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RESEARCH PAPER

Simple and Sensitive Paper-based Colorimetric Biosensor for Determining Total Polyphenol Content of the Green Tea Beverages

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Abstract A simple paper-based colorimetric biosensor based on immobilized tyrosinase and 3-methyl-2-benzothiazolinone hydrazone (MBTH) was developed for assessing total polyphenol content (TPC) of various green tea beverages. The patterned biosensing zone of the paper-based biosensor showed a sensitive response to catechin (a typical green tea polyphenol) by generating pink color adducts that can be captured for further color image analysis using a scanometric method. This color change can be connected to the TPC of the samples. The analytical performance of the biosensor in scanometric set-up was optimized. The biosensor showed a response time of 13 min in the linear range between 0.08-1.03 mM of catechin ($r = 0.9979$). The detection limit (LOD) of the biosensor was 0.071 mM while reproducibility was found at 3.11% RSD (relative standard deviation). TPC of green tea beverages was assessed by the biosensor, and the results were in accordance with the UV/Vis spectrophotometric method.

Keywords: biosensor, tyrosinase, MBTH, TPC, green tea, scanometric

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1. Introduction

Tea as a beverage is one of the famous and the most commonly consumed product in the world and is known to contain polyphenols, alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatiles, minerals, and other compounds [1]. From several epidemiological studies, one can deduce that polyphenols from tea exhibit advantageous effects on human health due to high antioxidant capacities which is capable for protecting cells from damaging effects of reactive oxygen species and reducing risk of few kinds of cancer and disease [2-4]. More than 96 polyphenols were already known to be contained in 41 green teas and 25 fermented teas [5]; while gallic acid and some catechins such as (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG) were considered as the major functional compounds among them [6]. It was known that catechins (flavan-3-ols) constituted up to 90% of green tea polyphenols [7].

Liquid chromatography (HPLC) has been mostly used as standard method for determining polyphenolic compounds of green tea samples [8,9]. In this regard, the selectivity of polyphenol detection can be improved by employing liquid chromatography-mass spectrophotometer (LC-MS) [10]. However, HPLC and LC-MS were critiqued as high-cost instrument with time-consuming and tiresome procedures [11,12], while UV-Vis spectrophotometer offered simpler procedure and cheaper cost for determining polyphenol in green tea samples [13,14]. In addition, the classical, spectrophotometric-based Folin-Ciocalteu (FC) method has mostly been used for determining TPC of tea beverages such as green tea [14,15], black tea [16,17], and white tea [18]. However, since the FC reagent can also react with aromatic amine and sugar [19] that usually contained in tea beverages, the obtained TPC values of FC method may be

overestimated. Hence, the development of the specific method for TPC determination is demanded.

One alternative method that can be considered for determining TPC is using chemical sensor or biosensor in a colorimetric setting, as the method is already applied to tea [20] and other beverages such as coffee and wine [21,22]. The development of colorimetric biosensor for the TPC determination of green tea beverages can be done by employing tyrosinase, a binuclear copper enzyme which catalyzes the oxidation of monophenol to catechol and also the oxidation of catechol to its corresponding quinone [23]. This quinone can be further reacted with chromogenic reagent such as MBTH to form the pink to red colored adduct which can be detected visually or spectrophotometry [23,24]. To develop colorimetric biosensor, these enzyme and chromogenic reagent can be immobilized on solid support membrane such as synthetic or natural film as a sensing probe. For example, tyrosinase and MBTH were immobilized onto Nafion/silicate and chitosan films to construct a sensing probe for biosensor with fiber optic spectrophotometer system. By using this setting, several phenolic compounds can be detected and quantified [25]. Tyrosinase and MBTH were also immobilized onto filter paper [21,26] to construct paper-based biosensor that is simpler and cheaper than film-based optical biosensor. In this platform, the color change of biosensor after reacted with polyphenol was captured by smartphone camera or flatbed scanner and measured by using image processing program. By using this setting, TPC of various wine samples [21] and the availability of phenolic compounds in wastewater [26] could be determined properly. However, this biosensor platform has not been applied for TPC determination of green tea beverages.

The current work was meant to develop paper-based colorimetric biosensor with scanometric method that allows TPC determination of green tea samples on the field, where a spectrophotometer is not available or affordable as in the remote area or tea plantation. Here, the biosensor was fabricated by immobilizing tyrosinase and MBTH onto the patterned paper. It exhibited a sensitive response to catechin by developing a visible, pink-colored adduct that can be consecutively scanned and quantified by a flatbed scanner and color image analysis program. The fabrication process of biosensor, the search of optimum condition for the analytical procedure, and its application for determining TPC of green tea beverages are clearly discussed.

2. Materials and Methods

2.1. Chemicals

Tyrosinase (EC 1.14.18.1, lyophilized ≥ 2000 unit/mg) and (±)-catechin hydrate were purchased from Sigma-Aldrich (USA), while MBTH was obtained from Fluka (UK). Phosphate buffer solution (PBS) at pH 7.0 for diluting tyrosinase and MBTH was made by adjusting 0.2 M KH_2PO_4 with 0.2 M NaOH. Both chemicals were obtained from Merck (Germany). L-Ascorbic acid anhydrous (vitamin C) was purchased from Sigma-Aldrich (USA). FC reagent, Na_2CO_3, and ethanol were supplied from Merck (Germany). All chemicals used in this current work were analytical grade.

2.2. The biosensor fabrication

The development of polyphenol biosensor was done by immobilizing bioreagent (tyrosinase and MBTH) onto patterned filter paper that has a circular shape with 0.8 cm internal diameter as a biosensing zone, as it can be seen in Fig. 1A. The circular pattern was created by applying the screen-printed technique using rubber-based ink (Sunrise, Indonesia) over the filter paper sheets (Whatman CAT No.1095.093, Merck UK) on both sides to make a circular shape with the total wall thickness in both side around 0.2 mm, so that this circular pattern has hydrophobic properties. The bioreagent was dropped (5 μL) onto the biosensing zone and allowed to dry for 10 min at room temperature (25°C) to construct the polyphenol biosensor. For TPC determination, sample or standard polyphenol solution is individually transferred (5 μL) onto the biosensor.

2.3. The preparation and optimization of the bioreagent solution

The bioreagent was prepared in PBS at pH 7.0. Fresh solution of tyrosinase (500 unit/mL) and MBTH (6.5, 13, 26, and 52 mM) in PBS were mixed at different volume ratios (1:1; 1:2; 2:1; 1:3; and 3:1) to yield a series of bioreagent solution which was then dropped to different biosensing zones. For optimization study, a freshly made of catechin solution (0.52 mM) was independently applied (5 μL) to each zone. Afterward, the color change of each zone was scanned and quantified. The bioreagent solution that gave the highest color intensity was selected for the following fabrication process.

2.4. Analytical procedure

In all cases, a flatbed scanner (CanoScan, LIDE 110, Japan) was employed to capture the color change of the biosensing zone, after addition of the standard (catechin) or sample solution onto the biosensing zone. Here, the color picture mode with 300 dpi resolution was applied for the scanning process. To obtain analytical signals, the color intensity of scanned images (as JPEG files) was measured by using ImageJ 1.5 for Windows (https://imagej.nih.gov/ij/). The color intensity value (mean red, green, and blue or

RGB) of the circled-area of each biosensing zone was corrected with that of a biosensing zone with no standard or sample addition (blank). All of the experimental procedures were performed in triplicate.

2.5. TPC determination of green tea samples

Various green tea beverages from local market of Jember, Indonesia were employed to determine the practical use of the developed polyphenol biosensor. TPC of green tea samples was calculated by using a calibration curve of catechin (0.017-0.947 mM) and expressed as catechin equivalent in mM (mM CE). The TPC values of green tea samples were then compared with that of the standard FC method. Here, the TPC method described by Magalhães *et al.* [27] was adopted with slight modification. To the 5.0 µL sample solution, 10.0 µL of FC reagent was added and allowed to stand for 5 min. Next, 100.0 µL of 7.4% Na_2CO_3 was added to the mixed solution. After 60 min, the absorbance of the mixed solution was read at 740 nm. Similarly, TPC of green tea samples was calculated based on the calibration curve of catechin.

2.6. Statistical analysis

The results of analyzed TPC from the green tea beverages samples using the proposed biosensor was compared with the TPC results obtained by FC method by employing independent *t*-test [28]. To determine the association of the obtained TPC values of both methods, the correlation analysis was carried out [6].

3. Results and Discussion

3.1. The biosensing zone

In the proposed method, the polyphenol biosensor was constructed by patterning a filter paper sheet with black circular shape with hydrophobic rubber-based ink by screen printing technique. Filter paper is chosen as solid support for biosensor as it is available abundantly, cheap, easy to manage, and disposable [21]. Here, the circular pattern as biosensing zone can be multiplied and cut in the desired numbers of biosensing zone, such as three biosensing zone as used in this work for triplicate measurements. The hydrophobic black circular pattern served as a liquid barrier to form a microwell, where the bioreagent was immobilized to the circle area as a biosensing zone. By using this biosensing zone, the measurement of color change was performed in a fixed volume and constant area, while in the previous work [21] the area of the generated color formed was found to be inconsistent although the constant volume of the sample solution was applied, since no hydrophobic pattern was used. This might be due to the fact that direct spotting of the bioreagent (tyrosinase and MBTH) solution on the filter paper surface makes bioreagent diffusion form inconsistent, as in manual liquid spotting on the surface of thin layer chromatography plate. In another study [26], the similar bioreagent was dropped on the square-cut (1×1 cm^2) filter paper to construct the phenolic biosensor which is also no guaranty of a constant sample volume that covers the area of filter paper.

3.2. The biosensing scheme

The enzymatic oxidation of catechin by tyrosinase to yield catechin-quinone followed by color-forming reaction of this catechin-quinone with MBTH was served as biosensing mechanism of the colorimetric-based polyphenol biosensor as given in Fig. 1B. As expected, the colorless biosensing zone was immediately turned into pink color upon the addition of catechin solution (Fig. 1A). Although this color change is visually detected by normal eye, however, for the analytical purposes, the color change can be quantified by measuring the color intensity as RGB values.

3.3. The optimum bioreagent solution

As described earlier, tyrosinase solution was mixed homogenously with a series of MBTH solutions to obtain different bioreagent solutions which were then independently immobilized onto different biosensing zones. Afterward, 0.52 mM catechin was added (5 µL) to those biosensing zones. As shown in Fig. 2, the bioreagents with MBTH concentration at 51 and 102 mM generated the highest color intensity among others. While both of the bioreagents gave the relatively similar color intensity, the bioreagent containing 51 mM MBTH was selected to reduce the chemical use. Thus, a mixed solution of 500 unit/mL tyrosinase and 51 mM MBTH at equal volume was used as the bioreagent.

3.4. The optimum pH

For studying catechin oxidation by tyrosinase, Jimenez-Atienzar *et al.* [29] suggested that the pH of the system should be above pH 7.0, while Munoz-Munoz *et al.* [30] used pH 7.0 for the quite similar study. Based on the stereochemistry of tea's catechins, the best substrate for tyrosinase is epicatechin, while the next best substrate for the enzyme above is catechin [30] as it was used in the current work. Hence, to reconsolidate those works, the epicatechin solutions at pH 7.0 and 7.5 were tested toward the developed biosensor in the current work. As depicted in Table 1, no significant result ($df = 8$ and $\alpha = 0.05$) was observed between the different pH of the applied epicatechin solutions. Based on this finding, we used catechin at pH 7.0 for polyphenol standard by following Jimenez-Atienzar

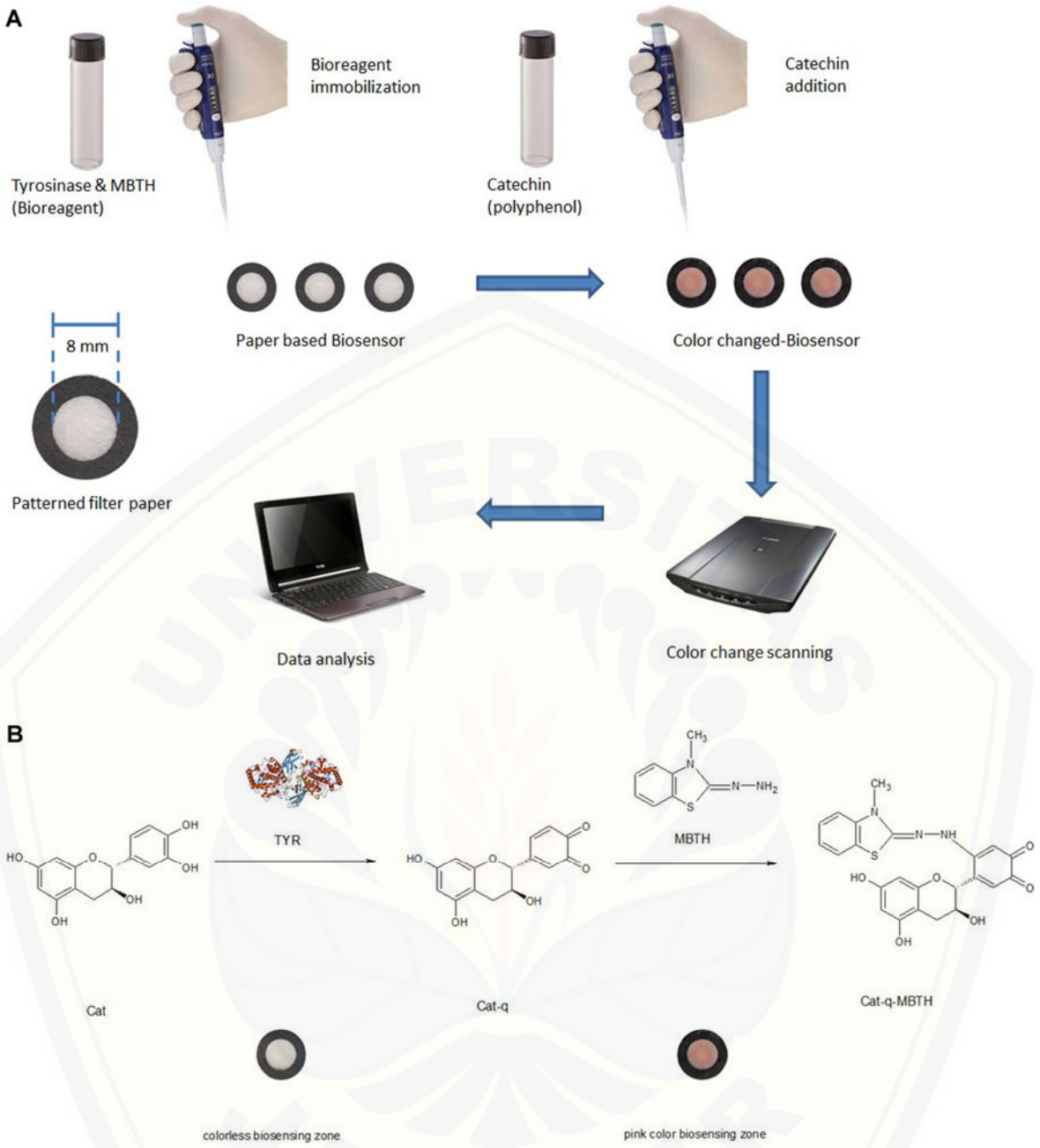

Fig. 1. (A) The overview of the current study showing the single biosensor, fabrication process, typical color change of biosensor after reacted with catechin (polyphenol), and data acquisition using scanometric method, and (B) the proposed enzymatic oxidation of catechin (Cat) by tyrosinase (TYR) followed by coupling reaction of its corresponding quinone (Cat-q) with 3-methyl-2-benzothiazolinone hydrazone (MBTH) to form the pink color adduct (Cat-q-MBTH) at biosensing zone.

et al. [29] and also for the economical reason, as catechin is cheaper than epicatechin.

3.5. Response time

To determine response time, 0.52 mM catechin was applied (5 µL) to polyphenol biosensor. Then, color intensity value of the biosensing zone as biosensor response was noted every min until stable response was achieved. Fig. 3A showed that the biosensor response was firstly stabilized at 13 min. Hence, this response time was used for the following measurements.

The response time of the developed biosensor was slightly slower than that shown by the related paper-based chemical sensor [20]. Instead of tyrosinase, sodium periodate was used to oxidize polyphenols, while MBTH was also used as reagent dye to detect the oxidation adduct

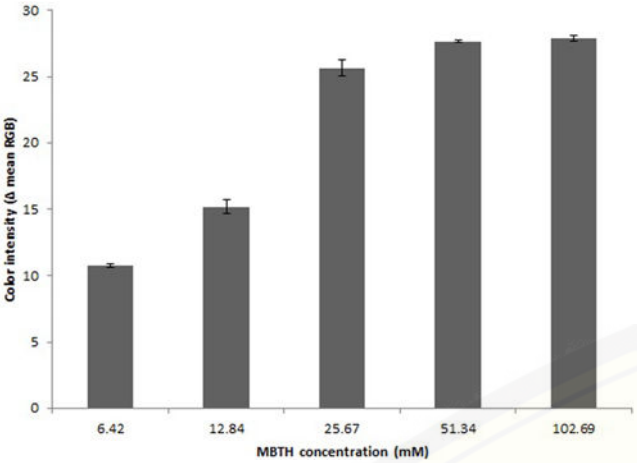

Fig. 2. The color intensity of the polyphenol biosensors at various 3-methyl-2-benzothiazolinone hydrazone (MBTH) concentrations with a fixed concentration of tyrosinase at 500 unit/mL, after addition of 0.52 mM catechin (n = 3).

Table 1. The obtained color intensity data after addition of 1 mM (-)-epicatechin at different pH in the range of 2-12 min (n = 3)

Time (min)	Color intensity (mean RGB)		P*
	pH 7.0	pH 7.5	
2	203.115 ± 2.201	199.814 ± 2.559	0.0602
4	205.506 ± 2.488	203.347 ± 3.050	0.2550
6	207.030 ± 2.248	205.245 ± 3.307	0.3476
8	207.955 ± 1.903	206.876 ± 3.497	0.5616
10	206.899 ± 2.452	208.025 ± 3.860	0.5971
12	207.250 ± 2.192	209.508 ± 4.207	0.3182

*Results were obtained by independent *t*-test, with *t* value (t_{tab}) of 2.306 (df = 8 and α = 0.05).

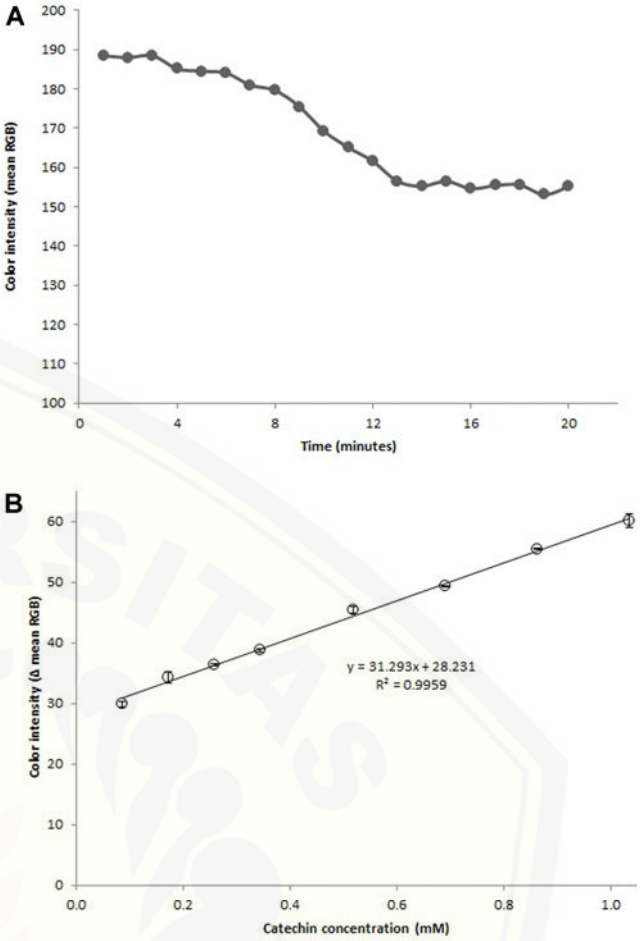

Fig. 3. (A) The observed color intensity of the polyphenol biosensor at 0-20 min after addition of 0.52 mM catechin (n = 3) and (B) The calibration curve of catechin solution at 0.086-1.034 mM (n = 3) constructed by the polyphenol biosensor.

(quinone). It was reported that sodium periodate was also used as an oxidizing agent for phenolic acid [31] and flavonoid [29] in the kinetic studies of tyrosinase, and it was shown that an intensely faster oxidizing capability of sodium periodate than tyrosinase [23,29,31].

As the response time of the biosensor was applied for TPC determination, therefore it is determined the overall time required for a TPC analysis. However, in term of the operating time for conducting single analysis, the proposed method was proven to be faster than the standard method (FC) which typically needs 20 min to 2 h for single analysis by using similar standard polyphenol (catechin) [32-34].

3.6. Linearity and detection limit

Catechin was used as polyphenol standard for TPC determination of green tea samples as in other previous works [20,32,34]. Here, the calibration curve of catechin was constructed by plotting the catechin concentration against color intensity (Δmean RGB), as it can be seen in Fig. 3B. The biosensor gave a linear response at 0.086-1.034 mM catechin concentration with a coefficient of correlation (*r*) of 0.9979 (α = 0.05, n = 3). The sensitivity of biosensor that obtained from the slope of the linear curve was calculated to be 31.293 Δ mean RGB/mM catechin. The detection limit (LOD) of biosensor that calculated to be 0.071 mM was obtained from three times the standard deviation of response.

3.7. Repeatability and accuracy

A simulated sample containing 0.535 mM of catechin was used to determine the repeatability of biosensor. The result showed that the relative standard deviation (RSD) of six replicates was calculated to be 3.11%, suggesting that the developed method has good precision [35]. In addition, a recovery test suggested by Huber [35] was further used to

assess the performance of biosensor. Here, a simulated sample with initial concentration at 155.42 mg/L catechin equivalent (CE) was independently spiked with 50.996, 76.465, and 105.258 μg of catechin to represent 30, 45, and 60% of initial TPC value and analyzed with the developed biosensor. It was known that the recovery of catechin was found at 101-105%, suggested that the biosensor is accurate and has great potential for determining TPC in the green tea samples.

3.8. Selectivity

The effect of a potential interfering substance on TPC determination using the developed biosensor was examined. It is already reported that vitamin C is one of the most potent inhibitors for grape tyrosinase [36]. Therefore, vitamin C was selected as interfering substance on TPC determination. On the labels of some green tea beverages, it was found that vitamin C is added to the products at 200 ppm concentration per bottle, while other brands do not clearly state the amount of the vitamin C per bottle. Here, different concentration of vitamin C (0.57-2.84 mM in water) with 1:1 volume ratio of the simulated sample (catechin solution at 0.52 mM concentration), and its effect on the color intensity changes in biosensor was investigated.

Interference was computed by subtracting biosensor response of the vitamin C spiked-sample with that of initial sample (no vitamin C) divided by biosensor response of the initial sample. Fig. 4 showed that interference was found less than 5% for vitamin C at 0.57-2.27 mM concentration. Vitamin C at 2.84 mM concentration gave 6% interference signal on TPC determination of the simulated sample (Fig. 4) which is higher than that suggested (5%) by Yuwono and Indrayanto [37]. At this level, vitamin C may inhibit the catalytic activity of tyrosinase, thus affecting the enzymatic oxidation of catechin.

3.9. Stability

To evaluate the stability of the developed biosensor, various storage conditions such as room (25-28°C), chilling (2-8°C), and freezing (-20-0°C) temperatures were applied. The biosensor was individually placed in a well-capped cabinet at those storage conditions. Then, the biosensor response after catechin addition at 0.52 mM was monitored every day until 15% decrease of the initial response was achieved. It was known that the biosensor response was decreased more than 15% in a day when the biosensor was stored at room temperature (25°C) (data not shown), while the same response was observed in 5 and 9 days when it was stored at chilling (4°C) and freezing temperature (-4°C) respectively as depicted in Fig. 5. Thus, the biosensor response was stable up to 8 days when it was stored at freezing temperature.

3.10. Application in real samples

The paper-based, colorimetric biosensor based on immobilized tyrosinase and MBTH has been developed for the TPC detection in wines [21] and wastewater samples [26]. In those works, *L*-DOPA was used as standard phenol and the analytical performance was optimized by using this compound. This approach seems to be inaccurate as *L*-DOPA does not represent the major phenolic compounds of the selected samples. On contrary, in the current work catechin was used as standard phenol for optimizing the analytical parameters as this compound is already known to contain in green tea [9] and clearly identified as basic

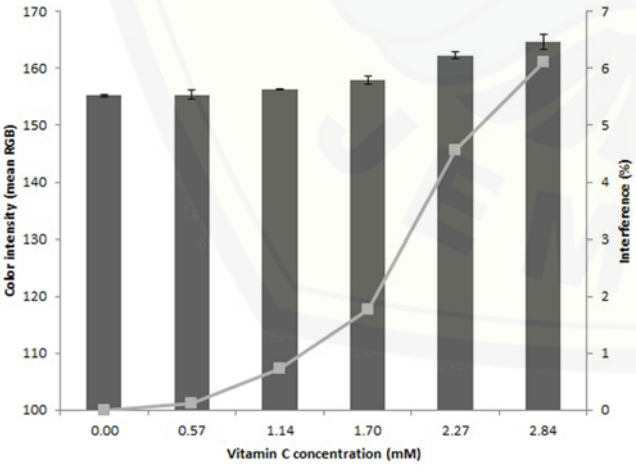

Fig. 4. The interference signal in 0.52 mM catechin measurement by the polyphenol biosensor after addition of vitamin C at various concentrations and the volume ratios at 1:1 (as the bar chart), and interference value as the curve.

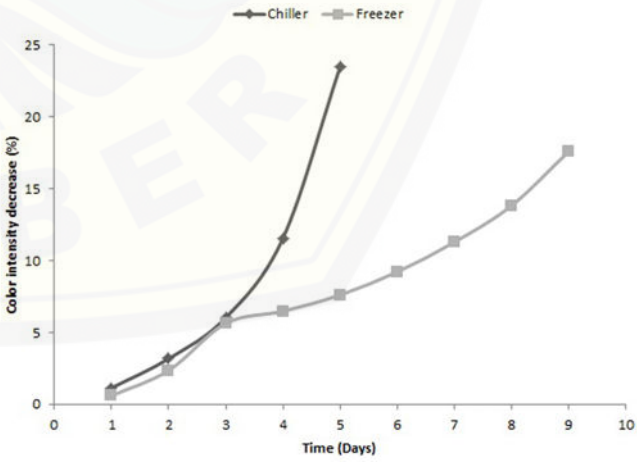

Fig. 5. The color intensity decrease of biosensors within 0.52 mM catechin (n = 3) addition after it was individually stored at chiller and freezer.

Table 2. The comparison study between the previous paper-based biosensors and the current biosensor

Parameter	Information	Reference
Dynamic range, standard phenol/ polyphenol	0–0.5 mM, L-DOPA	[21]
	0.001- 0.512 mM, L-DOPA	[26]
	0.086-1.034 mM, catechin	Current work
Detection limit	5 µM for L-DOPA	[21]
	0.032 mM for 4-chlorophenol and catechol and 0.128 mM for m-cresol and p-cresol	[26]
	0.071 mM for catechin	Current work
Accuracy study	Not available	[21]
	Not available	[26]
	The recovery of catechin was 101-105%	Current work
Selectivity study	Not available	[21]
	Not available	[26]
	Vitamin C as interference substance	Current work
Sample; the connection between standard and sample	Wine beverages, no clear connection between standard and samples	[21]
	4-chlorophenol, catechol, *m*-cresol, and *p*-cresol; no clear connection between standard and samples	[26]
	Green tea beverages; the selected standard represents the basic structure of the major compounds in samples	Current work
Stability, temperature	9 days, 4°C	[21]
	70 days, 4°C	[26]
	5 days, 4°C	Current work

structure of tea catechins (Table 2). Hence, TPC of green tea was computed and expressed as catechin equivalent as well as in other previous works [20,29,33]. Moreover, the selectivity and accuracy studies were performed toward the developed biosensor, while these studies were not carried to the analog, previous paper-based biosensors, as depicted in Table 2.

Table 3 presented the calculated TPC values of various green tea beverages by the polyphenol biosensor and UV-Vis spectrophotometer. It can be deduced that the result of both methods was in good agreement, as the statistical comparison (by using *t*-test) of the mean values obtained from both methods showed no significant difference (df = 4 and α = 0.05). Additionally, TPC values of both methods were highly correlated (r = 0.9893), as it can be seen in Fig. 6. Based on this finding, the developed method can be used for determining TPC of green tea beverages.

As it was already known that an ordinary flatbed scanner is used for obtaining analytical signal in the scanometric method, the electrical power consumption is extremely reduced. In addition, the use of a filter paper for the analytical device will make the method low-cost and more affordable than a cuvette or a microwell plate. Therefore, in terms of cost (both for fabrication and application), the biosensor with scanometric method was shown to be more

Table 3. Results of total polyphenol content (TPC) (mM CE) of various green tea beverages determined by the polyphenol biosensor and the UV/Vis spectrophotometer (n = 3, α = 0.05)

Sample	Polyphenol biosensor	Spectrophotometer	t_{cal}
K	0.747 ± 0.017	0.802 ± 0.005	0.174
L	0.403 ± 0.006	0.376 ± 0.022	2.000
M	0.557 ± 0.015	0.537 ± 0.003	2.194
N	0.448 ± 0.007	0.447 ± 0.003	0.170

*Results were obtained by independent *t*-test, with *t* value (t_{tab})of 2.776 (df = 4 and α = 0.05).

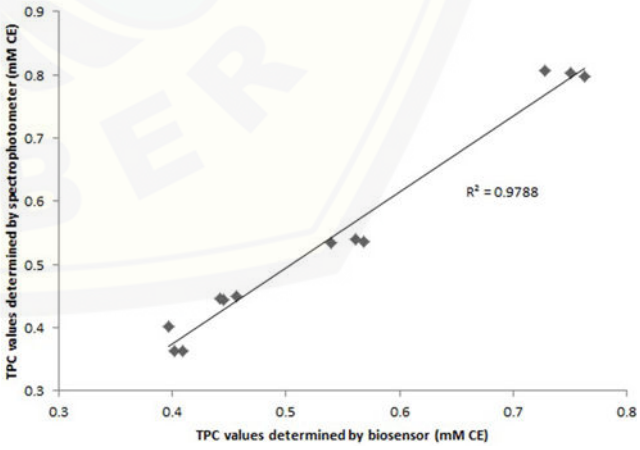

Fig. 6. The correlation of total polyphenol content (TPC) values (mM CE) of various green tea beverage samples obtained by the polyphenol biosensor compared to the UV/Vis spectrophotometer.

economical than the spectrophotometric method. Additionally, the use of handphone camera along with free apps such as color lab® or color grap® can also be applied to the developed method so that it can be applied for simple field analysis. Hence, the developed method can be used for TPC determination of green tea samples and is suitable for the field application (*e.g.* tea plantation) or in a poor lab setting, where a spectrophotometer is not affordable.

4. Conclusion

In this work, a paper-based colorimetric biosensor for measuring TPC content in the green tea beverages was developed by immobilizing tyrosinase and MBTH onto circular patterned-paper. The biosensor showed sensitive response to catechin, a basic structure of the green tea polyphenols, and being optimized for TPC determination in a scanometric set-up based on this compound. The proposed method provides a simple, inexpensive, and reliable method for determining TPC of green tea beverages; hence, it opened possibility for its application in a field trip study or remote area where the experimental resource is limited.

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The authors declare that they have no conflict of interest. Neither ethical approval nor informed consent was required for this study.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Karori, S. M., F. N. Wachira, J. K. Wanyoko, and R. M. Ngure (2007) Antioxidant capacity of different types of tea products. *Afr. J. Biotechnol.* 6: 2287-2296.
2. Sano, J., T. Ogawa, S. Inami, F. Ishibashi, K. Okamatsu, H. Kamon, K. Seimiya, G. Takagi, S. Sakai, A. Nomura, and K. Mizuno (2003) Effect of green tea intake on the development of coronary artery disease. *J. Am. Coll. Cardiol.* 41: 531.
3. Uesato, S., Y. Kitagawa, M. Kamishimoto, A. Kumagai, H. Hori, and H. Nagasawa (2001) Inhibition of green tea catechins against the growth of cancerous human colon and hepatic epithelial cells. *Cancer Lett.* 170: 41-44.
4. Vinson, J. A., K. Teufel, and N. Wu (2004) Green and black teas inhibit atherosclerosis by lipid, antioxidant, and fibrinolytic mechanisms. *J. Agric. Food Chem.* 52: 3661-3665.
5. Lin, L. Z., P. Chen, and J. M. Harnly (2008) New phenolic components and chromatographic profiles of green and fermented teas. *J. Agric. Food Chem.* 56: 8130-8140.
6. Nadifiyine, S., C. Calas-Blanchard, A. Amine, and J. L. Marty (2013) Tyrosinase biosensor used for the determination of catechin derivatives in tea: correlation with HPLC/DAD method. *Food Nutr. Sci.* 4: 108-118.
7. Lee, L. S., S. H. Kim, Y. B. Kim, and Y. C. Kim (2014) Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. *Molecules*. 19: 9173-9186.
8. Bae, I. K., H. M. Ham, M. H. Jeong, D. H. Kim, and H. J. Kim (2015) Simultaneous determination of 15 phenolic compounds and caffeine in teas and mate using RP-HPLC/UV detection: Method development and optimization of extraction process. *Food Chem.* 172: 469-475.
9. He, X., J. Li, W. Zhao, R. Liu, L. Zhang, and X. Kong (2015) Chemical fingerprint analysis for quality control and identification of Ziyang green tea by HPLC. *Food Chem.* 171: 405-411.
10. Yi, T., L. Zhu, W. L. Peng, X. C. He, H. L. Chen, J. Li, T. Yu, Z. T. Liang, Z. Z. Zhao, and H. B. Chen (2015) Comparison of ten major constituents in seven types of processed tea using HPLC-DAD-MS followed by principal component and hierarchical cluster analysis. *Lebenson Wiss Technol.* 62: 194-201.
11. Abdullah, J., M. Ahmad, L. Y. Heng, N. Karuppiah, and H. Sidek (2007) An optical biosensor based on immobilization of laccase and MBTH in stacked films for the detection of catechol. *Sensors*. 7: 2238-2250.
12. Dolinsky, M., C. Agostinho, D. Ribeiro, G. De Souza Rocha, S. Girão Barroso, D. Ferreira, R. Polinati, G. Ciarelli, and E. Fialho (2016) Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables. *J. Culin. Sci. Technol.* 14: 1-12.
13. Diniz, P. H., M. F. Barbosa, K. D. de Melo Milanez, M. F. Pistonesi, and M. C. de Araújo (2016) Using UV–Vis spectroscopy for simultaneous geographical and varietal classification of tea infusions simulating a home-made tea cup. *Food Chem.* 192: 374-379.
14. Wang, X., J. Huang, W. Fan, and H. Lu (2015) Identification of green tea varieties and fast quantification of total polyphenols by near-infrared spectroscopy and ultraviolet-visible spectroscopy with chemometric algorithms. *Anal. Methods*. 7: 787-792.
15. Cleverdon, R., Y. Elhalaby, M. McAlpine, W. Gittings, and W. Ward (2018) Total polyphenol content and antioxidant capacity of tea bags: comparison of black, green, red rooibos, chamomile and peppermint over different steep times. *Beverages*. 4: 15.
16. Tadesse, A., A. Hymete, A. A. Bekhit, and S. F. Mohammed (2015) Quantification of total polyphenols, catechin, caffeine, L-theanine, determination of antioxidant activity and effect on antileishmanial drugs of ethiopian tea leaves extracts. *Pharmacognosy Res.* 7: S7-S14.
17. Bhebhe, M., T. N. Füller, B. Chipurura, and M. Muchuweti (2016) Effect of solvent type on total phenolic content and free radical scavenging activity of black tea and herbal infusions. *Food Anal. Methods*. 9: 1060-1067.
18. Castiglioni, S., E. Damiani, P. Astolfi, and P. Carloni (2015) Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas. *Int. J. Food Sci. Nutr.* 66: 491-497.
19. Box, J. D. (1983) Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* 17: 511-525.
20. Hidayat, M. A., F. Jannah, and B. Kuswandi (2016) Development of paper based sensor for the determination of total phenolic

content in green tea beverages. *Agric. Agric. Sci. Procedia*. 9: 424-430.
21. Arciuli, M., G. Palazzo, A. Gallone, and A. Mallardi (2013) Bioactive paper platform for colorimetric phenols detection. *Sens Actuators B Chem.* 186: 557-562.
22. Fernandes, S. C., S. K. Moccelini, C. W. Scheeren, P. Migowski, J. Dupont, M. Heller, G. A. Micke, and I. C. Vieira (2009) Biosensor for chlorogenic acid based on an ionic liquid containing iridium nanoparticles and polyphenol oxidase. *Talanta*. 79: 222-228.
23. Muñoz, J. L., F. García-Molina, R. Varón, J. N. Rodriguez-Lopez, F. García-Cánovas, and J. Tudelaa (2006) Calculating molar absorptivities for quinones: Application to the measurement of tyrosinase activity. *Anal. Biochem.* 351: 128-138.
24. Mutti, F. G., R. Pievo, M. Sgobba, M. Gullotti, and L. Santagostini (2008) Biomimetic modeling of copper complexes: a study of enantioselective catalytic oxidation on *D* -(+)-catechin and *L*-(−)-epicatechin with copper complexes. *Bioinorg. Chem. Appl.* 2008: 762029.
25. Abdullah, J., M. Ahmad, L. Y. Heng, N. Karuppiah, and H. Sidek (2006) Chitosan-based tyrosinase optical phenol biosensor employing hybrid nafion/sol–gel silicate for MBTH immobilization. *Talanta*. 70: 527-532.
26. Şenyurt, Ö., F. Eyidoğan, R. Yılmaz, M. T. Öz, V. C. Özalp, Y. Arıca, and H. A. Öktem (2015) Development of a paper-type tyrosinase biosensor for detection of phenolic compounds. *Biotechnol. Appl. Biochem.* 62: 132-136.
27. Magalhães, L. M., M. A. Segundo, S. Reis, J. L. F. C. Lima, and A. O. S. S. Rangel (2006) Automatic method for the determination of Folin−ciocalteu reducing capacity in food products. *J. Agric. Food Chem.* 54: 5241-5246.
28. Amatatongchai, M., W. Sroysee, S. Laosing, and S. Chairam (2013) Rapid screening method for assessing total phenolic content using simple flow injection system with laccase based-biosensor. *Int. J. Electrochem. Sci.* 8: 10526-10539.
29. Jiménez-Atiénzar, M., J. Cabanes, F. Gandía-Herrero, and F. García-Carmona (2004) Kinetic analysis of catechin oxidation by polyphenol oxidase at neutral pH. *Biochem. Biophys. Res. Commun.* 319: 902-910.
30. Munoz-Munoz, J. L., F. García-Molina, M. Molina-Alarcón, J. Tudela, F. García-Cánovas, and J. N. Rodríguez-López (2008) Kinetic characterization of the enzymatic and chemical oxidation of the catechins in green tea. *J. Agric. Food Chem.* 56: 9215-9224.
31. Muñoz, J., F. Garcia-Molina, R. Varon, J. N. Rodriguez-Lopez, P. A. García-Ruiz, F. García-Cánovas, and J. Tudela (2007) Kinetic characterization of the oxidation of chlorogenic acid by polyphenol oxidase and peroxidase. Characteristics of the o-quinone. *J. Agric. Food Chem.* 55: 920-928.
32. Büyükbalci, A. and S. N. El (2008) Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. *Plant Foods Hum. Nutr.* 63: 27-33.
33. Katalinic, V., M. Milos, T. Kulisic, and M. Jukic (2006) Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.* 94: 550-557.
34. Stevanato, R., S. Fabris, and F. Momo (2004) New enzymatic method for the determination of total phenolic content in tea and wine. *J. Agric. Food Chem.* 52: 6287-6293.
35. Huber, L. (2007) *Validation and Qualification in Analytical Laboratories*. 2nd ed., pp. 145-147. CRC Press, Boca Raton, FL, USA.
36. Queiroz, C., M. L. Mendes Lopes, E. Fialho, and V. L. Valente-Mesquita (2008) Polyphenol oxidase: characteristics and mechanisms of browning control. *Food Rev. Int.* 24: 361-375.
37. Yuwono, M. and G. Indrayanto (2005) Validation of chromatographic methods of analysis. *Profiles Drug Subst Excip Relat Methodol.* 32: 243-259.