

## Antioxidant Capacity and $\alpha$ -Amilase Inhibition by Avocado (*Persea americana* Mill) Peel and Red Ginger (*Zingiber officinale* var. Rubrum) based Functional Drink

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### ABSTRACT

Avocado peel (*Persea americana* Mill) and red ginger (*Zingiber officinale* var. Rubrum) proved to have antidiabetic properties. However, there has been no research on the analysis of antidiabetic activity of the combination of avocado peel and red ginger in functional beverages. This study aims to make functional beverages from the combination of avocado peel and red ginger. Total phenolic content, antioxidant capacity, inhibition of the  $\alpha$ -amylase activity, and public preferences towards functional beverages (organoleptic) were then evaluated. Functional beverages were made by brewing the powdered avocado peel and red ginger with 200 ml of hot water (96°C) for 5 minutes. The results showed that the increase of avocado peels amount in the combination could increase total phenolic content. The high antioxidant capacity was also found in samples with high content of avocado peels. The results of inhibition of  $\alpha$ -amylase enzyme activity did not show any significant differences. Organoleptic test results showed that there were no significant differences in each sample. The combination of avocado peel and red ginger has the potential to be an antidiabetic functional beverage.

**Key words:** Avocado, red ginger, total phenolic, antioxidant,  $\alpha$ -amylase

### INTRODUCTION

In the last twenty years the number of people diagnosed with diabetes has drastically increased throughout the world that it is considered as a worldwide epidemic disease [1]. This is reflected by total population with diabetes that reaches as high as 415 million [2]. In Indonesia, the population suffering diabetes in 2013 is doubled that figure in 2007, and it is observed throughout the archipelago. The number of diabetics in Indonesia reaches 10 million and ranks seventh largest in the world. The number is likely to be even increased as the prevalence of diabetes in Indonesia reaches 6.9% [3].

Many efforts have been suggested to treat the diabetes, such as diet management, physical exercise, blood sugar levels regular controlling, and pharmacological therapy [4]. Nowadays, pharmacological therapy is still an option to deal with diabetes [5]. However, pharmacological therapy has disadvantages and may results in side effects such as nausea, vomiting, constipation, hypoglycemia, and nervous system abnormalities [5,6]. An alternative effort to manage diabetes is to consume herbs and spices that have

antioxidant and antidiabetic activity [7]. Several plants reported to have antioxidant and antidiabetic activity are red betel (*Piper crocatum* Ruiz & Pav.), Cinnamon (*Cinannamomum burmanii* Blume), salam (*Syzygium polyanthum*), mulberry (*Morus canva*), green tea (*Camellia sinensis*), green grass jelly (*Premna oblongifolia* L. Merr), avocado (*Persea americana* Mill), and red ginger (*Zingiber officinale* var. Rubrum) [8–14]. Anggorowati et al. [15] reported that herbal tea made of avocado leaves dried for 30 minutes at 40°C has an antioxidant content of 24.863  $\mu\text{g/mL}$ . Avocado seeds are also reported to have high antioxidant activity [16]. Furthermore, avocado peels which regarded as waste are reported to have antioxidant and antidiabetic activity [17,18]. Methanol extract of avocado peel has been reported to have antioxidant activity of 9467  $\mu\text{g/mL}$  [19] and  $\alpha$ -amylase inhibition activity of 36.02  $\mu\text{g/mL}$  [20]. However, these studies are only limited to the examine the leaves extract in the form that are not readily consumable, and hence further processing is required.

The present study, thus, aims at exploring alternatives preparation of avocado peel as functional drinks by combining the peel

extract with various composition of red ginger, so that the the resulted drink has optimum antioxidant and antidiabetic activity for diabetic patien to consume. This research includes: manufacture of functional drinks, determination of total phenolics, testing of antioxidant capacity,  $\alpha$ -amylase enzyme inhibitio assay, and summed up with organoleptic tests to asses the acceptability of functional drinks.

## MATERIALS AND METHODS

### Materials

Peel of ripe avocados, red ginger, aquades (Merck),  $\alpha$ -amylase (1,4- $\alpha$ -D-Glucan-glucanohydrolase), methanol (Merck), starch (Merck), iodine (Merck), folin ciocalteu (Merck), sodium carbonate (Merck), gallic acid (Merck), phosphate buffer, DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich).

### Functional drink preparation

Avocado peel is washed thoroughly with running water, drained for 10 minutes at ambient temperature. Avocado peel is cut into small pieces and then dried with at temperature of 60°C for 24 hours. Dried avocado peel was crushed with a blender into powder. The same thing was done for red ginger. Samples consisting of 5 grams mixture of avodaco and red ginger were put into tea bags. The avocado(A):red ginger (G) ratio were 100%:0% for AG1, 90%:10% (AG2), 80%:20% (AG3), 70%:30% (AG4), 60%:40% (AAG5), 50%:50% (AG6), and 0%:100% (AG7). Functional drinks are made by brewing the tea with 200 mL of hot water (96°C) and let stand for 5 minutes. Samples were allowed cool until 25°C prior to analysis.

### Total phenolic determination

The total phenolic test was carried out following the method presented by Kusumawati & Yogeswara [21]. Briefly, a total of 0.2 mL of the functional drink was put in a test tube and 0.2 mL of the Folin-ciocalteu reagent was added. The solution was vortexed and incubated for 6 minutes, followed by addition of 2.1 mL of 5% sodium carbonate. The mixture was votexed and incubated for 30 minutes. The absorbance of the solution was measured at 760 nm using a biochrom spectrophotometer. Gallic acid of various concentrations was employed as standard. Phenolic levels are expressed in units of mg equivalent gallic acid/g sample (mg GAE/g).

### Antioxidant assay

Briefly, 0.5 ml DPPH solution (10 mg/L) was put into a test tube and 20  $\mu$ L of the functional beverage test solution was addedthen added, followed by addition of 480  $\mu$ L methanol. The mixture was votexed then incubated at room temperature for 30 minutes. The colour change was measured at 517 nm with a biochrom spectrophotometer. For standard, gallic acid was used at various concentrations. The antioxidant capacity is expressed in units of mg equivalent gallic acid/ml sample (mg GAE/mL). Measurement of antioxidant activity is calculated by the following formula:

$$\% \text{inhibition} = \frac{\text{Abs. Control} - \text{Abs. Sample}}{\text{Abs. Control}} \times 100 \quad (1)$$

### $\alpha$ -amilase inhibition assay

The  $\alpha$ -amylase activity test was carried out by the iodine starch method [22]. Ten  $\mu$ L of the  $\alpha$ -amylase solution (0.025 mg/mL) was mixed with 390  $\mu$ L phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) by varying functional beverage ratios. After 10 minutes of incubation at 37°C, 100  $\mu$ L of 1% starch solution was added, and the incubation was prolonged for 1 hour. Next, 0.1 mL of 1% iodine solution was added, followed by addition of 5 mL of distilled water. The absorbance was measured at 565 nm. Determination of blanks, samples, starch and  $\alpha$ -amylase was carried out under the same reaction conditions. Inhibition of enzyme activity is calculated by the following formula:

$$\% \text{inhibition} = \frac{(\text{Abs. Sampel} - \text{Abs. Blank})}{(\text{Abs. Blank} - \text{Abs. Control})} \times 100 \quad (2)$$

### Organoleptic test

Organoleptic test by 25 untrained panelists who were students of Dhyana Pura University, Bali. The panelist's seats were determined and placed in sufficient space to let them apart. The panelists were requested to fill a consent form prior to test. Then, the panelists were given a questionnaire test of preference of functional drink samples with the code AG1, AG2, AG3, AG4, AG5, AG6, AG7. The panelists assessed the functional drinks and filled out the preference test questionnaire (scale is described in Table 1). Panelists were required to drink mineral water first before switching to the next sample [23].

**Table 1.** Preference scale

Preference	Score
hardly preferred	1
less preferred	2
moderately preferred	3
preferred	4
strongly preferred	5

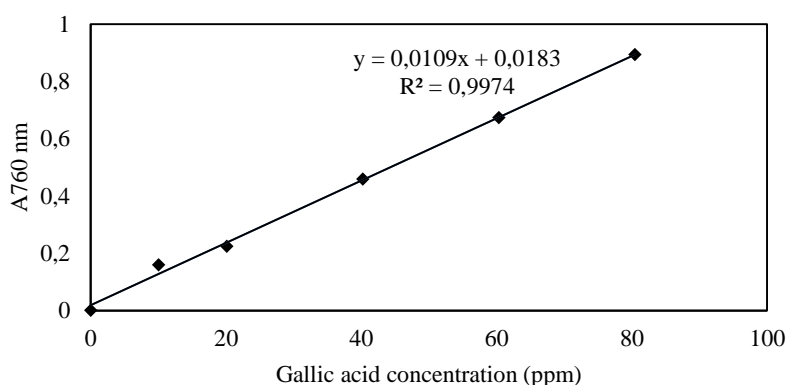
**Data analysis**

All analyzes were carried out in triplo. All results are displayed as mean  $\pm$  SD. Data were processed by using SPSS 22. The data obtained were analyzed by analysis of variance (ANOVA) at the level of  $\alpha = 0.05$ . Should there was a significant difference ( $p < 0.05$ ),

analysis was continued with the Tukey test. Pearson correlation test was performed to determine the relationship between total phenolic, antioxidant capacity, and inhibition of  $\alpha$ -amylase enzyme activity.

**RESULTS AND DISCUSSION****Total phenolic**

The total phenolic content of combined avocado peel and red ginger functional drinks is calculated by the linear regression equation obtained from the standard gallic acid calibration curve in concentration range of 0 to 80 ppm. The equation of the line is  $y = 0.0109x + 0.0183$  with an  $R^2$  value of 0.9974. The combine avocado peel and red ginger functional drinks have a significantly different total phenol content, as seen in Table 2.

**Figure 1.** Calibration curve of Gallic acid**Table 2.** Total phenolic acid concentration

Sample code	Avocado:red ginger ratio	Total phenolic (mg GAE/100mL)
AG1	100 : 0	0.33 $\pm$ 0.10 <sup>b</sup>
AG2	90 : 10	0.34 $\pm$ 0.46 <sup>b</sup>
AG3	80 : 20	0.32 $\pm$ 0.11 <sup>b</sup>
AG4	70 : 30	0.31 $\pm$ 0.02 <sup>b</sup>
AG5	60 : 40	0.27 $\pm$ 0.04 <sup>ab</sup>
AG6	50 : 50	0.22 $\pm$ 0.04 <sup>ab</sup>
AG7	0 : 100	0.12 $\pm$ 0.01 <sup>a</sup>

Note: Total phenolic is expressed as the average $\pm$ SD of the measurements. Superscript denotes significant difference tested by Turkey test ( $P < 0.05$ ).

Table 2 shows that ANACOVA ( $p < 0.05$ ) test revealed significantly different values for each samples, whereas further analysis by Tukey method showed that the AG1-AG6 sample did not have significant phenolic total differences,

except sample AG7 has a significant total phenolic difference.

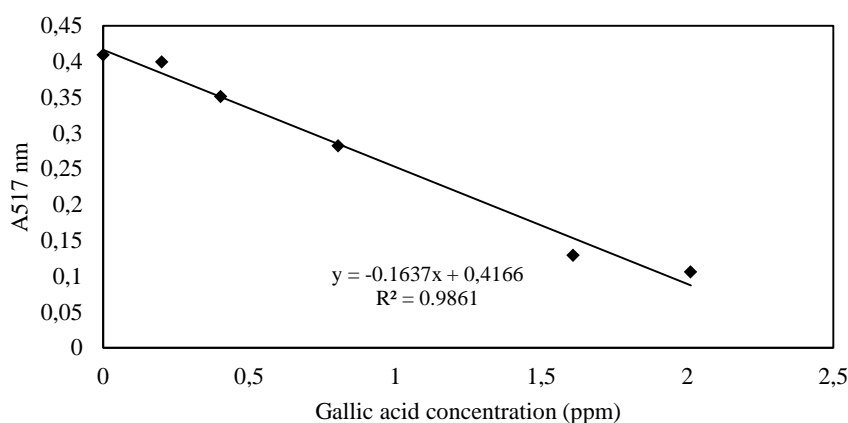
High phenolic content was found in samples AG1-AG4 (0.31-0.34 mg GAE/100 mL), while the lowest was found in the AG7

sample ( $0.12 \pm 0.01$  mg GAE/100 mL). The low value of total phenolic content in the sample is due to the fact that many active compounds in red ginger cannot dissolve with water solvents [24]. Phenolic compounds such as tannin, gingerol, shogaol are more easily dissolved with ethanol or n-hexane solvents [25-27]. This is supported by a report which revealed that the total phenolic content of red ginger, dried 24 to 48 hours prior to ethanol extraction is higher than those extracted by water [28]. The phenolic content of red ginger in this study is lower than those reported by Murad and Mustapha [29] who came up with  $101.6 \pm 0.6$  mg GAE/100 g for the sample prepared by a juice extractor. It turns out the selection of

extraction process correlates with the extraction efficiency [30], [31].

### Antioxidant capacity

The antioxidant capacity of avocado-based functional drinks based on red ginger is calculated based on the linear regression equation obtained from the standard calibration curve for gallic acid at a concentration of 0-100 ppm. The equation of the line is  $y = -0.1637x + 0.4166$  with a value of  $R^2 = 0.9861$ . Avocado-based functional drinks versus red ginger have antioxidant capacity, the results of which can be seen in Table 3.



**Figure 2.** Standard curve of Gallic acid antioxidant activity

**Table 3.** Analysis of antioxidant activity

Sample code	Avocado:red ginger ratio	Antioxidant capacity (mg GAE/100mL)
AG1	100 : 0	$73.79 \pm 17.82^b$
AG2	90 : 10	$80.37 \pm 9.17^b$
AG3	80 : 20	$69.89 \pm 18.55^b$
AG4	70 : 30	$65.86 \pm 4.45^b$
AG5	60 : 40	$57.93 \pm 7.15^{ab}$
AG6	50 : 50	$49.93 \pm 8.26^{ab}$
AG7	0 : 100	$21.70 \pm 2.73^a$

Note: Antioxidant activity is expressed as the average  $\pm$  SD of three measurements. Superscript denotes significant difference tested by Turkey test ( $P < 0.05$ ).

Table 3 shows that ANACOVA ( $p < 0.05$ ) test revealed significantly different values for each samples, whereas further analysis by Tukey method showed that the AG1-AG6 sample did not have significant

differences in antioxidant activity, except sample AG7 has a significant antioxidant difference.

High antioxidant capacity was found in the AG1-AG4 sample (65.86 - 80.37 mg

GAE/100mL), while the lowest was found in the AG7 sample ( $21.70 \pm 2.73$  mg GAE/100mL). This is because avocado peels being studied have phenolic compounds such as catechins, hydroxybenzoic acid, hydroxycinnamic acid, flavonoids, and procyanidins [13]. Flavonoid compounds on avocado skin are more soluble in water [32]. Gingerol compounds in red ginger extracts act as antioxidants. Gingerol is a compound that is not soluble in water. Therefore, the addition of the red ginger ratio does not increase the antioxidant content of the red ginger combination drink compared to the

avocado peel [24,33]. In addition, a positive correlation between antioxidant activity and total phenolic influences the antioxidant capacity in functional drinks [30].

#### **$\alpha$ -amylase inhibition**

The activity of the  $\alpha$ -amylase enzyme was observed by measuring the decrease in blue color intensity in the iodine-starch complex due to the starch hydrolysis by  $\alpha$ -amylase enzyme [34]. The results of the inhibition of the  $\alpha$ -amylase enzyme can be seen in Table 4.

**Table 4.**  $\alpha$ -amylase inhibition

Sample code	Avocado:red ginger ratio	Inhibition percentage (%)
AG1	100 : 0	$3.83 \pm 0.28^a$
AG2	90 : 10	$4.76 \pm 0.11^a$
AG3	80 : 20	$7.15 \pm 1.29^a$
AG4	70 : 30	$4.87 \pm 0.48^a$
AG5	60 : 40	$6.44 \pm 1.90^a$
AG6	50 : 50	$7.46 \pm 3.06^a$
AG7	0 : 100	$8.56 \pm 1.91^a$

Note:  $\alpha$ -amylase inhibition is expressed as the average  $\pm$ SD of the measurements. Superscript denotes significant difference tested by Turkey test ( $P < 0.05$ ).

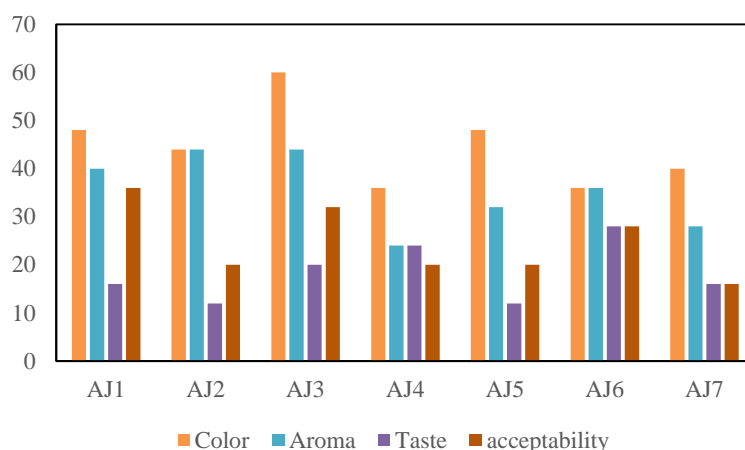
Table 4 shows that the analysis using ANOVA ( $p < 0.05$ ) showed significantly different values for each sample. Further tests by the Tukey method showed that each sample did not have a significant difference in inhibition of the activity of the  $\alpha$ -amylase enzyme.

Inhibition of the activity of  $\alpha$ -amylase enzymes of combined avocado peel and red ginger showed no significant difference. This is because the phenolic compounds of the two ingredients, namely flavonoids, do not contribute to the inhibition of the activity of the  $\alpha$ -amylase enzyme due to inefficiency in the extraction as compared to other extraction methods [35]. Avocado peels and red ginger have the potential to inhibit the activity of the  $\alpha$ -amylase enzyme. Avocado peel has been investigated to have  $\alpha$ -amylase enzyme inhibitory value of 36.02 mg/mL [20]. Inhibition of the activity of the  $\alpha$ -amylase by red ginger

was reported to be 3.14 mg/mL [36]. Apparently, because the two ingredients have the same high effect, a synergistic effect was observed when the two were combined. This is in line with research reported by Syahrir et al. [37] which states that the combination between active ingredients can show a synergistic.

#### **Organoleptic test**

The organoleptic test conducted in this study is a preference test where the assessed attributes are color, aroma, taste, and acceptability of avocado peel-red ginger derived functional drink. The organoleptic test was carried out on 25 untrained panelists. Normality test show that the data were not normally distributed with the sig.  $< 0.05$ , therefore the SPSS test was continued to the Frequency test. The level of preference for functional drinks can be seen in Figure 3.



**Figure 3.** Functional drink organoleptic test

It can be seen from Fig 3 most panelists (60%) preferred AG3 sample that has slight brown color. In terms of aroma, 44% of panelists favoured AG2 and AG3 samples. On the opposite, most panelists didn't like the overly pungent aroma released by red ginger. About 28% of panelists said they like the taste of functional drinks in the AG6 sample from which it can be inferred that the panelists preferred the dominant flavor of ginger. The overall acceptability test shows the AG1 domination with 36% of panelists votes.

## CONCLUSION

Functional drinks made from avocado peels and red ginger show significant total phenolic levels, antioxidant activity, and amylase inhibitory activity at all combination ratios. Organoleptic test revealed variability of panelists preference, with an overall favourite goes to the AG1 sample (100%: 0%) as voted by 36% panelists. This functional drink has the potential to be used as an alternative treatment for diabetes mellitus. Further studies are needed to find out the mechanism of action of the compounds contained in these functional drinks.

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