Polyphenols Recovery from Tropical Fruits (Pink Guava) Wastes via Ultra-Filtration Membrane Technology Application by Optimum Solvent Selection

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ABSTRACT: *Wastes generated from the processing of some fruits still contain large amounts of chemical components such as polyphenols, which are deemed as very effective antioxidants. This study involves the characterization of wastes generation by pink guava processing and selection of the best solvent that will allow us to extract the excess polyphenols via ultra-filtration membrane technology. The wastes were gathered from a juice processing factory in Sitiawan, Perak, Malaysia. The results are conclusive of the fact that the wastes contain quite a large percentage of polyphenols. The highest total polyphenol content was observed in mixture of methanol-water (60% concentration) and pure water as solvents, respectively for 1g of waste per using 25mL of each solvent. This phenomenon is also directly proportional to extraction time and duly concluded that pure water is the effective solvent for retrieving polyphenols from pink guava processing wastes.*

KEYWORDS: *Fruit waste; Membrane technology; Polyphenols extraction; Antioxidant; Solvent.*

INTRODUCTION

The global concern regarding nutrition and increases awareness in healthy lifestyles, especially in the context of antioxidants has led to quite a number of investigations regarding the chemistry of natural antioxidants and their purported benefits. Some of the benefits of antioxidants are its ability to reduce or eliminate degenerative diseases. It is also common knowledge that fruits and vegetables are rich in antioxidants [1]. Despite its benefits, the processing of fruits to produce juice generates a significant amount of waste; and it is not beyond the realm of possibilities that these wastes still contain an appreciable amount of polyphenols[1]. The extraction and reprocessing of these wastes could also serve as secondary source for foodstuff and polyphenols [2-5].

The chemical composition of guavas, as one of the tropical fruits, are wholly dependent on their cultivation, ripeness and the picking season [6]. A common guava nutritional content includes vitamins A and C [7], as well as sugar, protein, and a mineral combination with antioxidants such as polyphenols[8], lutein, zeaxanthine

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and lycophene [9]. It is also a well-established fact that guava has higher contents of polyphenols and vitamin C than either lychee or papaya [10]. Moreover, it is also known that guava is the best source of food-grade pectin. In terms of total content of vitamin C, white guava (1.43 mg AA/100 g FW) is proven superior to pink guava (0.72 mg AA/100 g FW) [11]. *Dube et al.* [12] concluded that polyphenols in white guava is inversely related to firmness compared to pink guava, however, the content of polyphenols in white guava is considerably higher. Additionally, *Kong et al.* [13] posited that the residual wastes from pink guava processing came mostly from peels and seeds. Recent studies confirmed that certain polyphenols such as quercetin, epigenin, gallic acid, tannins and ß-carotene are present in wastes from pink guava processing [14].

A study proved that solvent extraction is capable of isolating usable compounds from wastes generated by the fruit processing industry [15]. Alternative methods are also effective, such as precipitation, adsorption, and electrolysis. Research is currently being conducted on the principal techniques of polyphenol extraction from plants [16], and their studies indicated that extraction using ethanol and hexane is viable for the extraction of carotenoids in food. Meanwhile, *Puri et al.* [17] determined that ethanol is the best solvent for extracting polyphenols from peels of citrus fruits. It can be concluded from their studies that solvents are effective vis-à-vis extraction. The factors that contribute to solvent extraction are solvent, pH, temperature, number of steps, and volumes of solvent and particle size in the samples [18]. Furthermore, due to membrane separation techniques' high efficiency in different industries [19-21], the certain membrane technologies were analyzed with the aim of purifying polyphenols compounds. For instance, *Beltran et al.* [22] explored the recovery of antioxidants from grape products by using supercritical fluids and membrane technology, and confirmed that ultra-filtration membrane technologies are viable for the separation of polyphenols from grape seeds. *Gonder et al.* [23] also developed several protocols for purifying polyphenols compounds from pulp and paper mill wastewaters using NF hybrid membrane technologies. Membranes are highly effective in process separation due to its ability in controlling permeation rates. Study on characteristic properties of crude pineapple waste extract for bromelain purification by membrane processing represented that two stage UF/MF membrane could extract up to 57% at temperature of 20–25 °C and at pH 7 in extract operational condition [24]. In other investigation, valuable compounds from Purple Sweet Potato (PSP) were extracted by using UF/MF membrane. The results showed that the highest extraction rate was achieved by presence of cross-flow and rotating disk filtration modules application [25].

Based on the effectiveness of polyphenol component on lifestyle health cycle, the current research-based study is focused on extraction of polyphenol from pink guava wastes. This work is divided into two main parts; the first is to gather and characterize wastes from pink guava processing, followed by using the result to determine the best solvent for retrieving natural antioxidant or polyphenol from these wastes via ultra-filtration membrane technology.

EXPERIMENTAL SECTION

Equipment and methods

The wastes were collected by non-probable random sampling method from three different pink guava juice-processing factories in Sitiawan, Perak, Malaysia. Samples were gathered from various sources, such as the sieves, refiners, and decanter waste tanks. These samples were immediately packed into a closed plastic container and stored in a fridge condition (ACSON International Refrigerator- Malaysia) to prevent mildew or oxidation. The samples were kept in storage for twelve weeks; these samples are assumed to be representative of the properties and quality of the wastes as a whole.

The experiments were divided into three phases; the first phase involves determining the dry weight, total soluble solid, total sugar, protein, minerals, vitamins and antioxidants of the wastes generated from pink guava processing (the tests were conducted by experts from Biopharmaca Research Centre under Research and Community Service Institution of Bogor Agricultural University), followed by analyzing recovery methods of polyphenol via five different solvents: methanol, ethanol, water, acetone, and acetonitrille. The last phase involves selecting the best solvents via the utilization of membrane technology.

Characterizations of pink guava processing wastes

Sample Preparation

A total of 10 g of sample was retrieved from the sieves, refiners, and decanter waste tanks, respectively.

These samples were equally mixed with 750ml of pure water in suspension phase to determine the total soluble solid, sugar, protein, minerals, vitamins and antioxidants.

For measuring the dry weight, 10 g of premixed samples (unadulterated in water) were placed onto a dry crucible and left in an oven for an hour at a temperature of 105º C. Afterwards, the samples were placed into desiccators and allowed to cool to room temperature. Then, the samples were weighed and the values recorded almost immediately. This process was repeated until the recorded weight remains constant. Ultimately, the constant weight was subtracted from the weight of the crucible when it is empty to determine the actual weight of the sample.

Methods

SNI[(1)] 01-2892-1992, particle 3.1, SNI 01-2892-1992, particle 2.1 and SNI 01-2891-1992, particle 7.1 standards were executed to determine the total sugar, reducing sugar and total protein, respectively. The Atomic Absorption Spectroscopy (AAS) measured the value of Iron (Fe) and Calcium (Ca), and Tannin, via the Titrimetry method. Furthermore, High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan, 2004) was utilized to determine the content of vitamin A, Ascorbic acid, Epigenin and Beta Carotene with same condition in the other related studies [26, 27]. Finally, the total soluble solid was determined by a Spectrophotometer (Thermo pectronic enesys 20, 2008), and was also used to measure the contents of Quercetin and Gallic Acid.

Recovery of Extracted Polyphenol

Sample Preparation

The extraction of polyphenols from samples of pink guava processing wastes was conducted separately, with methanol (99.8%, Merck, Germany), ethanol (99.9%, Merck, Germany), acetone (99.9%, Merck, Germany), acetonitrille (99.9%, Merck, Germany) and water acting as solvents. A total of 1.5 g of each sample was mixed with 25 mL of solvent, and shaken at ambient temperatures for 2 hours. Afterwards, the extracts were centrifuged by IEC MediSpin centrifuge (Thomas Scientif-USA), and filtered by Whatman filter papers (No 1, General electric, USA). Two solvents with higher affinities and different concentrations were also used for this purpose, while clear extract were used to analyze the Total Polyphenol Content (TPC).

The optimal extraction time was determined by mixing the extracts with the optimum solvent and shaken for 10, 30, 60, 90, 150, 180, and 240 minutes at ambient temperatures.

Recovery of Polyphenols

A commercial tubular Ultra-Filtration (UF) membrane type polyethersulphone ES 209 (supplied and manufactured by PCI Membrane Technology, USA) was used to recover polyphenols. The specification of used UF membrane is presented in Table 1. Prior to its usage, the membrane was soaked overnight in water to eliminate the impurities that might have lingered from the mechanized process, or additives used for stabilization and the wetting of the membranes. The UF experiment was carried out via continuous retentate recycling.

The system consisted of a filtration membrane model ES 209 UF that is connected to a feed reservoir. The steps involved in the experiment were initial water flushing to remove the storage solution and the measurement of water flux, followed by the filtration of feed pink guava waste processing solution, and finally, flushing and cleaning with water. During the operation stage, the feed stream was pumped through the UF tubular membrane. The retentate, including the species that were excluded by the membrane pores, went on via the recirculation loop back to the feed, while permeating solvents and solutes were transported through the membranes' pores, gathered on the shell side of the UF tubular membrane, and left to permeate the reservoir. The Ultra filtration tubular membrane was started up with the permeate ports closed, allowing the cross velocity to be established prior to permeate withdrawal, with both the feed inlet valve and the retentate being left wide open. After the pump was switched on, the inlet valve opened, and the retentate valve was slowly closed, which establishes suitable conditions. As the volume of the permeate increases, the concentration of the polyphenols is expected to increase as well. The UF experiment was carried out under continuous retentate recycling. The resulting permeate

This method employs the Luff-Schoorl method (Basoglu and Uylaser, 2000). SNI is an Indonesian National Standard and the standard is nationally applicable in Indonesia only. It was formulated by the Technical Committee and defined by BSN or Badan Standard National. http://websisni.bsn.go.id/index.php?/sni_main/sni/index_simple.

Table 1: the specification of applied UF membrane.

Membrane Type	Material	Max. pH Range	Maximum Pressure (bar)	Max temp (°C)	Apparent Retention Character	Hydrophilicity	Solvent Resistance	Applicable Module(s)
ES209	Polyethersulphone	1.5-12	30	80	9,000 MW	2	++	B1

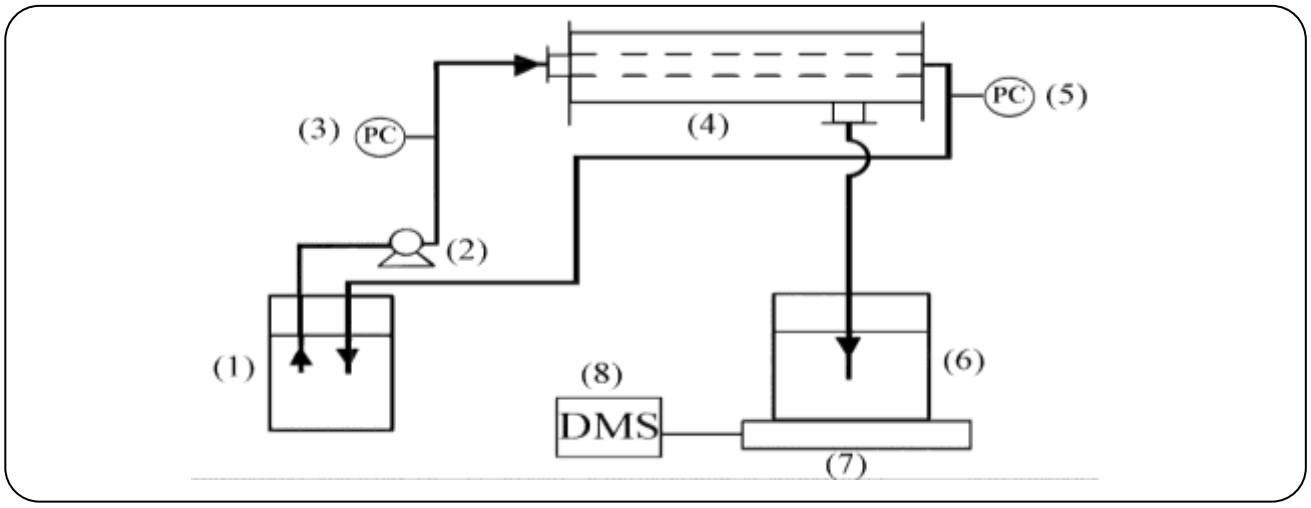

Fig. 1: Schematic diagram of membrane filtration device: (1) feed reservoir, (2) peristaltic pump, (3) inlet pressure gauge, (4) UF tubular membrane with the membrane housing (32.5 cm) (The molecular weight cut off (MWCO) is 9000 and the diameter is 1 cm with 150 cm of length), (5) outlet pressure gauge, (6) permeate collection reservoir, (7) balance AND GF 400 (model 77200-6, Japan) and (8) data logging. Master flex flexible tubing was used for all connections. The equipment also had a flow rate indicator and a valve for pressure control.

was continuously removed until the desired Volume Concentration Ratio (VCR) was realized. Additionally, the process recovery was conducted by pumping the feed stream extract solution through the UF tubular membrane to the level of the desired Trans Membrane Pressure (TMP). All of the data was collected and recorded by a computer via a data logger, and each experiment was repeated at least thrice in order to ensure accuracy and reproducibility.

Measurement of total polyphenols content and recovered polyphenols

The Total Polyphenol Content (TPC) was determined using the colorimetric Folin–Ciocalteau (supplied by Sigma-Aldrich, Germany) assay[28] . The oxidation of polyphenols with the Folin-Ciocalteau reagent included reactions with the mixture of $H_3PW_{12}O_{40}$ and $H_3Pmo_{12}O_{40}$ acids in the alkaline medium. This method combines 200 µL of the extract sample with1500 µl of 0.25 N Folin-Ciocalteau reagents, which were well mixed with a Vortex (model M 37610-33, supplied by Barnstead International in Malaysia). Then, the mixture was in turn reacted with 1500 µL of 1 N Na_2CO_3 solution for 3 minutes at a temperature of 22°C. alternatively; the solution was incubated at ambient temperatures in the dark for 90 minutes in order to form a mix of blue oxides. The absorbance was taken at 725, 735, 750 and 765 nm by a spectrophotometer (Thermo Spectronic Genesys 20 model 4001/4, made in USA, 2008) in order to obtain optimum results. Due to a wide spectrum of polyphenol compounds, gallic acid (supplied by Sigma-Aldrich, Germany), with a calibration curve of 0.1-1.2 mg/mL was utilized as a standard unit for the determination of TPC. If the measured absorbance value exceeded the linear range of the standard curve, it was diluted. The level of water flux was measured relative to the amount of water pumped into the membrane system.

RSULTS AND DISCUSSION

Characterizations of pink guava processing wastes

The results showed that the dry weight of the samples as 28.42%, which is indicative of the fact that the samples

still contain high amounts of water. However, this could also be due to the increased growth of microorganisms, which ultimately resulted in the overall decrease of organic nutrients such as sugar and proteins.

Currently, there is no standard information regarding characteristics of pink guava or their wastes in Malaysia. The only data that are available is kept by MARDI (Malaysian Agricultural Research and Development Institute)[29], detailing Dietary Fiber Powder (DFP) from pink guava by-product. This implies that it possesses suitable hydrating properties as a food ingredient, due to its high amount total dietary fiber content of 76.1 g per 100g. However, it should also be noted that some studies indicated that the nutrients in guava varies according to its agricultural conditions [12, 30].

This experiment indicated the absence of sugar contaminants, due to the fact that the samples were taken from wastes and microorganisms that were degrading the sugar. The results are also indicative of another fact: the high content of polyphenol in wastes generated by pink guava processing, such asquercetin (0.40mg/100g), gallic acid (8.7mg/100g) and tannin (62.6mg/100g). These values are in agreement with previous studies [8, 13, 31, 32], which prove that both guava fruit and wastes are rich in antioxidants. The results also showed that the pink guava wastes contain various components such as Ascorbic acid (0.2mg/100g), Iron (0.0013% w/v), Calcium (0.029% w/v) and Vitamin A (0.5IU/100g) which are in agreement with previous research studies [7]. The next section discusses the extraction of polyphenols. Thus, the study on the extraction of polyphenol components was carried out as follows.

Extraction of polyphenols

Absorbance

The characteristic spectra of all of the solvents (methanol, ethanol, acetone, acetonitrille and water) between 700 and 800 nm were investigated in this study. When solvents measured the absorption spectra with different polarities, it was discovered that these solvents modified the position, intensities, and shapes of the absorption bands. The spectra exhibited very broad maxima, shifting at around 725 nm, and became even broader and more nearly flat apart from the peak area at 765 nm in all of the solvents. Acetone was most responsive, while water, ethanol, acetonitrile and

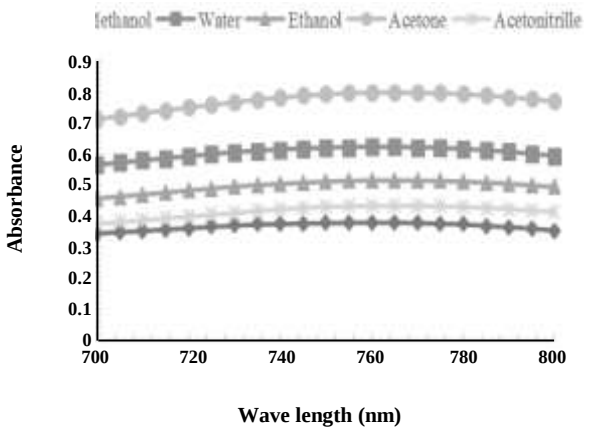

Fig. 2: Absorption spectra produced by Gallic acid and solvents at 700-800 nm.

methanol were the lower responsive to the colorimetric assay in all of the spectrum ranges, respectively. Color intensities was dependent on the formation of blue molybdotungs to phosphate, which were the result of the oxidation of phenols by a yellow molybdotungs to phosphoric heteropoly anion reagent [33]. The response of polyphenols was in tandem with the number of polyphenols groups.

Determination types of solvent for extraction

The total polyphenol content varies in response to the different materials' organic and water solvents (Table 2). The yield of extracted polyphenols varies from 0.03 to 0.07mg/g, 0.05 to 0.12mg/g, and 0.04 to 0.10 for the decanter, refiner and sieve, respectively. The highest TPC values were obtained by methanol and water in all of the samples, where the extraction capacity of the former was two times higher than acetone, acetonitrille and ethanol solvents. Previous studies also indicated that the most widely used solvent for extracting polyphenol substances are methanol and their water mixtures , which is in accordance with the polarity of the solvent and solubility of polyphenols in methanol [34, 35]. This is due to the fact that it is more effective at extracting polyphenols linked to polar fibrous matrices[36].

Accordingly, the effectiveness of different concentrations of methanol on TPC was examined. The TPC levels increases by increasing the concentration of methanol until it reaches 60%. At higher concentrations, the TPC levels declines. The greatest recovery was

Table 2: Total phenolic content sample from decanter, refiner and siever using different solvents.

Solvent	Linear equation	R^2	Decanter (mg/g)	Refiner (mg/g)	Siever (mg/g)
Acetone	y = 6.3736x + 0.0416	0.9955	0.03	0.06	0.04
Acetonitrile	y = 3.9524x – 0.0499	0.9975	0.03	0.05	0.04
Ethanol	y = 5.3721x – 0.0394	0.9943	0.03	0.06	0.04
Methanol	y = 3.6789x – 0.0157	0.9992	0.07	0.12	0.10
Water	y = 4.7354x – 0.0676	0.9953	0.04	0.08	0.08
Methanol-Water (20%)	y = 3.7739x + 0.0102	0.9747	0.07	0.16	0.10
Methanol-Water (40%)	y = 7.1072x – 0.1011	0.9878	0.07	0.12	0.09
Methanol-Water (50%)	y = 6.3688x – 0.1324	0.9927	0.12	0.14	0.12
Methanol-Water (60%)	y = 4.5651x – 0.0413	0.9892	0.15	0.22	0.14
Methanol-Water (70%)	y = 6.1253x – 0.0851	0.9701	0.11	0.12	0.13
Methanol-Water (80%)	y = 5.7316x + 0.0576	0.9926	0.09	0.15	0.09

achieved by concentrations between 50% and 60% (v/v) of methanol, which were 0.15 mg/L for decanters, 0.22 mg/L for refiners, and 0.14 mg/L for sieves, due to the lower polarity of the solvent [37]. Thus, the extraction by 60% methanol/water as solvents significantly affects the total extractable polyphenol of the extracts. Consequently, the polarity and solubility rate of polyphenols in presence of methanol/water solvent increase in concentrations lower than 50% and higher than 60%. This statement also is in agreement with previous studies [38, 39].

Effect ratio of waste/solvent (60% methanol/water) and extraction time on TPC

When the effect ratio of waste/solvent was analyzed, it was discovered that it influences the extraction of polyphenol (Fig. 3). The highest amount of polyphenol extracted was with a 25 mL mixture of 60% methanol/water, at a ratio of 1 for wastes gathered from sieves (0.174 mg/g) and refiners (0.135 mg/g), respectively. This trend is repeated for all wastes, demonstrating no significant changes at higher ratio of solvents. This is consistent with the mass transfer theory; where the concentration gradient between solid and liquid, which is made up of polyphenols, is greater when a higher solvent to solid ratio is utilized. The two aspects that control the extraction process were the equilibrium from a concentration that dissolves in the solvent and mass transfer rates. The solvent-to-solid ratio has a positive effect on extraction via the significant increase of total polyphenols almost linearly with the solvent's ratio [40].

It is observed that time influences the extraction of polyphenols. Fig. 4 shows the TPC-obtained changes from wastes of pink guava, based on different extraction times. The highest extraction was achieved for a refiner and a sieve at duration of 180 minutes, while it was detected at duration of 150 minutes for the decanter. Since there are no significant changes beyond the equilibrium condition, 150 minutes was selected as the optimum time for the extraction of polyphenol from the wastes of pink guava processing. It is oriented in a single direction, possessing the maximum-recorded effect ratio and concentration of solvents for higher polyphenols extraction.

Effect of extraction time on TPC

It is observed that time influences the extraction of polyphenols. Fig. 4 shows the TPC-obtained changes from wastes of pink guava, based on different extraction times. The highest extraction was achieved for a refiner and a sieve at duration of 180 minutes, while it was detected at duration of 150 minutes for the decanter. Since there are no significant changes beyond

the equilibrium condition, 150 minutes was selected as the optimum time for the extraction of polyphenol from the wastes of pink guava processing. It is oriented in a single direction, possessing the maximum-recorded effect ratio and concentration of solvents for higher polyphenols extraction. However, the optimum extraction time is different compared to other ingredients, due to difference in chemical structures. For example, three hours is required to extract polyphenols from dried sage [41], whereas only 10 minutes is required to extract polyphenols from peanut skins [42].

Selection of solvent for recovery of polyphenols

Effects of solvents on permeate flux

Since the highest extraction was realized by methanol/water at 60% and pure water as solvents, the permeate flux between them was compared and demonstrated (Fig. 5). The permeate flux by pure water increased from 9 kg/m^{2}h to 30 kg/m^{2}h, which is twice than 60% methanol/water. The effect of solvents in the permeate flux was profound, due to the fact that the concentration of phenolics was higher for methanol/water, at 60%. Moreover, permeate was removed through the UF membrane where all impurities remained, adjacent to the membranes' surface. However, the layer of solvent became more concentrated in the dissolved and suspended materials, next to the membranes' surface (boundary layer) for all solvents. The results, as well as another study [43], are indicative of the high level of ultra-filtration process' sensitivity to critical concentrations of the high molecular weight of the components.

Effects of TMP and feed flow rate on permeation flux

In continuous particle accumulation, particle concentrations reached its maximum near the membranes' surface, and a particle cake layer is formed between the membrane and the polarization layer. A possible fouling mechanism could be obtained by evaluating the transient flux's decline of pink guava waste processing ultra-filtration at different operating conditions. The declination of the flux with time at different TMPs and feed flow rates are shown in Fig. 6.

The average permeation rate is therefore expected to be higher when the pressure for both water and 60%methanol/water solvents are increased. It is also

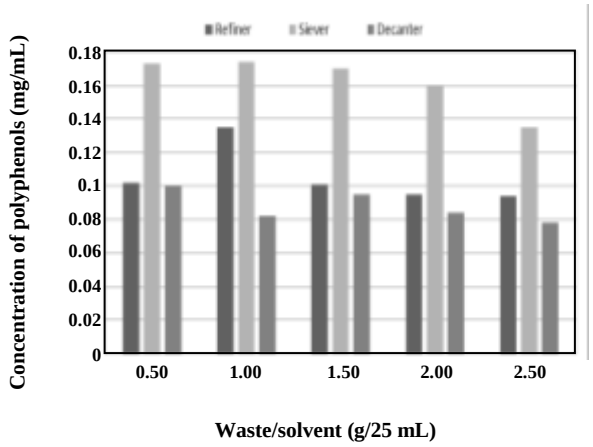

Fig. 3: Concentration of polyphenols Vs. waste/solvent ratio (60%methanol/water).

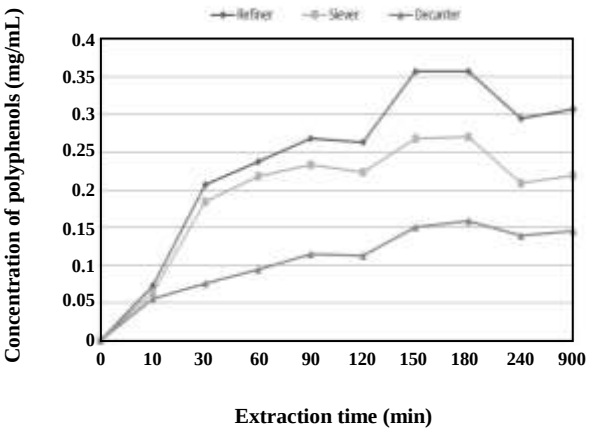

Fig. 4: Concentration of polyphenols Vs extraction time.

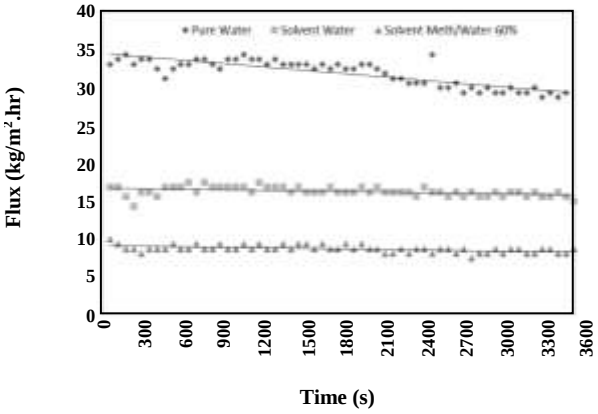

Fig. 5: The effect of methanol/water 60 %, water solvents and pure water on permeation rate vs. time.

noticed that when the TMP's pressure is increased from 2 to 3.4 bar, the permeation rate was enhanced from 0.6 to 2 g/sec for 60% methanol/water, and 5 to 7 g/sec for pure water. This can be explained as follows; TMP acts as a driving force for permeation, increasing the value of TMP raises the rate of permeation. The separation of polyphenolics and sugar via ultra-filtration are also rather similar[44] . The increment of permeation flux influences the rate of deposition, and fouling is directly proportional to TMP pressures. It would compress the rejected solute into a denser fouling layer via the increase of fouling resistance. Giving more attention to the associated trends for water solvent it can be found that with increase of initial pressure the permeation rates is decreasing with passing the time. This phenomenon is due to physical interaction between UF membrane and polyphenol components. By increasing the initial pressure from 2 bar to 2.97 and 3.4 bar, respectively, collision of chemical components with membrane within filtration processing, could be accumulated. Consequently, the permeation rate in first stage performed the highest value and followed decreasing trend by passing the operational time.

Additionally, the trend of permeation rate is highly stable at lower TMPs, while the permeation rate declined at the highest TMP levels for both solvents. The permeation rate declines at a TMP level of 3.4 bar region in the first 1400 seconds of pure water, and in the first 1000 seconds for 60% methanol/water. The declination of the rate of permeation is attributed to the adsorption of colloidal species and the built up of the polarization layers' concentrations.

Effect of TMP on recovery of polyphenols

The rate of recovery is between 0 to 100%, and it is a parameter of economic importance; usually, the commercial membrane process is often designed to realize the highest possible recovery value. However, the recovery also influences the membranes or process performance. The recovery or yield is defined as the percentages of fraction between permeate, which passes through the membrane and the initial feed flow. The combination effect of TMP and the recoveries of polyphenols on permeate for water and methanol/water 60% as solvents was studied.

Although water has higher levels of recovery regarding to lower levels of TMPs (59.2%), maximum

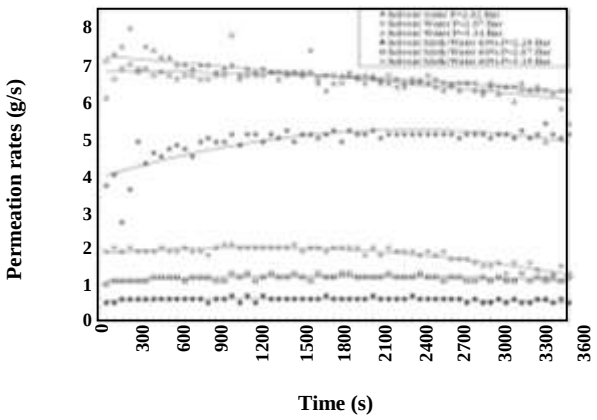

Fig. 6: The effect of TMP and flow rate on permeation rate Vs. time.

polyphenol recovery was almost 75 % for both 60% methanol/waterand water solvents at same pressure condition (3.4 bar) (Fig. 6). This trend precipitates a high deposition rate and the higher-pressure compresses the rejected solute, leading to the thickening of the fouling layer, enhancing the effect of fouling. As previously mentioned, the phenolic content is directly proportional to them ethanol/water solvent, due to the enhancement of phenolics concentration, which decrease the recovery levels of both flux and polyphenols. In agreement with current statement, related study regarding the separation of Polyphenolics and Sugar by ultra-filtration, it is found that the flux declined more rapidly for higher flux concentrations during the beginning [44].

CONCLUSIONS

The results showed that the variation of the polyphenol contents depends on the solvents' conditions and concentrations. The study indicated that the available TPC in wastes from pink guava processing varies from 0.03 to 0.12 mg/g of all solvents, where a mixture of 60% methanol/water being regarded as the best solvent. The effect ratio of waste/solvent (25mL 60% methanol/water) to total polyphenols content is higher when using 1g of waste. Moreover, the extraction time of 150 minutes was selected as the optimum time for polyphenol extraction from pink guava processing wastes. Polyphenol recovery, enhanced by TMP, increases for both mixture of methanol/water 60% and pure water solvents, the latter demonstrating the best results. Although the value of extracted

polyphenol is not suitable for industrial phase, this study indicates research potential of new applied method.

Abbreviations

PSP	Purple Sweet Potato
UF	Ultra Filtration
MF	Micro Filtration
NF	Nano Filtration
AAS	Atomic Absorption Spectroscopy
TPC	Total Polyphenol Content
VCR	Volume Concentration Ratio
DFP	Dietary Fiber Powder
TMP	Total Membrane Pressure

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