science*, *41*(2), 102–103.

Pricilia, Grace., 2015. Validasi Metode Analisis untuk Penetapan Kadar Parasetamol dalam Sediaan Tablet Secara Spektrofotometri Ultraviolet. *Ejournal.Unsrat.Ac.Id*, *4*(4).

Phillips, K., 2006. Difference in Folate Content of Green and Red Sweet Peppers (Capsicum annuum) Determined by Liquid Chromatography−Mass Spectrometry. *ACS Publications*, *54*(26), 9998–10002.

Permata, Asiska., 2018. Penetapan Kadar Vitamin C dengan Spektrofotometri UV-Vis Pada Berbagai Variasi Buah Tomat. *Jurnal.Univrab.Ac.Id*.

Razali, N., Mat-Junit, S., 2012. Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of Tamarindus indica L. *Elsevier*.

Rosmainar, Lilis, Widia Ningsih, Ni Putu Ayu, Haula Nanda., 2018. Penentuan kadar Vitamin C Beberapa Jenis Cabai (Capsicum sp.) Dengan Spektrofotometri UV-VIS. Jurnal Kimia Riset Volume 3 No. 1.

Tosun, I., Ustun, N., 2008. Physical and chemical changes during ripening of blackberry fruits. *SciELO Brasil*.

Tyagi, S., Sahay, S., Imran, M., 2017. Pre-harvest factors influencing the postharvest quality of fruits: A review. *Researchgate.Net*, *23*(4), 1–12.

Ullah, S., Hussain, A., Ali, J., 2012. A simple and rapid HPLC method for analysis of vitamin-C in local packed juices of Pakistan. *Cabdirect.Org*.

Utami, N., Sutanto, S., 2020. Pengaruh berbagai metode ekstraksi pada penentuan kadar flavonoid ekstrak etanol daun iler (*Plectranthus scutellarioides*). *Journal.Unpak.Ac.Id*.

Verdiana, M., Widarta, I., 2018. Pengaruh jenis pelarut pada ekstraksi menggunakan gelombang ultrasonik terhadap aktivitas antioksidan ekstrak kulit buah lemon (Citrus limon (Linn.) Burm F. *Ojs.Unud.Ac.Id*.

Verdiana, M., Widarta, I., 2018. Pengaruh jenis pelarut pada ekstraksi menggunakan gelombang ultrasonik terhadap aktivitas antioksidan ekstrak kulit buah lemon (Citrus limon (Linn.) Burm F. *Ojs.Unud.Ac.Id*, *7*(4), 213–222.

Wahyulianingsih, W., 2016. Penetapan kadar flavonoid total ekstrak daun cengkeh (*Syzygium aromaticum* (L.). *Jurnal.Farmasi.Umi.Ac.Id*.

Wahyuni, Y., Ballester, A. R., Sudarmonowati, E., Bino, R. J., and Bovy, A. G., 2013. Secondary metabolites of Capsicum species and their importance in the human diet. *Journal of Natural Products*, *76*(4), 783–793.