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Printed Low-Cost Microfluidic Paper-based Analytical Devices for Quantitative Detection of Vitamin C in Fruits

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Abstract. Microfluidic paper-based analytical device has been fabricated and subsequently applied to determine the Vitamin C level of the non-seasonal fruit i.e pineapples, oranges, guavas, and apples in Indonesia. µPAD was prepared by creating a hydrophobic barrier on Whatman Chromatography paper No. 1 in a certain pattern printed using Xerox Colorcube 8580 DN-2. 0.05 µl of 2,6-Dichlorophenolindophenol was dispersed into a detection area on the µPAD devices prior to the addition of 0.2 µl of fruits juice samples. The barrier allowed the fruits juice to flow and alter the color of the reagents through a redox reaction. Vitamin C in fruit juice altered the color of 2.6-Dichlorophenolindophenol reagent into a pinkish color. The changing of the reagent color was then processed using Image J software to determine the RGB intensity level. The results showed that the average amounts of Vitamin C in pineapples, oranges, guavas, and apples are 0.05%, 0.05%, 0.08%, and 0.04% respectively.

1. Introduction

Vitamin C levels determination has been generally carried out by the volumetric titration method. This method requires some reagents which later help the researchers to indicate the level of vitamin C through the color changing [1]. The color changing itself derived from the redox reaction between vitamin C and the reagent [2]. The color changing is known as the basic principle in relevant to Vitamin C levels determination as well as the other quantitative-based researches determination. The titration method requires a lot amount of both reagents and samples which could reduce the effectiveness of a research study, indicated as high-cost research.

µPAD is a renewable methodology that has been developed since 2004 [3], and in 2007 the first µPAD was introduced [4]. Having the same principle as the titration, this new methodology has been known by its effectiveness used in research studies, such as µPAD to determination of metal in water [5], µPAD for detection of benzoic acid in food [6], µPAD for Glucose and Nitrite [7], µPAD for determination of alcohol content in whiskey samples [8]. µPAD has been known as a method that requires only a couple microliter-amounts of both reagents and samples. The color changing, which was generated by the redox reaction, also occurred on a paper-based (µPAD) device. µPAD has a lot more advantages, such as the innovative analytical method, also the low-cost of making the test kit (µPAD). Moreover, µPAD is a method that can continue to be developed in the future [9–12].

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There were few several studies found about the application of µPAD to quantitatively determine the levels of vitamin C in non-seasonal Indonesian fruits. Therefore, the determination of vitamin C levels using the µPAD method on non-seasonal fruits in Indonesian was observed in this study. The fruits used in this study were pineapple, orange, guava, and apple. The study aimed to describe the effectiveness of µPAD on determining the vitamin C level of Indonesian fruits.

2. Material and Methods

2.1 µPAD preparation

The µPAD was prepared according to Carillho [13] with a different pattern of µPAD. The µPAD pattern was designed with CorelDraw 2017 software and printed on Whatman Chromatography paper No. 1 (WhatmanTM, GE Healthcare, UK) using the Xerox Colorcube 8580 DN-2 type T2B047382 printer with Solid Ink Colorcube 8580 DN(USA). The printed-paper was heated at 120°C for 60 seconds and kept in a desiccator to reach room temperature.

2.2 Standard and Reagents Preparation

The standard concentration of vitamin C made in this study was prepared according to the study conducted by Jangsun [14] with several modifications. The standard concentrations of vitamin C used in the study were 0%, 0.01%, 0.02%, 0.04%, 0.05%, 0.08%, 0.1%. The reagents (2,6-dichlorophenolindophenol (DCPIP), Sodium hydrogen carbonate, and ascorbic acid) were purchased from Merck. The standards were dispersed on the µPAD to observe the color. The standard-µPADs were photographed and observed on Image J software to determine the RGB intensity level.

2.3 Samples Preparation

The samples were obtained from pineapple, orange, guava, and apple which are the non-seasonal fruits in Indonesia and were purchased from the local market. The preparation of the samples was carried out according to Janghel [15] with slight modifications. The samples were obtained from the separation of juice and pulp of the fruits and were weighed for 100 grams each sample.

2.4 Vitamin C Levels Determination

Quantitative determination of vitamin C levels was observed according to Songjaren [16] study with some modifications in the design of the µPAD device (Figure 1.). The DCPIP reagent, as much as 0.05 µl, was dripped in the detection zone prior to the dripping of the fruit samples and the color changed was observed.

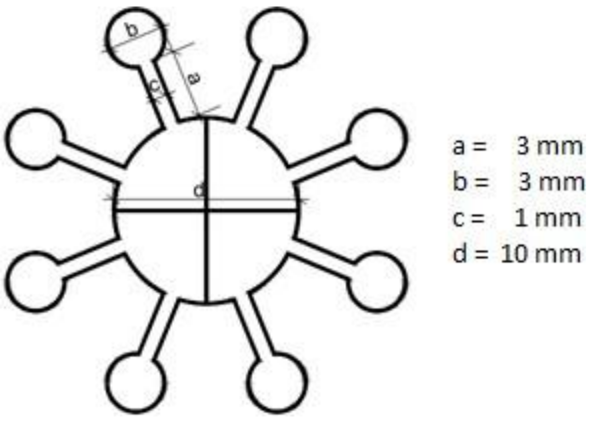

Figure 1. The design of the µPAD

The color changes shown on µPAD devices were photographed and observed. The photos of color changed on µPAD device was observed according to Orava [17] with some modifications. The photo was taken perpendicularly to the distance of 14-15 cm, without using the addition of lights as lights, the focus was arranged to make the object clearly visible. All settings were kept constant for taking photos of all samples. Then the photographs were then processed with Image J software to determine the RGB intensity level.

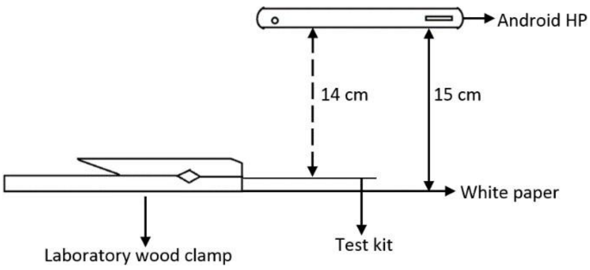

Figure 2. The imaging setup

2.5 Data Analysis

The data was obtained from the RGB intensity levels of the standard and the samples in five replications each. The Vitamin C levels of samples were determined from the RGB intensity level which was the closest to RGB standard. The RGB result between the standard and the samples were analyzed using Euclidean Distance method on Microsoft Excel 2016 program according to the research done by Anggraeni [18] with slight modification.

3. Result and Discussion

The data obtained from observing the standard RGB was presented in Figure 3. Figure 3 showed that the blue color decreased as the level of vitamin C increased, especially on 0.02% and 0.04%. The decreased of blue color led to a purple color which generated by the increased of the red color as the vitamin C level increased. Meanwhile, the green color of the standard was gradually increased as the vitamin level increased.

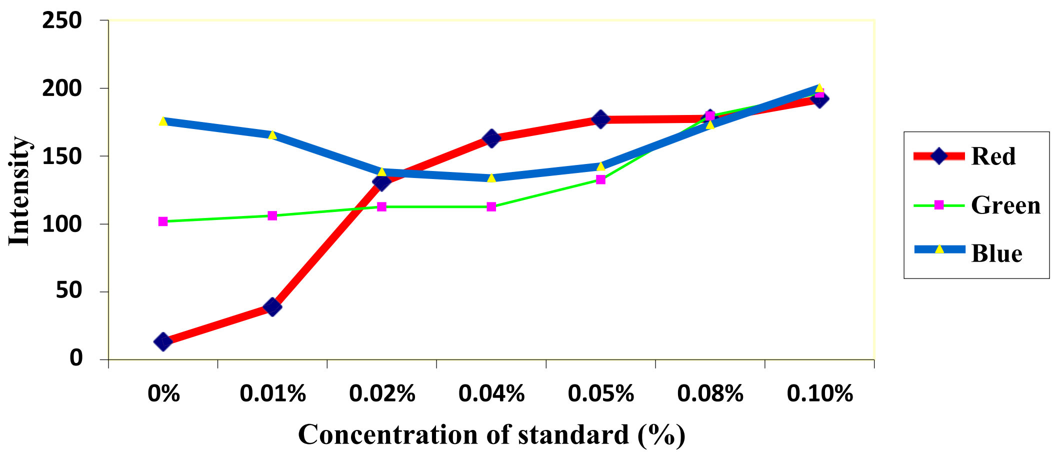

Figure 3. Relationship between Concentration and Intensity

From Figure 3 it is known that the intensity level of red increased while the intensity of the blue color decreases and returns to increase. This is because the DCPIP reagent, which is initially blue, changes to the color of pink and finally the color that is formed fades because of the higher standard concentration is given. The change in the color of the DCPIP reagent which indicates increasing vitamin C levels refers to the Grzelakowska study [19] that the lower the pH value, the increase in acidity and the decrease in pH causes the DCPIP reagent to fade.

3.1 Quantification of Vitamin C

The vitamin C determination of the samples was determined by the samples RGB intensity levels compared to the RGB intensity level of the standard. The standard RGB intensity level was presented in Figure 4. Figure 4 showed that each sample color changed was similar to the standard on some certain concentration.

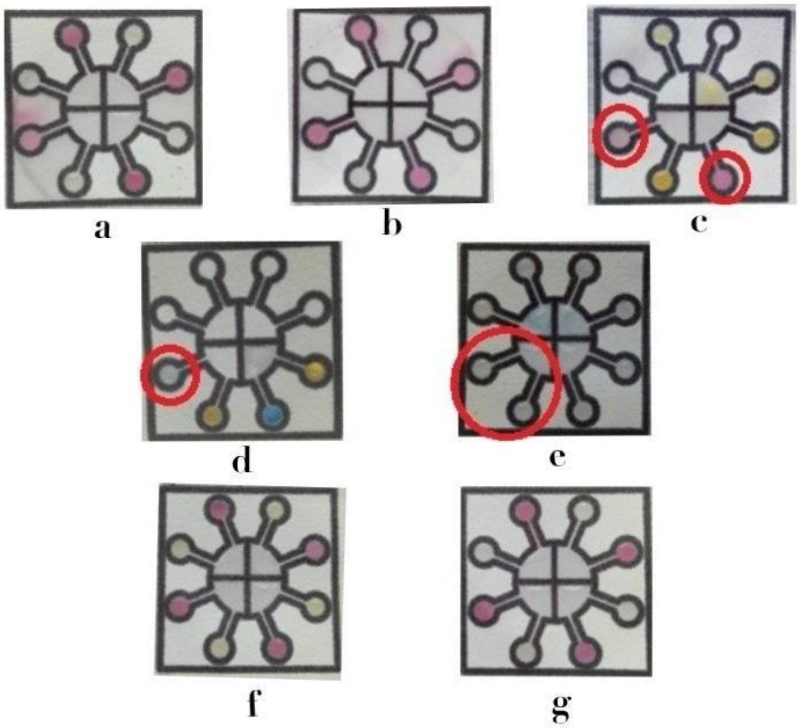

Figure 4. a. Color changed on µPAD obtained from the pineapple sample; b. Color changed on µPAD obtained from the orange sample; c. Color changed on µPAD obtained from 0.05% standard (in red circles); d. Color changed on µPAD obtained from guava sample (in red circle); e. Color changed on µPAD obtained from 0.08% standard (in red circle); f. Color changed on µPAD obtained from apple sample; and g. Color changed on µPAD obtained from 0.04%standard.

Determination of vitamin C level of the samples was done by comparing the color changed (RGB intensity levels) obtained from samples and from the standard. The RGB intensity levels of the standard and the samples were presented in Table 4 and Table 5 respectively. It was shown that each of the RGB intensity level of the sample was compared to the standard in near range of RGB intensity level. The RGB of pineapple was compared to the 0.05% standard which was in near range with the pineapple. The orange RGB level was compared to the same 0.05% standard as the pineapple. The RGB level of apple sample was more likely to be the lowest compared to the other samples. Whereas the guava tended to have the highest vitamin C since it was compared to the RGB level of 0.08% standard. Instead of comparing the data through a table, a figure of a chart was prepared to present the RGB curve of the samples and the standard. It was presented in Figure 5.

Table 1. The RGB Intensity level of standard

Concentration	Intensity		
	Red	Green	Blue
0%	12.905±0.22	101.542±0.28	175.782±0.11
0.01%	38.576±1.5	105.764±0.56	165.51±0.33
0.02%	130.766±0.2	112.336±0.47	138.196±0.19
0.04%	162.695±0.3	112.289±0.1	133.726±0.6
0.05%	176.783±0.2	132.413±0.78	142.362±0.11
0.08%	177.413±0.1	179.33±0.23	172.776±0.1
0.10%	191.882±0.24	196.23±1.25	200.143±0.19

Table 2. The RGB intensity levels of Sample

Reagent	Sample	Red	Green	Blue
2.6-d	Pineapple	176.852 ± 0.3	131.4327 ± 0.8	142.2815 ± 0.4
	Orange	176.979 ± 0.12	121.3655 ± 0.2	138.999 ± 0.13
	Guava	178.136 ± 0.2	178.4985 ± 0.6	171.86 ± 0.5
	Apple	163.0353 ± 2.01	112.225 ± 0.36	134.6595 ± 0.33

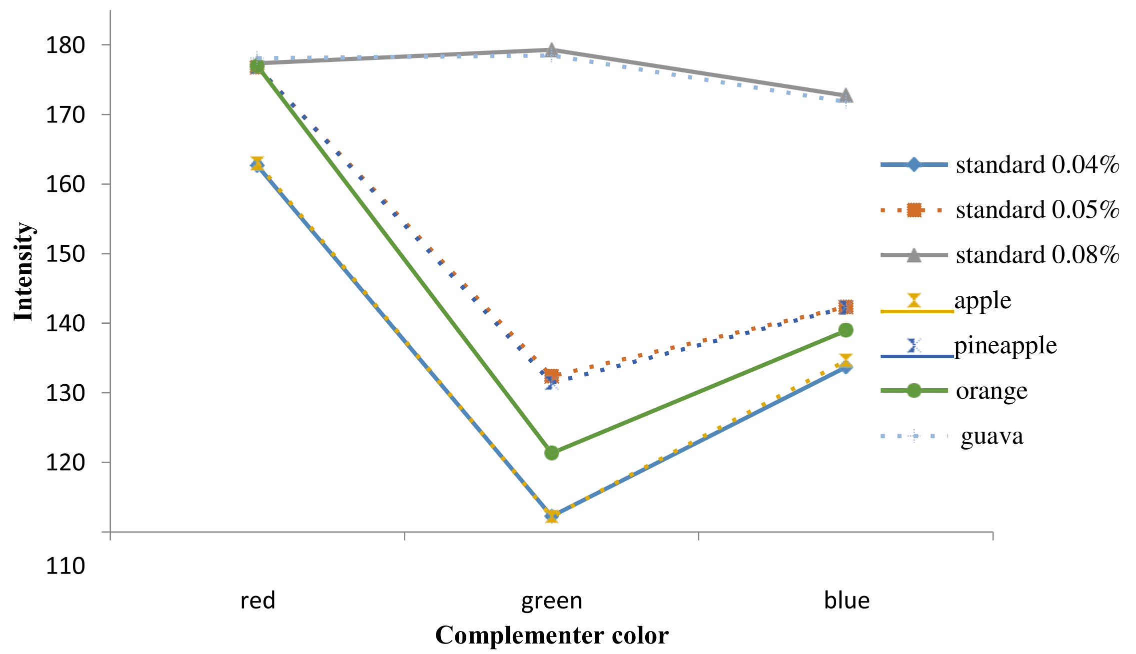

Figure 5. The relationships between complex color and RGB intensity level of sample and standard.

Though the RGB value could determine the vitamin C level of the samples, a statistical analysis was done to observe the nearest distance between both samples and standard. The statistical analysis was done on Euclidean distance analysis. It is a method in which the distance or difference between the RGB sample and RGB standard is observed [20]. The smallest distance obtained from Euclidean distance analysis was considered as the similarity of the two different variables (samples and standard) [21]. The Euclidean distance was calculated as follows:

$$Euclidean = \sqrt{(R_2 - R_1)^2 + (G_2 - G_1)^2 + (B_2 - B_1)^2}$$

where R2, G2, and B2 were RGB intensity level of µPAD obtained from the sample; and R1, G1, and B1 were RGB intensity level of µPAD obtained from the standard. The Euclidean Distance of each sample RGB compared to the standard was presented in Table 6.

Table 6 presented that the smallest distance/difference for the pineapple was on the 0.05% standard while the further distance was on 0% and 0.01% of standard. It was also shown that pineapple and orange shared the same vitamin C level, which was 0.05%. The Euclidean distance indicated that guava had the most vitamin C level (0.08%) among the samples while the lowest vitamin C level obtained was from the apple (0.04%) which had Euclidean distance 1.433 and 0.966, respectively.

Table 3. Euclidean Distance of RGB of Each Samples and Standard

Standard	Pineapple	Orange	Guava	Apple
0%	169.983	168.147	182.316	156.027
0.01%	142.544	140.919	157.504	128.389
0.02%	50.053	46.220	88.061	32.463
0.04%	25.300	15.226	77.951	0.996
0.05%	0.986	3.369	54.734	25.610
0.08%	56.784	33.780	1.433	78.503
0.10%	88.162	62.934	36.101	110.350

As observed through the color intensity and levels of similarity through Euclidean distance, it were concluded that pineapple and orange samples that are shown to be close to standard complementary values is 0.05%, this can be concluded that the levels of vitamin C contained in pineapple and orange are 0.5%. The results of complementary color intensity and Euclidean distance indicated by guava samples approached the value of complementary colors of the standard 0.08% so that it was concluded that the vitamin C content of guava fruit was 0.08%. Whereas for vitamin C levels of apple samples by 0.04% seen from the intensity of complementary color and the smallest Euclidean distance, the sample approaching the intensity of the value of complementary color standard concentration is 0.04%.

4. Conclusion

Vitamin C levels in fruit samples can be determined using the µPAD method on the basis of a redox reaction where the results obtained are compared with the standard results obtained. The µPAD method can be used as a new alternative method for determining vitamin C in fruit samples that is cheaper, faster and easier to use.

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