Agustin *et al. J. Sci. Technol. Res. Pharm.* (2022) 2: (2)p10-22
https://doi.org/10.15294/jstrp.v2i2.694068
ISSN 2776-0685

Journal of Science and Technology Research for Pharmacy

RESEARCH Open Access

Utilization Of Avocado (*Persea Americana* Mill.) Seeds And Cinnamon (*Cinnamomum Burmannii*) As A Functional Powder Drinks

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ABSTRACT

Background: Functional powder drinks are drinks that if consumed can have a positive influence on the health of the body. Avocado seeds and cinnamon have bioactive compounds where these compounds have antioxidant properties that are good for our bodies.

Aim: This research aims to find out the physical quality and antioxidant activity of functional powder drinks from avocado seeds and cinnamon.

Method: Methods and types of research conducted are quantitative research using descriptive design. Testing parameters include tests of physical quality, qualitative and quantitative flavonoid tests as well as antioxidant activity tests using UV-Vis Spectrophotometers.

Result: The results of physical quality tests showed functional beverage powder has a smooth texture, a whitish brown color, a distinctive smell of spices, and a distinctive sweetness of spices with a pH of 6,569. Qualitative tests of flavonoids showed the results of functional powder drinks positive containing flavonoid compounds due to the presence of red color formed, then continued with quantitative tests flavonoids obtained flavonoid levels of 12.201%.

Conclusion: Antioxidant test results in functional powder drinks showed an IC_{50} value of 128,422 mg/L which belongs to the category of moderate-level antioxidants.

Keywords: avocado seeds, cinnamon, functional powder drinks, physical quality, flavonoid, antioxidant activity

INTRODUCTION

Health is very important for all humans because, without good health, every human being will find it difficult in carry out his daily activities [1]. In everyday life, the simplest or easiest thing to do in maintaining health is to consume foods and drinks that can improve health, one of which is by consuming functional drinks that are good for health [2]. Functional drinks are drinks that contain nutritional or non-nutritional elements and if consumed can have a positive influence on the health of the body [3], functional drinks can be made from a variety of plants, one of the plants that can be processed into functional drinks is avocado seeds (*Persea americana* Mill.) [4].

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https://doi.org/10.15294/jstrp.v2i2.694068
ISSN 2776-0685

Avocado seeds have bioactive compounds such as phenolic derivatives namely flavonoids, polyphenols and others, where these compounds have antioxidant properties that are good for our bodies. Antioxidants can control blood glucose levels through the mechanism of improving pancreatic function in producing insulin [5]. Avocado seeds have antioxidants with an IC_{50} value of 31.5 ppm [6], avocado seeds have a slightly bitter taste and smell so that it can be combined with other ingredients as flavors and fragrances in beverage formulations [4].

The fragrant aroma of cinnamon (*Cinnamomum burmannii*) is usually used as a flavor, aroma and at the same time can be used as medicine [7], the flavonoid content of cinnamon is 1.75 – 2.61 mg/g [8]. Some studies explained that cinnamon plant is an antimicrobial, antifungal, anti-inflammatory, anti-allergic, and anticancer effect [9]. Cinnamon extract contains compounds that have the potential to be antioxidants, such as phenols and flavonoids [10].

Functional powder drinks tested based on physical quality include tests of organoleptic, pH, qualitative and quantitative flavonoid, also antioxidant activity [11]. Indonesian National Standard number 01-4320-1996 describes the physical requirements of traditional powdered drinks that are normal in color, smell normal and typical spices, and have a normal taste and typical spices [12]. One component of functional drinks that have physiological functions for the body is antioxidants [13]. Measurement of antioxidant activity by DPPH method (2,2-diphenyl-1-picrylhydrazil) is a simple method and uses samples in small amounts within a short time [14].

Based on the above description, the author wants to utilize avocado seed waste so that it can be processed into beverage products with the addition of star anise as flavors and fragrances made into functional powder drinks that are efficacious as antioxidants. Therefore, the author is interested in conducting research with the title "Utilization of Avocado (*Persea americana* Mill.) Seeds with Cinnamon (*Cinnamomum burmannii*) As A Functional Powder Drinks".

MATERIAL AND METHODS

This research is a descriptive type with a quantitative descriptive design because it describes or describes a sample objectively with data in the form of numbers or guessed data. The variables used in this study were the single variables of the physical quality and antioxidant activity of functional beverages from the waste of avocado seeds (*Persea americana* Mill.) and cinnamon (*Cinnamomum burmannii*). In this study, the materials, tools and procedures used are as follows:

a. Materials

The main raw materials used are the simplisia of avocado seeds (*Persea americana* Mill.) and cinnamon (*Cinnamomum burmannii*). Other ingredients are sucrose, filter paper, aquadest, buffer pH 4.7, and 10, Mg powder, HCl 37%, methanol p.a, quercetin, $AlCl_3$ 10%, CH_3COOH 5%, DPPH (2.2- diphenyl-1-picrylhydrazil).

b. Equipment

The Analytical balance (Labex), 18-mesh sieve, blender (Fomac-Miller Machine FCT-Z500, pH meters (Hanna HI 8010), beaker glass (Iwaki Pyrex), magnetic stirrer, hotplate stirrer (Thermo scientific), thermometers, drip pipettes, test tubes (Iwaki Pyrex), tube racks, vortex

https://doi.org/10.15294/jstrp.v2i2.694068
ISSN 2776-0685

(Thermo scientific), spatula, volumetric glass (Iwaki Pyrex), glass funnels (Iwaki Pyrex), volumetric pipettes (Iwaki Pyrex), volumetric flask (Iwaki Pyrex), cuvettes (Purshee), and UV-Vis spectrophotometers (Raptor).

c. Method

This research was conducted with three stages, namely the first stage is the manufacture of functional beverage powder, the second stage is testing functional beverage powder and the third stage is processing and analysis of data that can be.

1) Functional Powder Drinks of Avocado (*Persea americana* Mill.) Seeds with Cinnamon (*Cinnamomum burmannii*)

Extraction of avocado seeds and cinnamon refers to Anggoro (2018) modification, which is the simplisia powder of avocado seeds and cinnamon are heated with aquadest at temperature of 90 ^{o}C for 15 minutes and strained, then evaporated until thick. The composition of functional beverage powders can be seen as follows.

Table 1. Functional Powder Drinks Composition

Composition	Sum
Avocado seeds	40%
Cinnamon	35%
Sucrose	25%

(Source: Rohmayanti modification *et al*., 2019)

The process of making functional powder drinks refers to the modification of Rifkowaty *et al.* (2019), avocado seeds extract and cinnamon mixed according to treatment, sucrose is added to the juice mixture. The mixture of sucrose and extract is heated over a steady low heat while continuing to stir until crystals form. The resulting crystals are smoothed with a blender and sedated with a sying of 18 mesh so that functional beverage powder is obtained and further analysis is carried out.

2) Functional Powder Drinks of Avocado (*Persea americana* Mill.) Seeds with Cinnamon (*Cinnamomum burmannii*) Testing

1. Physical Quality

a) Organoleptic test

Organoleptic testing is a means of testing using the human senses as the primary tool for measuring the power of reception to products that include smell, color, taste and texture (Akmal, 2013).

b) pH test

Measured pH sample using pH meters that have been calibrated first using a pH buffer of 4, 7, and 10 then the sample of 8 grams is dissolved in 20 mL aquades, then dipped the pH meter electrode into the sample and awaited until the reading number becomes stable. The pH measurement is replicated three times and calculated on average (Haqiqi, 2016).

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2. Qualitative Test

A total of 4 grams of the sample is dissolved in 40 mL of methanol p.a, then taken 10 mL and put into a test tube. Added to the sample in the form of magnesium powder 2 mg and given 3 drops of concentrated HCl. The sample is shuffled and observed changes that occur. Tests were conducted with three replications, the formation of red, yellow or orange in the solution showed the presence of flavonoids (Purwati, 2017).

3. Quantitative Test

a) Total Flavonoid Levels

The manufacture of a standard solution of 200 ppm quercetin is done by weighing as much as 10 mg of quercetin, dissolved into methanol p.a and added in 50 mL volumetric flusk, mixing until homogeneous [6].

Determination of the maximum wavelength of quercetin is done with a standard solution of 200 ppm quercetin obtained, then made into a 100 ppm quercetin solution. A total of 1 mL of 100 ppm quercetin solution, added with 1 mL of 10% $AlCl_3$ and 8 mL of CH_3COOH 5%. The solution is incubation for 30 minutes, absorbance is measured at wavelengths of 350-500 nm [16].

The manufacture of calibration curves is done by making serial solution levels of 40, 60, 80, 100, and 120 ppm. A total of 1 mL of serial solution levels of each concentration are entered, reacted with 1 mL $AlCl_3$ 10% and 8 mL CH_3COOH 5%. The sample was silenced for 30 minutes, a series absorption reading of levels using UV-Vis spectrophotometry at maximum wavelength [17].

Determination of total flavonoid levels in functional beverage samples is done by making a concentration solution of 800 ppm, then vortex for 10 minutes at a speed of 3000 rpm. Then taken as much as 1 mL sample is inserted in a test tube and added 1 mL $AlCl_3$ 10% and 8 mL CH_3COOH 5%. The sample is then incubation for 30 minutes, absorbance measured at maximum wavelength [17].

Flavonoid levels are calculated using linear regression equations based on calibration curves resulting from UV-Vis spectrophotometer readings. Absorbance data obtained from measurements is entered into linear regression equations as y and x values as raw solution concentrations. The linear regression equation is expressed by the formula: y = bx + a with y = absorbance, a = interception, x = concentration (ppm), b = slope (slope).

The absorbance results of the sample measurement are entered into linear regression. Absorbance of the sample as y, so that the total flavonoid levels obtained are expressed as the mg amount equivalent of quercetin (QE) in each gram of the sample [17] :

$$\% \text{ Total Flavonoids} = \frac{C \times V \times Fp}{m} \times 100\%$$

Information:

C = Concentration of quercetin (ppm)

V = Total sample volume (L)

https://doi.org/10.15294/jstrp.v2i2.694068
ISSN 2776-0685

Fp = Dilution factor
M = Sample weight (g)

b) Antioxidant Activity

Determination of maximum wave display DPPH is done by making a concentration of 50 ppm. The standard solution is then placed into a dark bottle, incubation in a dark room for 30 minutes. The DPPH solution is determined by its maximum absorption wavelength using UV-Vis spectrophotometer at a wavelength (λ) of 400-600 nm [18].

The manufacture of quercetin solution as a comparison is done by making a concentration of 25 ppm. Then dilution is done to add methanol p.a so that the solution is obtained with concentrations of 2,4,6,8 and 10 ppm. A total of 2 mL of 50 ppm DPPH solution is added with each series of 1 ml of quercetin solution. The solution is homogenized and incubation in a dark room for 30 minutes. The sample measured its uptake with a UV-Vis spectrophotometer with the wavelength obtained [18].

Determination of antioxidant activity in functional beverage samples is done by making a solution of concentration samples of 200, 400, 600, 800, and 1000 ppm. The sample solution is then mix used vortex for 10 minutes at a speed of 3000 rpm, then taken as much as 1 mL from each concentration is inserted into the test tube and added 2 mL DPPH solution 50 ppm, then blanko solution is made with 1 mL methanol p.a and 2 mL DPPH solution 50 ppm. The solution returns for 1 minute at a rate of 2000 rpm, then incubation at lightproof indoor room temperature for 30 minutes. This solution is then measured at its absorbance at maximum wavelength [18].

$$\% \text{ Inhibition} = \frac{Standard\ absorbance - Sample\ Absorbance}{Standard\ absorbance} \times 100\%$$

IC_{50} value is a number that indicates the concentration of the test sample that provides immersion of 50% (able to inhibit or soak the oxidation process by 50%). The value of IC_{50} is determined by making a linear curve between the concentration of the test solution (x-axis) and % damping (y axis) so that the equation y = bx + a where y is % inhibition and x is the value of IC_{50} [19].

$$IC_{50} = \frac{50 - a}{b}$$

RESULTS

Organoleptic test

Organoleptic tests are performed to determine the physical quality of functional powder drinks including color, smell, taste and texture. The results of organoleptic tests can be seen in Table 2.

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ISSN 2776-0685

Table 2. Organoleptic Test of Functional Powder Drinks

Test Criteria	Result
Color	Whitish chocolate
Smell	Typical spices
Taste	Typical spice, sweet
Texture	Fine powder

Based on the data above, the results of organoleptic tests of functional beverage powders have a whitish brown color, a distinctive smell of spices, a distinctive sweet taste of spices and with a smooth texture.

The pH test

The pH test is done to determine the level of acidity or numbness in a functional drink. The results of the pH test can be seen in Table 3.

Table 3. pH test in functional beverages

Testing	Result	Category
pH	6,569	Weak acid

Based on the data above, the pH result of functional drinks has a pH of 6,569 which includes weak acids.

Flavonoid Test

Flavonoid tests are conducted to find out the presence of flavonoid content in samples of functional drinks that have the potential as antioxidants. Flavonoid tests are qualitative and quantitative flavonoid tests.

a. Qualitative Test of Flavonoids

Qualitative tests of flavonoids are conducted to determine the presence of flavonoids in functional powder drinks. The results of the flavonoid qualitative test can be seen in Table 4.

Table 4. Flavonoid Qualitative Test

Sample	Result	Interpretation
Simplisa Avocado Seeds and Cinnamon	Deep Red	+
Functional Drinks	Red	+

Based on the data above showed positive results of flavonoids in functional powder drinks characterized by the presence of red after testing.

b. Total Flavonoid Quantitative Test

Quantitative tests of total flavonoids were conducted to determine the levels of flavonoids in functional powder drinks analyzed using UV-Vis Spectrophotometer at maximum wavelength (λ maks) of 418 nm. The results of the total flavonoid quantitative test can be seen in Table 5.

Table 5. Flavonoid Quantitative Test

Concentration (ppm)	Absorbance	Flavonoid Levels (%)
800	0,624	12,201%

Based on the data above showed that the total flavonoid levels in the functional powder drinks was 12,201%.

Antioxidant Activity Test

The antioxidant activity test was conducted to determine the antioxidant properties in functional beverage samples analyzed using a UV-Vis Spectrophotometer with a solution of DPPH (2.2- diphenyl-1-picrylhydrazil) at a maximum wavelength (λmaks) of 518 nm with an absorbance of 0.781 and used quercetin comparison. The results of the antioxidant activity of functional drinks are as follows in Table 7.

Table 7. IC_{50} Value Measurement Results from Functional Beverage Samples

Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (mg/L)	Antioxidant Properties
200	0,367	53,009		
400	0,272	65,216		
600	0,187	76,014	128,422	Moderat
800	0,092	88,220		
1000	0,031	95,988		

Based on the data above shows that the IC_{50} value of functional beverage samples amounted to 128,422 mg/L which shows that functional powder drinks have moderate antioxidant properties.

DISCUSSION

In this study, functional powder drinks from avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) that have been made tested with organoleptic test parameters, pH tests, flavonoid qualitative tests, flavonoid quantitative tests, and antioxidant activity tests. The purpose of the test is to find out the physical quality and antioxidant activity of functional drinks from avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*).

https://doi.org/10.15294/jstrp.v2i2.694068
ISSN 2776-0685

The stage of making functional beverage powder begins by weighing the simplisia weight of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) as in Table 1. The infusions or sample samples are made by heating the material and solvent aquadest with a ratio of 1: 10, then heated at a temperature of 90 ^{o}C for 15 minutes using a water handler. The excess obtained is then fertilized to half of the initial volume, then made powder by mixing sucrose and stir until crystallized [19].

This extraction stage is used 1:10 extraction comparison because according to [20], this comparison is the best against flavonoid compounds. The addition of sucrose aims as a sweetener at the same time so that the crystallization process can then be made beverage powder in order to improve quality and facilitate storage so that its functional properties for health can be maintained properly. The fine crystals are then smoothed with 18 mesh sieve, using 18 mesh sieve because the degree of smoothness produced is higher so that the powder dissolves faster in water. The degree of smoothness indicates uniformity of the results of grinding or spreading rough and smooth fractions. The smoother the powder, it will quickly dissolve in water because the surface of the powder that comes into direct contact with the solvent is wider while the rougher the powder, the longer it takes to dissolve because the more cells must be penetrated by the solvent [15].

The first test conducted was an organoleptic test, obtained the result that the functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) has a whitish brown color, new typical spices, the sweet taste of spices with a smooth texture. The results obtained are in accordance with SNI 01-4320-1996 on the quality standards of traditional powder drinks that explain that traditional powder drinks must have normal colors, smells and normal flavors typical of spices [12].

The pH test on functional drinks is performed using a pre-calibrated pH meter. Calibration is part of the maintenance of the tool, which aims to ensure that the measurement results of the tool are acceptable and fall within the required validation range. Calibration of pH is carried out using a standard buffer solution reference material at acidic, alkaline and neutral conditions [21]. Test results from three replications showed functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) had a pH of 6,569 which showed functional drinks had weak acidic properties due to the presence of flavonoid content in them. It is suggested that flavonoids are a group of polyphenols so it has the chemical properties of phenol compounds that are acidic so they can dissolve in bases and have antioxidant properties [22].

Qualitative flavonoid tests were conducted using Mg powder and HCl 37%, the addition HCl in flavonoid tests used to hydrolyze flavonoids into their aglicons. The flavonoids are compounds that contain two aromatic rings with more than one hydroxyl group. The addition of Mg powder and HCl is to reduce the benzopiron nucleus contained in flavonoid structures so that red or orange flavilium salts are formed [23]. Based on the results of qualitative flavonoid tests on the simplisia powder of avocado (*Persea americana* Mill.) seeds, cinnamon (*Cinnamomum burmannii*), and functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) as a comparison is known to contain flavonoids in the presence of discoloration to deep red and functional drink samples undergo a discoloration change from orange to red, the results of qualitative tests of red flavonoids indicate that the sample is positive to contain flavonoid

https://doi.org/10.15294/jstrp.v2i2.694068
ISSN 2776-0685

compounds [24].

Total flavonoids are the total amount of secondary metabolite compounds derived from a plant. The total content of flavonoids is measured based on the presence of quercetin in plant extracts because quercetin is the most active substance in flavonoids so quercetin represents other flavonoid compounds [6]. Quantitative tests of total flavonoids were conducted with a UV-Vis Spectrophotometer at a maximum wavelength of 418 nm. The wavelengths used for quantitative tests of flavonoids total between 350-500 nm [16]. Long setting Maximum waves are done to find out at what wavelength produces the maximum absorption value in the sample, so that the measurement results are accurate and minimize errors [18]. The solvent used is methanol p.a because methanol is polar. Methanol is a liquid that easily enters the cell through the cell wall of the material, so that the metabolites of the sunder contained in the cytoplasm will be dissolved in the solvent and the compound will be extracted perfectly. Flavonoid compounds are polar compounds because they have a number of sugars that are bound, therefore flavonoids are more likely to dissolve in polar solvents. According to the principle of polarization, a compound will dissolve in a solvent that has the same polarity [25].

Quantitative tests of total flavonoids were conducted to look for flavonoid levels in functional beverage samples measured quantitatively by the aluminum chloride method using a standard solution of quercetin, so that the results were calculated as milligrams of QE (Quercetin Equivalent) per gram dw (dry weight) sample of functional beverages. Quantitative tests of total flavonoids were conducted with the addition of 1 mL of 10% $AlCl_3$ and 8 mL CH_3COOH 5% at every 1 ml of sample solution concentration to be tested. Azizah *et al.* (2014), suggested that the total flavonoid content was determined based on a colorimetric reaction that is, after the sample was reacted with $AlCl_3$ in an acidic medium. The addition of $AlCl_3$ in the sample can form a complex between aluminum chloride and quercetin resulting in a shift in wavelength towards visible and characterized by the solution producing a more yellow color. The function of adding acetic acid to maintain wavelengths in visible (visible) areas.

Samples containing flavonoids will react with $AlCl_3$ the function of $AlCl_3$ reagent is to form a reaction between $AlCl_3$ and flavonoid groups forming complex forms between hydroxyl groups and neighboring ketones or with neighboring hydroxyl groups. $AlCl_3$ solution will react with ketone groups in C-4 and OH groups on C-3 or C-5 in flavon compounds or flavonols to form stable yellow complex compounds. Compounds used as standards in determining flavonoid levels are quercetin, because quercetin is a flavonoid flavonol group that has a keto group on C-4 atoms and also a hydroxyl group in neighboring C-3 and C-5 atoms [17]. The reaction between flavonoids and $AlCl_3$ is shown in Figure 1.

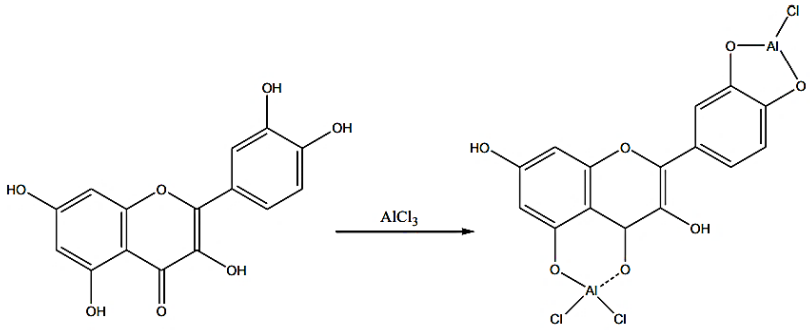

Figure 1. Complex formation reactions of flavonoids and $AlCl_3$ [22]

Flavonoid extracts sampled functional beverages obtained are yellowish brown. The results of total flavonoid testing on flavonoid extracts of functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) samples in Table 5 showed that the flavonoid content was 12,201%.

Functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) have flavonoid content that has the potential to be used as antioxidants. The antioxidant activity of functional beverages is expressed as Inhibitor Concentration 50 (IC_{50}), it is defined as concentration antioxidant compounds needed to reduce free radical activity by 50%, where the smaller the IC_{50} value, the higher the antioxidant activity. Determination of antioxidant activity is done by the method of DPPH (2,2-diphenyl-1-picrylhydrazyl) by utilizing DPPH free radical compounds in polar solvents such as methanol to test antioxidant compounds in dampening free radicals [26]. The capture of DPPH free radicals by antioxidants will cause a reduction of DPPH compounds causing the purple color to fade and the yellow diphenyl-picrylhydrazine complex which is non-radical (Izza *et al.*, 2016). The reaction between DPPH and antioxidants (AH) can be seen in Figure 2.

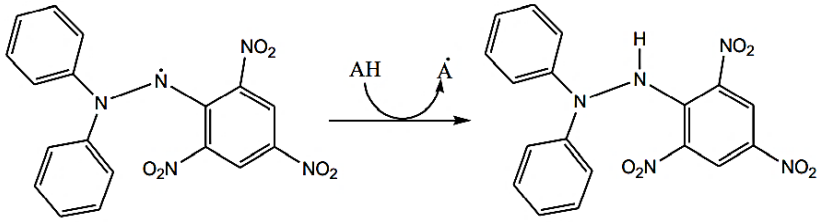

Figure 2. Antioxidant reactions with free radicals (DPPH)
(Arifin and Ibrahim, 2018)

This study used quercetin as a comparison because quercetin is a flavonoid compound of the type of flavonol and flavon, where flavon compounds have antioxidant properties [22]. Testing the value of antioxidant activity in the sample was conducted in the presence of incubation for 30 minutes after adding DPPH. The purpose of incubation of the sample solution and DPPH is so that the DPPH can react perfectly with the sample. During the incubation process there is a reaction between antioxidant compounds with DPPH radicals characterized by a discoloration of the purple solution in DPPH to a yellow to clear color. This change occurs when all the electrons in the DPPH

free radical become paired, then the color of the solution changes from deep purple to bright yellow and absorbance at maximum wavelength will be reduced [28].

The results showed that the IC_{50} value of quercetin amount 7,349 mg/L, while the IC_{50} value of the functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) was 128,422 mg/L. Rahmi (2020), suggested that the division of the category of antioxidants of a natural ingredient (raw material) that has IC_{50} less than 50 mg/L can be categorized as a very powerful antioxidant, 100-150 mg/L as a moderate antioxidant and if more than 200 mg/L is categorized as a weak antioxidant, so functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) have the potential as a moderate antioxidant.

CONCLUSION

Based on the research that has been done by the author, the conclusions obtained are avocado (*Persea americana* Mill.) seeds which is a waste can be used as functional powder drinks along with cinnamon (*Cinnamomum burmannii*) which have functional value because they contain antioxidants that are good for the body. Physical quality test results obtained that functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) has a whitish brown color, smells typical of spices, sweetness typical of spices, smooth texture and has a pH of 6,569 which belongs to the weak acid group. Flavonoid test results obtained for qualitative flavonoid tests showed positive containing flavonoid compounds due to the presence of red color formed after reacting and quantitative tests of flavonoids totally obtained flavonoid levels of 12,201%. Antioxidant test results in functional powder drinks of avocado (*Persea americana* Mill.) seeds and cinnamon (*Cinnamomum burmannii*) show IC_{50} value of 128,422 mg/L which belongs to the category of moderate levels of antioxidants.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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