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CHARACTERIZATIONS AND EXTRACTION OF POLYPHENOLS FROM RESIDUAL PULP OF PINK GUAVA AS SOURCE OF ANTIOXIDANTS

Lilis Sukeksi and Maya Sarah
Department of Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Medan, Indonesia
lilissukeksi79@yahoo.com

ABSTRACT

Pink guava (Psidium guajava L.) is a tropical fruit rich in high-profile nutrients such as polyphenols, which act as antioxidants. After processing pink guavas for fruit juice, the residual pulp still contains a large amount of polyphenols. Our previous study indicated that the total polyphenol content of residual pulp pink guava after processing ranged 0.03 to 0.12 mg/g. The aim of present study is to find the best solvent for the extraction of polyphenols. The highest total polyphenol content was obtained using methanol as a solvent and the second highest was obtained using water as a solvent. A mixture of methanol and water at a ratio of 60% increased the total polyphenol content. The best extraction time was 180 minutes and a ratio of 1 g of pulp waste to 25 ml of 60% methanol/water solvent yielded the highest polyphenol content. The Folin-Ciocalteau method was used to determine the total polyphenol content.

Keywords: residual pulp, polyphenols, antioxidant, extraction, solvent.

1. INTRODUCTION

In recent years there has been a significant increase in the consumption of many types of real tropical fruit juice due to their nutritive value. In tropical countries, the guava is the most significant fruit in industrial juice extraction. The guava is a good source of many antioxidants such as polyphenols and β-carotenes [1, 2, and 3]. It also has a significant amount of vitamins like ascorbic acid [3,4] and is a great source of many important minerals such as phosphorous, calcium, and iron [3, 5]. Furthermore, the guava can be made into many forms of food products because it has a distinctive and strong aroma. It is used to produce many varieties of processed juice products through clarification, concentration, canning and blending with other juices. The guava can also be processed into desiccated powder, jam, jelly and cheese.

The processing of fruits and vegetables in food processing plants has resulted in high quantities of both liquid and pulp waste. The residual pulp contains primarily biodegradable organic matter, which represents a major disposal problem. The disposal has resulted in significant environmental problems due to microbial spoilage. However, this residual pulp that is often used as feed or fertilizer is a lost source of valuable nutrients. The residual pulp, which results after processing in a factory, still contains a large quantity of antioxidants such as polyphenols compounds. To reduce the amount of disposal at processing plants, procedures should be employed such as in-plant treatment, reuse of residual pulps and several other forms of processing. The residual pulps could be utilized in new products or adapted according to processing methods [6]. Developments in this direction aim to change residual pulps resources into bioenergy, foodstuff and value-added bio products.

The residual pulps produced from pink guava processing can also be used to make available sources of many natural additives and foodstuff ingredients [6, 7]. Studies have been conducted on residual pulps from fruit processing as a possible source of antioxidants [1, 6, and 8]. Polyphenol compounds such as polyphenol acids and flavonoids from fruit, found in the residual pulp from pink guava processing, have been shown to be beneficial as antioxidants [9]. A polyphenol antioxidant is an antioxidant that has polyphenol as its sub-structure. These compounds, which have over 4000 distinctive species, are important to human health. They may be instrumental in fighting oxidative stress that causes certain degenerative and cardiovascular diseases [10].

Based on investigation, some agro-industrial processing byproducts or residual pulp have been proven to be effective sources of polyphenol antioxidants. Residual pulp, if used effectively, could be the most important source of polyphenol compounds. Pizzichini [11] developed several protocols for purifying polyphenols compounds from olive oil mill waste water using membrane technologies. The pink guava residual pulp also can be used as a source of antioxidant dietary fiber, as shown by the results of recent research by [12]. Processing valuable compounds from fruit or vegetable residual pulp is necessary in order to minimize the increasing amount of this waste. Methods should be developed for the complete use of fruit processing residual pulp on a large-scale and at realistic price levels.

Indonesia is a tropical country that produces various types of fruits, such as guavas, snake fruit (salak), mangosteens, sapodillas, durians, etc. In Indonesia, guavas are among the leading fruits of North Sumatera that are distributed nationally. Sumatera Utara is a province in Indonesia and a part of area plantation that produce guava fruit. Up to this point in time, agricultural products from fruits and vegetables have only been consumed in fresh form. The use of fruits and vegetables has many constraints, due to the fact that fruits or vegetables are perishable and cannot be stored for a long time. This factor has hindered farmers from increasing their production capacity. Moreover, problems also arise

at concurrent harvesting times so that the public cannot accommodate production if produce is only to be consumed fresh. This causes selling prices to decrease and results in losses for farmers.

Therefore, this study aims to utilize the very high potential of pink guava as an industrial product and the residual pulp that results from these industrial processes, which can be used as downstream products that have economic value.

For this reason, we investigated the potential of pulp waste from fruit processing for the extraction of natural antioxidants or polyphenols. The residual pulp from pink guava processing that still contains polyphenols was extracted using some organic solvents and pure water. Many valuable natural polyphenols have been extracted by using organic solvents, such as methanol, ethanol, acetone, etc. This research examines the use of organic solvents and water for the extraction of polyphenols.

2. METHODOLOGY

a) Collection of samples

Pink guavas were collected from public orchards in the Province of North Sumatera, after pressed for the extraction of juice the residual pulp was analyzed to determine the polyphenol content. All samples were immediately packed into sealed plastic containers and stored in a freezer. The time between freezing and analysis of the sample was 1 week. Improper storage of residual pulp can result in it rapidly losing its potency, making them ineffective for purposes of extraction. Therefore, residual pulp must be stored properly and refrigerated to prevent mold or oxidation.

b) Characterization of samples

This subchapter will explore the characteristics of pink guava processing residual pulp samples prior to the extraction of polyphenols. These characteristics include the dry weight, and amount of antioxidants contained in the pink guava residual pulp.

i. Dry weight (DW)

Dry weight (DW) is the material that remains after removal of water or moisture content. It represents the water present in field samples. The most common way to find DW is through the evaporation of water contained in samples. Before sampling, a crucible was prepared by first soaking it in distilled water and then drying it at 105 ^{0}C. Next, the crucible was placed in the desiccator at room temperature and its weight recorded using a microbalance (AND GF 400, model 77200-6-, Japan). 10 g of the sample was weighed out on a digital balance and placed on the dry crucible. The weight of the empty crucible was subtracted from the weight of the sample on the dry crucible in order to find out the weight of the sample before the drying process. The sample was carefully dried in the crucible and left in the oven (Memmert model 600) for a period of 1 hour at 105 ^{0}C. After 1 hour, the sample was placed inside the desiccator to achieve room temperature. Then, the sample was weighed and its weight immediately recorded. This process was repeated until a constant weight was achieved. Lastly, the weight of the empty crucible was subtracted from the constant weight of the sample on the crucible. To get the percentage of the dry sample, the weight of the dry sample was divided by the weight of the wet sample and multiplied by 100 [13].

ii. Quercetin

Quercetin analysis was performed using a spectrophotometer. A standard stock solution of quercetin was prepared by dissolving exactly 10 mg sample in 100 ml ethanol. A standard calibration curve was measured at different concentrations of the standard stock solution. The absorbance was measured by the spectrophotometer at 256 nm. The quantity of quercetin was then calculated using the calibration standard curve.

iii. Gallic acid

Spectrophotometry was used to identify the amount of gallic acid in residual pulp samples. The extracts of pink guava residual pulp samples were prepared using water as a solvent. This solvent was chosen because water shows high affinity for the removal of gallic acid from extracts and at the same time is safe for human consumption. About 1.5 grams of pulp waste sample was mixed with 25 ml of water solvent and shaken at room temperature for 3 hours. The extracts were centrifuged using MediSpin centrifuge and then filtered using Whatman filter papers. The clear extracts were then analyzed to measure the gallic acid content using the colorimetric Folin–Ciocalteau assay [15].

iv. Tannin

Titrimetric was used to determine the amount of tannin in the pink guava residual pulp samples. To prepare a sample solution, 3 g of the samples was added to pure water to make 250 ml of solution. The sample was left in volumetric flask at room temperature for 4 hours and was then filtered. A 0.1 N $KMnO_4$ solution was used for titration until the blue colored solution turned a green color. The sample was analyzed repeatedly.

v. Apigenin

Apigenin analysis was performed using HPLC. 2 g of sample were added to 100 ml of boiling water and kept on a rotary shaker for 5 minutes. The solution was quickly filtered when it was still hot using a vacuum. Then, 1 ml was taken from this extract and diluted with 25 ml of mobile phase. The diluted solution was filtered through a 0.45 μm membrane filter. A standard solution of apigenin was prepared by dissolving 4.72 mg of standard apigenin in 50 ml methanol. The clear extract or supernatant and standard solution were then transferred to vials and injected into the HPLC system. A Waters μ-bondapak C18 column (3.9 x 300 mm) was used for the apigenin analysis. Mobile phase was carried out using

70% water, 29.5% methanol, and 0.5% acetic acid. The amount of apigenin in the extract was calculated from the area under the curve corresponding to the respective peaks and their standard plots.

c) Extraction of polyphenols

The solvents selected for the extraction of polyphenols in this experiment, namely, methanol, ethanol, acetone, and acetonitrile, were purchased from Merck (Germany). These solvents were chosen because they show a high affinity for polyphenols that were to be removed from the extracts [14]. Purified water was prepared using an Elga Micromeg deionizer. Folin-Ciucalteu's phenol reagent standard quercetin, apigenin, tannin and gallic acid with a purity of 98-99% were purchased from Sigma-Aldrich (Great Britain) for the analysis of TPC. Meanwhile, sodium carbonate (Na_2CO_3), potassium permanganate ($KMnO_4$), and acetic acid (CH_3COOH) were purchased from Fluka (Germany). A spectrophotometer was used to measure the quantity of total polyphenol content. The optical absorbance was measured with a Thermo Spectronic Genesys20 or spectrophotometer model 4001/4, made in the USA, 2008. The spectrophotometer was equipped with data logging.

i. Sample preparation

Extracts from pink guava residual pulp samples were prepared using selected solvents, namely, methanol, ethanol, water, acetone and acetonitrile. 1.5 g of sample was mixed with 25 ml of a solvent and shaken at room temperature for 3 hours. The extracts were centrifuged using a MediSpin centrifuge type 3-16 PK serial number 126479, Germany, 2008 and filtered used Whatman filter papers. The clear extracts were then analyzed to determine the TPC.

ii. Measurement of total polyphenols content (TPC)

The Folin–Ciocalteau assay [15] was used to measure the polyphenol content of the solution extract. The Folin-Ciocalteau reagent is a mixture of $H_3PW_{12}O_{40}$ and $H_3PMo_{12}O_{40}$ acids in the alkaline medium. 200 µl of the clear extract sample was added with 1500 µl of 0.25 N Folin-Ciocalteau reagents and mixed well with a Vortex mixer (Barnstead International, model M 37610-33, Malaysia). The mixture solutions were allowed to react for 3 minutes and stand at 22 ^{0}C. 1500 µl of 1 N Na_2CO_3 solution was added and the solution was mixed well. The solution was incubated in the dark at room temperature for 90 minutes. A mix of blue oxides was formed as a reaction. The intensity of blue solution was measured using the spectrophotometer, Thermo Spectronic Product Genesys 20, 2008, made in USA. To calculate the amount of TPC, gallic acid was used as a standard curve. The ranges of calibration in the standard curve were from 0.1 mg/ml to 1.2 mg/ml. To determine the highest absorbance the solution of polyphenols has to be measured at different intensities. The absorbance was measured at 725, 735, 750 and 765 nm to obtain optimum results.

3. RESULTS AND DISCUSSIONS

a) Characteristics residual pulp of pink guava fruit

A sample of pink guava processing residual pulp was taken from a pink guava fruit juices processing. The residual pulp might contain bacterial and fungal growth since such waste is potential food for them.

As shown in Table-1, the dry weight of the sample was 28.42%, which indicates that the sample still contained a large amount of water. This could be due to an increased growth in microorganism that would ultimately result in a decrease of organic nutrients, especially of sugar and protein.

Results obtained from the analysis of polyphenol compounds characterize the sample as follows: 0.40 mg/100g quercetin, 8.70 mg/100g gallic acid, 62.60 mg/100g tannin, and no evidence of apigenin detected in this experiment.

Brasil *et al*. [16] found that tannins in natural pulp were 190 mg/100g. They concluded that the decreases (73.7%) in total polyphenols content (tannins) just after fining suggest that most of the polyphenols were precipitated by gelatin. According Bashir *et al*. [17] the polyphenols content in the pulp and peel of pink and white guavas progressively decreases with a decrease in flesh firmness. The decrease in polyphenols content in the white guava was much more marked than in pink guava. It can be thus being concluded that pink guava has more polyphenols content than white guava. The result in this experiment must be different because the work of Padula and Rodriguez-Amaya [18] indicated that different cultivars resulted in different amounts of vitamin and nutrition content.

Table-1. Dry weight and polyphenols contents residual pulp of pink guava fruit.

No	PARAMETER	RESULT	UNIT
1	Dry Weight	28.42	%
2	Quercetin	0.40	mg/100g
3	Gallic Acid	8.70	mg/100g
4	Tannin	62.6	mg/100g
5	Apigenin	Not detected	mg/kg

The results indicate a significant amount of polyphenol content in pink guava processing wastes and this is in agreement with previous studies [17, 19,20, and 21], which show that the guava as well as guava residual pulps has a higher amount of antioxidant content. Thus, a study of the extraction of polyphenol content was carried out as follows.

b) Extraction of polyphenols

The extraction of polyphenols is dependent on the dissolution of each polyphenol compound and its diffusion in external solvent medium. To determine the polyphenols content using spectrophotometer, absorbance is influenced by intensities of blue colour which changes

the measurement. So the first step was to observe the TPC by measuring the best absorption spectra at different polarity. After finding the best peak absorbance using spectrophotometric method, the extraction phase was initiated using selected solvents.

i. Absorbance

Different solvents show intensities and shapes of absorption due to the differences in polarity. The polyphenols content was determined based on the maximum peak obtained from absorption spectra performance. Figure 1 shows the characteristic spectra of the solvents used in this experiment. The solvents are methanol, ethanol, acetone, acetonitrile and water within the range of 700-800 nm. The spectra displayed broad maxima, which shifted around 725 nm and became broader and nearly flat at 765 nm. Acetone showed the highest spectra responses in all spectrum range for all solvent.

The intensity of colour is dependent on forming molybdotungstophosphate blue. The blue colour is a result of the oxidation of phenols by a yellow colour of molybdotungstophosphoric heteropolyanion reagent [15]. However, as seen in Figure-1 the highest absorbance for all solvent is at 765 nm. So absorbance at 765 nm was selected to determine the TPC.

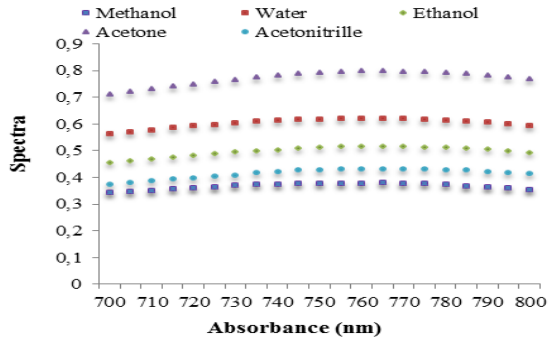

Figure-1. Absorption spectra produced by Gallic acid and solvents at 700-800 nm

ii. Determination of the best solvent for extraction

The yield of polyphenols was measured in term of TPC and the resulting TPC with different solvents is summarized in Table-2.

Table-2. TPC of sample using different solvent.

No	Solvent	R^2	TPC (mg/100g)
1	Acetone	0.9955	3.96
2	Acetonitrile	0.9975	3.88
3	Ethanol	0.9943	3.95
4	Methanol	0.9992	9.59
5	Water	0.9953	6.65

Many studies have been conducted to find the best solvent for extraction of polyphenols from fruit processing wastes. Response surface methodology was examined by Kong *et al*. [22] for the extraction conditions of polyphenols (Y1) and flavonoids (Y2) from pink guava processing wastes. They found the best conditions for extracting polyphenols using ethanol as a solvent.

The highest TPC values was obtained using methanol as a solvent, which yielded 9.59 mg/100g of sample. The TPC extraction value for methanol was around twice as high as that for acetone, acetonitrile and ethanol solvent. The second highest extraction solvent was water, which resulted in 6.65 mg/100g of sample. There were thus higher amounts of polyphenols in methanol and water extracts than in the others solvents. Methanol showed better characteristics as a solvent of polyphenols than water or other organic solvents.

These results show that the highest yield of polyphenols came from using methanol as a solvent. Solubility of polyphenols in solvents is determined by their polarity. Different solvents have different degrees of polarity with respect to polyphenols. For example, tannin, quercetin and gallic acid, all present in pink guava processing waste, are polar compounds. Therefore these polyphenols are soluble in methanol and water. On the other hand, other polyphenols such as apigenin are not detected in pink guava processing waste because apigenin is insoluble in methanol and water.

Another factor that influences the solubility of polyphenols is the interfacial tension of the solvent. This is related to the mass transfer process at the phase boundary of a solution. If the interfacial tension of a solvent increases, the solubility of polyphenols decreases along with the concentration of polyphenols.

Comparative studies in the past were carried out to find extractive efficiency of various solvents for TPC of mashua tubers. Chirinos *et al*. [23] used water, ethanol, methanol, acetone and hexane as solvents, testing their ability to extract antioxidant polyphenols from mashua tubers. In this study, they found that methanol and acetone were the most efficient solvents for the recovery of all the characteristics of TPC evaluated.

Canadanovic-Brunet *et al*. [24] and Turkmen *et al*. [25] found that methanol is the best solvent for catechin polyphenols extraction. These findings are in agreement with explanation of the work of polarity in solvents and the solubility of polyphenols compounds.

Lapornik *et al*. [26] compared the extracts from red currant marc, black currant marc and grape marc from plant byproduct using different solvents. They found that methanol has better characteristics than ethanol and water as a solvent for the extraction of polyphenols.

Lapornik [26] compared the extracts from plant byproducts using different solvents and extraction time. He extracted polyphenols from red currant, black currant and grape byproducts acquired after processing. The extracts were prepared using 70% ethanol, 70% methanol and water as solvents. Higher amounts of polyphenols were obtained from methanol and ethanol extracts than from water extracts. Methanol showed slightly better characteristics as a solvent of polyphenols than did

ethanol. Higher values of total polyphenols content was found in ethanol and methanol extracts than in water extracts due to the fact that methanol and ethanol are less polar solvent than water. This means that they are more efficient in the degradation of cell walls, which have no polar character, allowing polyphenols to be released from within cells.

Hussein *et al*. [27] and Kallithraka *et al*. [28] concluded that because of its polarity, methanol solvent is more effective at extracting polyphenols linked to polar fibrous matrices,

iii. Effect of mixing ratio between methanol and water as a solvent

We evaluated effect of a mixing ratio between methanol and water as a solvent, given the fact that methanol gives the best yield of extractable polyphenols and water gives the second best yield of extractable polyphenols. An additional reason for this line of inquiry is that water is safe for human consumption. Chirinos *et al*. [23] also studied the use of water as a solvent for the extraction of an extract with a high content of impurities (for example organic acids, sugars, soluble proteins), which could interfere in the identification and quantification of polyphenols. Obviously, using water as the solvent had a significant effect on the total extractable polyphenols of the extract. It can be concluded that every solvent has distinct specificities in extracting polyphenols compounds. Moreover, Khokhar & Magnusdottir [29] also found that water was a better solvent for extracting Zea polyphenols. Meanwhile, Kashiwada *et al*. [30] concluded that the most extensively used solvent for extracting polyphenols compounds are methanol and its water mixtures.Obviously, this experiment will evaluate how decreasing the amount of methanol by lowering the mixing ratio between methanol and water as a solvent affect the yield of TPC.

The effect of mixing ratio between methanol and water concentration as a solvent on extraction of polyphenols is presented in Table-3. The recovery of TPC increased with an increase in methanol concentration until the concentration reached 60%, after which, the recovery decreased with the increase in methanol concentration. The greatest recovery was achieved when using a methanol concentration between 50% and 60% (v/v).

Table-3. TPC yield using different mixing ratio of methanol and water.

No	Methanol/Water (%)	R^2	TPC (mg/100g)
1	20	0.9747	11.1
2	40	0.9878	9.63
3	50	0.9927	12.76
4	60	0.9892	17.10
5	70	0.9701	12.17
6	80	0.9926	10.86

As already mention in the literature review, the fundamental importance in process extraction is the solubility of the solute into the solvent. Polar polyphenols would be soluble in polar solvents and they each have different degree of polarity. Some of polyphenols have a hydrophilic and hydrophobic structure. Therefore, the presence of methanol in a ratio of 60% in this experiment will assist in penetrating the hydrophobic areas of extract from pink guava processing wastes. It means that, increasing methanol in the solvent results in an increase in polyphenols content. The decreasing yield of TPC when using more than 60% methanol/water as a solvent resulted from the increased interfacial tension of the solvent, which makes the mass transfer process difficult at the phase boundary.

Garcia *et al*. [31] found that an increase in the percentage of methanol has a positive influence on the extraction of polyphenols in fruit juices. The fruit used were strawberries, apples, and tangerines. This fact can be concluded that methanol has a suitable polarity for extracting polyphenols besides reducing polyphenol oxidase (PPO).

Chirinos *et al*. [23] saw that equal parts of water or amounts higher than 50% do not result in a higher quantity of extractable polyphenols from mashua tubers.

iv. Stability of polyphenols in methanol/water 60% as a solvent

The stability of polyphenols was determined for 5 different sets of time, namely 0, 60, 120, 180 and 240 minutes. The results are shown in Figure-2. The extract samples were stored at room temperature in both open and closed containers. The stability of polyphenols was measured in term of their peak of absorbance. Figure-2 shows the stability of polyphenols content during storage for all the extract samples. Among the extracted samples, the decreased stability of polyphenols does not show a significant difference between open and closed storage. Polyphenols compounds are formed by the joining of hydroxyl molecules. A polyphenol compound is stable when the total energy of the molecules together has lower energy than that of the molecules separate.

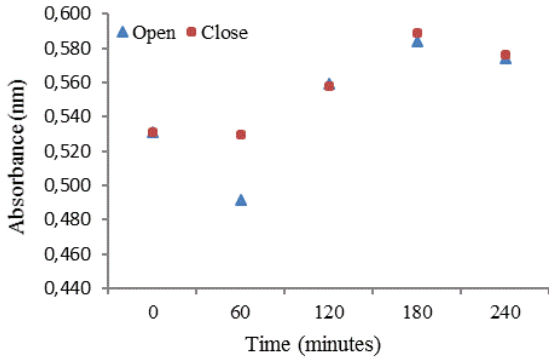

Figure-2. Stability of polyphenols content at opened and closed storing condition during analysis.

Fang and Bhandari [32] observed the stability of TPC in bayberries and the samples were stored in incubators with constant temperatures for 6 months. After 6 months it was found that the bayberries stored at 5 ^{0}C had a decreased in TPC of about 6-8%, those store at 25 ^{0}C had a decrease in TPC of about 6-9%, and those stored at 40 ^{0}C had a decrease in TPC of about 7-37%. Fang decided that degradation of polyphenols was related to phenolic compounds and suggested that bayberries be stored at temperature less than 25 ^{0}C.

v. Effect ratio of waste/solvent (25ml 60% Methanol/water) to TPC yield

Methanol mixed with water (60%) proved to be the best solvent for extracting polyphenols compounds at 17.10 mg/100g of samples. The experiment used 1.5 g waste/25 ml 60% methanol/water as a solvent. Given the optimal results obtained thus far, we decided to continue working with 60% methanol/water as a solvent.

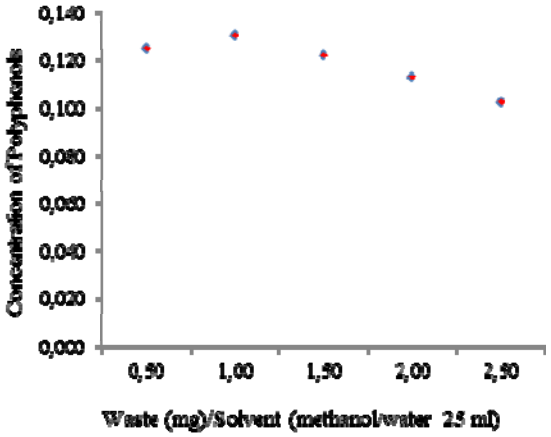

Figure-3. Concentration of polyphenols vs. ratio waste/solvent (25ml 60% methanol/water).

This section discusses how the ratios of waste to solvent affected TPC yield. 0.50, 1.00, 1.50, 2.00, and 2.50 mg of residual of pulp were dissolved in 25 ml of 60% methanol/water as solvent. Figure-3 shows how the waste/solvent ratio (25 ml 60% methanol/water) influenced the TPC yield.

The highest amount of TPC was obtained using 1 g of residual pulp. Al-Farsi & Lee [33] found that the solvent to solid ratio has a positive effect on extraction. They said that the increase in TPC is significant, and almost linear to with solvent ratio. Two factors that control the extraction processes are the solubility equilibrium in the solvent and mass transfer rate.

vi. Effect of extraction time to TPC yield

The effect of the extraction time was studied using 60% methanol/water as the extraction solvent. As shown in Figure-4 the TPC increased significantly when the extraction time was increased to 90 minutes. After 90 minutes, any increase in the extraction time did not significantly increase the TPC. The highest TPC resulted at duration for 180 minutes. Increasing extraction time resulted in increased TPC until equilibrium was reached. Therefore, 180 minutes was selected as the ideal time for the extraction of polyphenols from pink guava processing residual pulp.

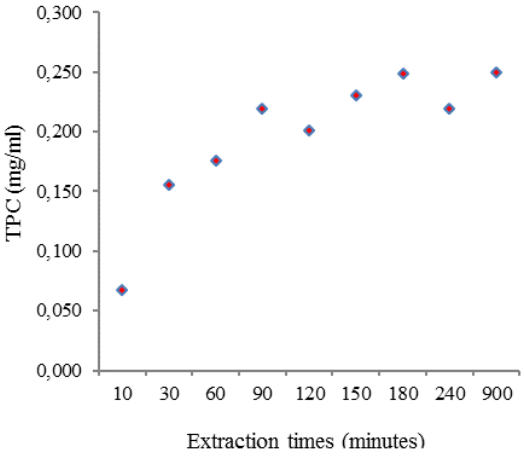

Figure-4. TPC vs. extraction time.

Chirinos *et al*. [23] evaluated the effect of the extraction time using acidified 90% methanol (0.1% HCl) as a solvent. He found the TPC increased significantly when the extraction time increased from 5 to 15 minutes, from 15 to 30 minutes and from 30 to 60 minutes. Flavonoid polyphenols significantly increase from 5 to 15 min and from 30 to 90 minutes.

Garcia [31] in his study of extraction time found that the amounts of extracted analyst were nearly constant during the different interval of time examined: 10, 15, 20 and 30 minutes. Durling *et al*. [34] found 3 hours to be the best extraction time for extracting polyphenols from dried sage and Nepote *et al*. [35] found 10 minutes to be the best time for extracting polyphenols from peanut skin.

According to the findings in these previous works, it can be concluded that the optimum extraction time depends on the equilibrium between solvent and solute.

4. CONCLUSIONS AND RECOMMENDATIONS

Pink guava processing residual pulp sample were characterized prior to the extraction of polyphenols. Its dry weight was 28.42% and the antioxidant content in the form of polyphenols was as follows: 0.40 mg/100g of quercetin, 8.70 mg/100g of gallic acid, 62.60 mg/100g of tannin, and no detectable trace of apigenin.

The study explored the application of four organic solvents and water to the extraction of polyphenol compounds from residual pulp pink guava processing. The study showed the TPC residual pulp of pink guava processing, varied from 3.s to 9.59 mg/100g. The highest TPC was obtained using methanol as a solvent and the second highest was obtained using water as a solvent. The highest polyphenol content resulted from a mixture of

methanol and water in different concentrations, where the optimal ratio was 60% methanol/water followed by 50% methanol/water. Variations in the TPC depended on the solvent conditions and concentration between solvents. The TPC was highest when using a waste/solvent ratio of 1 g of residual pulp to 25 ml of 60% methanol/water. 180 minutes was selected as the ideal time for polyphenol extraction.

Agroindustry byproducts are good sources of polyphenol compounds or antioxidants. While the use of polyphenol compounds as food antioxidants is interesting, practical aspects needs to be considered, such as the extraction efficiency, availability of sufficient raw material, and toxicity.

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