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Evaluation on physico-chemical properties of pink guava puree residue as bioresource

[1]Mhd Abd Kader, K. A., [1*]Ab. Aziz, N., [1]Aziz, A., [1]Talib, A. T., [1]Ahmad, N. H., [2]Tan, C. P. and [3]Kamarudin, S..

[1]Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2] Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3] Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

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Abstract

Pink Guava Puree (PGP) factory produces tons of residues from its unit operation [refiner (R), siever (S), and decanter (D)]. These residues represent a waste of nutrients and can contribute to environmental problem. However, it can benefit related industries if the properties of the residues are known. Thus, this research aims to determine the compositions of the residues from PGP processing factory in order to evaluate possible value-added by-product and energy sources. The residues from each unit operation were being tested for proximate composition, dietary fibre components, pH value, emulsifying activity (EA) and emulsifying stability (ES), carbon-to-nitrogen (C/N) ratio, and sugar analysis. The sugar content was analysed using High Performance Liquid Chromatography (HPLC-RI detector). Results showed that the residues have good composition of carbohydrate (11.82–12.18%), and thus potential as carbon source and can be a good substrate for fermentation. These residues may also benefit the food industry as a good source of dietary fibre (18.63–29.86%). The pH value for these PGP residues is 4; thus they were considered as acidic food by-product. The low pH value also contributed to the low EA and ES value other than the low content of protein in the sample. C/N ratio for PGP residue from R (46:1) is the lowest compared to the C/N ratio from S (84:1) and D (115:1). The amount of sugars detected in the PGP residues in descending order were fructose > glucose > sucrose.

Introduction

The agricultural and food industry produces large amount of biodegradable waste in the world. This has become a problem and challenge to food processing industries in managing their waste. Pelizer *et al*. (2007) stated that these residues are mainly organic; therefore, they must be handled properly. This is because their disposal may lead to potential environmental problems, in addition to losses of raw materials and energy, requiring significant investments in pollution control (Sousa and Correia, 2010). In Perak, 500 ha of pink guava are planted. The plantation produces 15% of the world's pink guava puree (SDBSB, 2006) with an annual harvest of almost up to 9 million kg since 2006. Out of the 9000 tons, almost 10% of the fruits are the scrubs and seeds or known as pink guava puree residues (Figure 1). Due to the high amount of residue produces, there have been ideas of turning the residues into value-added products. A lot of effort has been made into utilizing the agricultural waste to produce fertilizer, bioactive natural products, biosurfactant, biodiesel, and animal feeds. However, research on utilizing pink guava puree residues is still lacking. Agricultural wastes are mainly composed of hemicelluloses; cellulose, and lignin, and they support a sustainable slow release of carbon source, a suitable structure for the growth of microbial community, which made agricultural wastes become the most potential solid carbon sources (Yang *et al*., 2015). Based on this fact, this research aims to characterise the properties of pink guava factory residues which enable them to be used as bioresource.

*Corresponding author.
Email:*norashikin@upm.edu.my*

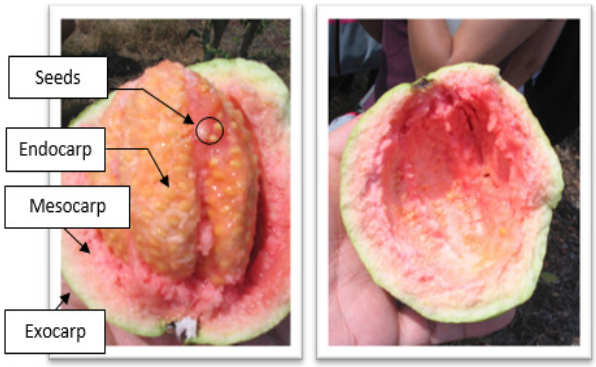

Figure 1. Pink guava fruit anatomy. Adopted from Vinnan (2009)

Materials and Methods

Pink guava puree residues

Pink guava puree production involves three steps of separation process, which are refining, sieving, and decanting. The schematic representation of pink guava puree processing is shown in Figure 2. The function of separation process is to produce the best looking pink guava puree. Residues from these separation units: 1) refiner (seed and coarse pulp), 2) siever (pulp), and 3) decanter (stone cell) were used in this work. All the three samples were supplied by the Sime Darby Beverages Sdn. Bhd. The samples were immediately stored in freezer after receiving. The pH was determined by using pH meter (SevenMulti, Mettler-Toledo, Switzerland).

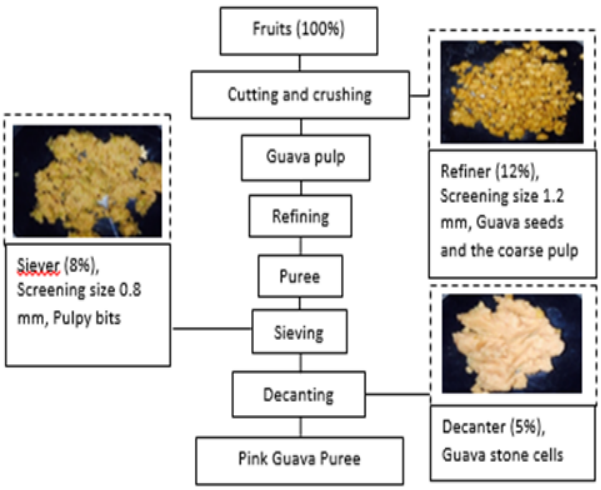

Figure 2. Pink guava puree processing. Adopted from Kong and Ismail (2011)

Proximate analysis

Moisture content was measured on the basis of the sample weight loss by oven drying at 110°C for 2 hours. Ash content was calculated from the sample weight after burning in furnace at 550°C for 2 hours. Fat content was obtained by using ether extraction method. Crude protein content was determined using conversion factor of 6.25. The crude fibre content was obtained by calculating the differences between the weights of dried residue sample to the weight of ash. The content of carbohydrate was determined using calculation based on all the proximate composition. The determination of Total Dietary Fibre (TDF), Soluble Dietary Fibre (SDF), and Insoluble Dietary Fibre (IDF) were done based on AOAC method 991.43 "Total, Soluble and Insoluble Dietary Fibre in Foods" (First Aztion 1991) and AACC Method 32-07.01 "Determination of Soluble. Insoluble and Total Dietary Fibre in Foods and Food Products" (Final Approval 10-16-91).

Lignocellulosic hydrolysis

The pink guava residue or sample was dried by using oven at 70°C. Then dried sample was cooled before being grounded by a TecatorCyclotec mill (1093 Cyclotec, FOSS, North America). Extraction of proteins, interfering phenolics, sugars, and other soluble compounds were performed with 100% acetone at 5°C and water at 60–70°C. The extraction process was repeated several times and its residue was dried for further process. Fiber Hot Extraction 2010 (Fibertec 2010, FOSS, Denmark) was used to determine acid detergent fiber (ADF), neutral detergent Fiber (NDF), and acid detergent lignin (ADL). Sodium lauryl sulphate and ethylenediaminetetraacetic acid (EDTA) or also known as neutral detergent solution was used to treat the earlier prepared residue sample. In this case, NDF was determined and the residue consisted of hemicelluloses, cellulose, and lignin.

After treating the residue sample with cetyltrimethylammonium bromide (CTAB) in sulphuric acid solution (also known as acid detergent solution), ADF was determined. The residue consisted of cellulose and lignin. Once the ADF was determined, the residue was extracted with 72% sulphuric acid. This residue contained only a fraction of acid-soluble lignin, and it was recovered by filtration on a glass crucible with an asbestos filter. The residue was dried at 70°C for a day to constant weight after washed. Then, the residue was weighed after being ash. The furnace was set at 525 ± 25°C for at least 5 hours. Lignin was calculated after correcting for mineral elements (Mandre, 2006).

The results were presented in percentage of the dry mass of plant material. Calculation of cellulose and hemicelluloses was made using the following equations:

$$\text{Acid detergent fibre} - \text{acid detergent lignin} = \text{cellulose} \quad (1)$$

Neutral detergent fibre – acid detergent fibre = hemicelluloses (2)

Emulsifying activity and emulsifying stability

Emulsifying activity (EA) and emulsion stability (ES) analysis were conducted according to Wu (2001) and Kuan *et al*. (2011) with modification. An amount of 0.2 g sample (refiner, siever, and decanter) was added into 10 mL of H_20 in 50 mL Falcon tube. Then, the sample suspension was homogenized by using Vortex-Genie 2 (Scientific Industries Inc., USA) at 2000 rpm for 2 min. 10 mL of palm oil was then added into the solution and the mixture was homogenized again. The solution was centrifuged (Eppendorf Centrifuge 5840 R) at 1500 × g at 30°C for 5 min. EA was calculated using equation (3) as height of emulsified layer over total height of mixture in tube. After that, the emulsion from previous procedure was heated at 80°C for 30 min. The solution was centrifuged again at 1500×g at 30°C for 5 min. ES was expressed as percentage height of remaining emulsified layer over the height of original emulsion layer.

$$EA\ (\%) = 100 \times \frac{heigh\ of\ emulsified\ layer}{total\ heigh\ of\ mixture\ in\ tube} \quad (3)$$

$$ES\ (\%) = 100 \times \frac{heigh\ of\ remaining\ emulsified\ layer}{total\ heigh\ of\ original\ emulsion} \quad (4)$$

Carbon-to-nitrogen ratio

Carbon was determined by finding the total organic of the sample. Firstly, a crucible was dried and weighted, followed by the addition of 2 g sample into it. The sample was then dried in the oven at 105°C for 24 hours. The dried sample was drawn from the oven (Shimaden SR93 Memmeert, Western Germany) and the weight of the dried sample with crucible was determined. Then, the dried sample with crucible was placed into a muffle furnace (VULCAN A-130, Canada). The temperature was set to be 550°C and the sample was left for 2 hours until no black specks were seen or until it is white. The burnt sample with crucible was left to cool down in a desiccator before being weighed. The weight at this point was recorded. The percentage of carbon was calculated as follows:

$$Ash\ (\%) = 100 \times \frac{weight\ initial - weight\ final}{weight\ final} \quad (5)$$

$$Volatile\ solids\ (\%) = 100 - Ash\ (\%) \quad (6)$$

$$Total\ organic\ carbon\ (\%) = \frac{volatile\ solids\ (\%)}{1.8} \quad (7)$$

Each sample was done in triplicate and the average results of the samples were recorded. Nitrogen was determined using Kjedahl method by using proximate analyser, Kjeltab™ 2300 distiller (FOSS, Denmark). 1.0 g of each sample in Kjedahl flask was added with 2 tablets of Kjeltabs Cu 3.5 (Fisher Scientific (M) Sdn. Bhd., Malaysia) and 2 mL of concentrated sulphuric acid. Then, the content was heated on a TecatorKjeltab digester (FOSS, Denmark) at 420°C for 1 hour. The solution in every Kjedhal flask changed from black to clear green colour and was left to cool for approximately 20 minutes. This was followed by distillation process using proximate analyser, the Kjeltab™ 2300 distiller (FOSS, Denmark). Every Kjedahl flask was diluted with 50 mL of 40% sodium hydroxide (NaOH) and 80 mL of distilled water. The distillation product was then transferred into a conical flask containing 10 mL of 2% boric acid with indicator. The mixture was then titrated with 0.1N hydrochloric acid (HCl), which turns pink colour to colourless. The percentage of nitrogen was calculated according to the following formula:

$$Kjedahl\ Nitrogen\ (\%) = \frac{(Vs - Vb) \times N \times 14.01}{W \times 10} \quad (8)$$

Where Vs (mL) volume of standardized acid used to titrate a sample; Vb (mL) is volume of standardized acid used to titrate a reagent blank; N is 0.1000 (normality of standard HCl, 14.01 is the atomic weight of nitrogen; W (g) is the weight of samples-standard; and 10 is the factor to convert mg/g to percentage.

Sugar analysis

The sugar standard solutions were prepared at concentration of 1 g/100 mL for each fructose (F), glucose (G), sucrose (S) and maltose (M), diluted with distilled water. The HPLC system was equipped with Shimadzu LC-6A pump, a RI detector (RI-1530) and a manual injector. The column used was Purospher ® STAR Amidogen (NH_2) (250–4.6 mm, 5 µm) (MERCK). The mobile phase used was 80% acetonitrile and 20% distilled water while the flow rate was 1.8 mL/min. The column was operating at room temperature (29 °C). The method for HPLC analysis to determine the sugar in pink guava puree residues were based on Hunt *et al*. (1977) and Yusof *et al*. (1988) with some modification. The sugar standards used were fructose, glucose, sucrose, and maltose in the concentration 1 to 5% (w/v). A calibration curve was obtained for each of the four sugars. Sugars in the samples were quantified by comparing peak areas of samples with those of the sugar standards. The extraction and preparation done prior to injection into HPLC was carried out according to Wills *et al*.

(1980). 10 g of the samples were heated with 100 mL of methanol on a steam bath (80°C) for 30 min. The mixture was filtered through Whatman No. 1 filter paper into a round bottom flask and the residue was re-extracted twice in 75 mL portions of methanol, and filtered. The filtrate was evaporated to about 10 mL under vacuum at 50°C in rotary evaporator. The volume was made up to 10 mL in a volumetric flask. The solution was then filtered through a Sep-pak C18 cartridge and a 0.45 µm membrane filter using a syringe. The injection volume was 10 µL.

Results and Discussion

Proximate analysis and pH value

The proximate composition of R, S, and D are shown in Table 1. Moisture content shows the highest percentage among all the components for all the three samples. PGP residue from S contained the highest moisture, whereas R contained the lowest moisture content which is (77.24%) and (67.46%). As the pink guava processed into puree, it will undergo several processes and various form of by-product can be produced. The residue in R will contain more seeds compared to others. Therefore, the amount of moisture content will vary along the production as the form of the by-product is different. The moisture content obtained was consistent with the result reported by Kong and Ismail (2011), which are ranging from 70–80%. Due to high moisture content, the residue must be dried to prevent the spoilage. The ash contents in R have the highest value (0.85% wet basis) compared to other sources, where S (0.70% wet basis) and D (0.62% wet basis). The high content of ash in R is due to the high content of lignin. Lignin was less soluble, so hydroalcoholic solvents could not extract them; thus produced a concentration effect on the studied by-products (Marin *et al*., 2007). Other than that, ash content had been reported to be associated with metallic constituents. Pink guava or guava has been generally reported to contain metallic constituents such as potassium, calcium, iron, phosphorus, manganese, sodium, copper and zinc (USDA, 2015). Fat content in the PGP residues from all the three sources were considered low (0.07%–1.20%) as compared to the apple pomace fat content (0.82%–1.43%) (Joshi and Attri, 2006). The highest fat content was from PGP residues in R (1.20% wet basis), followed by S (0.20% wet basis) and D (0.07) due to the seed content in the R sample. Fat content in PGP residues might increase if the residues are pre-treated—physically or chemically—before the analysis. The protein content for PGP residues from R, S, and D were ranging between 1.01%–1.78% (wet basis). The results obtained were almost similar with the protein content in apple pomace which was ranging between 1.03%–1.82% (wet basis) (Joshi and Attri, 2006). Apple pomace has been utilized using Solid State Fermentation (SSF) to produce crude protein for animal feed (Joshi and Attri, 2006). The amount of crude fiber of R (16.71% wet basis) was higher than S (9.01% wet basis) and D (11.72% wet basis). This is because the guava seed and the coarse pulp residues produced after the cutting and crushing (Refiner) process was a fibrous material that contains high lignocelluloses or dietary fiber content of lignin, hemicelluloses, and cellulose (Ouensanga *et al*., 2003). S contained the lowest amount of crude fiber which is 9.01% of wet basis. The total carbohydrate content in D is the highest with 12.19% of wet basis followed by 11.84% of wet basis in S and 12% of wet basis in R. The carbohydrate content is considered low and cannot be a good energy source.

Table 1 Physico-chemical composition in PGP residues (wet basis)

Components (%)	Refiner (R)	Siever (S)	Decanter (D)
Moisture Content	67.46 ± 1.13	77.24 ± 0.17	74.39 ± 0.42
Ash	0.85 ± 0.02	0.70 ± 0.04	0.62 ± 0.01
Fat	1.20 ± 0.25	0.20 ± 0.01	0.07 ± 0.06
Crude Protein	1.78 ± 0.10	1.01 ± 0.03	1.01 ± 0.10
Crude Fiber	16.71 ± 0.13	9.01 ± 0.02	11.72 ± 0.27
Carbohydrate	12.00	11.84	12.19
TDF	29.86 ± 0.79	18.63 ± 1.01	23.30 ± 0.49
IDF	28.58 ± 1.29	15.79 ± 0.30	19.19 ± 0.28
Hemicellulose	9.72 ± 1.16	5.70 ± 0.94	4.42 ± 0.39
Cellulose	10.77 ± 0.52	7.48 ± 1.08	11.74 ± 0.36
Lignin	40.02 ± 0.55	4.94 ± 0.79	4.57 ± 0.18
SDF	1.61 ± 0.26	1.02 ± 0.24	1.67 ± 0.33
NDF	60.51 ± 1.05	18.12 ± 0.58	20.73 ± 0.16
ADF	50.79 ± 0.61	12.41 ± 0.89	16.31 ± 0.43
Types of Sugar			
Fructose	0.78 ± 0.02	1.89 ± 0.02	2.18 ± 0.00
Glucose	0.49 ± 0.00	1.11 ± 0.05	1.28 ± 0.03
Sucrose	0.07 ± 0.00	0.37 ± 0.00	0.61 ± 0.02
Maltose	n.f	n.f	n.f

*Results are expressed as mean ± standard deviation (n=3); TDF, Total dietary fibre; IDF, Insoluble dietary fibre; SDF, Soluble dietary fibre; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; n.f, Not found.

The study on pH value of the samples was important as it is one of the factors that affect the growth of microorganisms. Cameotra and Makkar (1998) reported that different microorganisms produced biosurfactants at different pH medium, ranges from 4.5 to 10.5, depending on the behaviour of the microorganisms. Results have shown that all the samples have 4 pH value. From literature, any

samples with pH value lower than 4.5, are considered as acidic food products. The results were consistent with the results obtained by Jagtiani *et al*. (1988), who reported their pH values to ranging between (3.2–4.1) for eight selected guava varieties. The pH value for the residues is suitable for the growth of microorganisms in the biosurfactant production.

Lignocellulosic hydrolysis

Lignocellulosic materials, which are commonly related to dietary fibre studies, offer a lot of benefits that can be utilized as bioresource. The result obtained for crude fibre was not consistent or quantitatively related to dietary fibre (Kay, 1982). This is because there was residue left after extraction with dilute alkali and dilutes acid. The method recovers only 50 to 80% of cellulose, 10 to 50% of lignin, and 20% of hemicellulose. Inconsistent results were obtained; thus it cannot be used as a method for the determination of fibre (Southgate and Spiller, 2001). Thus, lignocellulosic hydrolysis was used to evaluate the fraction of fibre content. Table 1 shows the results of total dietary fibre (TDF), insoluble dietary fibre (IDF), soluble dietary fibre (SDF), acid detergent fibre (ADF), and neutral detergent fibre (NDF). IDF is not soluble in water while SDF is soluble in water. IDF—which consist of hemicelluloses, cellulose, and lignin—is usually added in the dietary fibre as it passes through the digestive system to help in reducing the risk and occurrence of haemorrhoids and constipation (Dhingra *et al*., 2012). SDF consist of pectin, gums, mucilage, etc. SDF can be utilized as functional ingredients in food industry. ADF is consists of cellulose and lignin. It is measured as residue after extracting the by-product with a hot dilute sulfuric acid solution of detergent, CTAB. NDF consists of hemicelluloses, cellulose, and lignin which are part of food remaining after extraction with a hot neutral solution of the detergent sodium lauryl sulfate. PGP residues from R gave the highest value on TDF (29.86%) and IDF (28.58%) compared to other sources while value of SDF for all sources are low (1.02%–1.67%) compared to the IDF value. The high value of TDF in R may be due to the screening size during the puree processing. The bigger the screen size, the more skins and seeds of pink guava trapped in the screener and affect the fibre composition in those three sources. From Table 1, NDF value of PGP residues from R, S, and D contained the highest percentage of lignocellulosic material compared to ADF as it consists of all fibre components.

The result in Table 1 shows the composition of dietary fibre in PGP residues. It shows that PGP residues contained high content of lignin (4.57%–40.02%) followed by cellulose (7.48%–11.74%) and hemicellulose (4.42%–9.72%). As stated earlier, PGP residues from D contain stone cells while PGP residues from R contain coarse pulp and seeds. These components in PGP residue from those sources contribute to the high content of lignin and cellulose (Jagtiani *et al*., 1988). PGP residues contain both cellulose and hemicellulose fractions which are potential to be used as a source of fermentable sugars.

Emulsifying activity and emulsifying stability

EA and ES of PGP residues were determined to evaluate the functionality of residues, which makes them suitable as emulsifiers without any pre-treatment needed. Table 2 shows the experimental result of EA and ES percentage. A thermodynamic balance between the emulsion phase is assured by an emulsifier, which decreases interfacial tension and keeps the emulsion stable (Dybowska, 2008). The percentage of EA for all the samples of PGP residues were very low (ranging from 2.67% to 3.33%) compared to other fibrous materials (okara, corn cob, wheat straw, and rice husk) reported by Kuan and Liong (2008), where they obtained high EA which are ranging from 40% to 60%. However, for ES results, the value obtained was quite high in comparison to the studies of EA and ES by Wu (2001) for corn gluten meal. The EA and ES were attributed to the soluble polysaccharide fractions or soluble dietary fibre (SDF), containing a pectin-like structure composed of galacturonan backbone of homogalacturonan and rhamnogalacturonan branched by β-1,4-galactan and α-1,3- or α-1,5-arabinan chains (Nakamura *et al*., 2006; Kuan and Liong, 2008).

Table 2 Functional and Physiological properties of PGP residues

Functional and Physiological properties	Refiner (R)	Siever (S)	Decanter (D)
EA (%)	3.33 ± 1.15	2.67 ± 1.15	3.33 ± 1.15
ES (%)	66.67 ± 28.87	83.33 ± 28.87	66.67 ± 28.87

*Results are expressed as mean ± standard deviation (n=3);
EA, emulsifying activity; ES, emulsion stability

The SDF stabilizes the oil droplets by steric repulsion, whereas the long hydrophilic polysaccharide chains create a thick hydrated layer, which prevents the droplets from coming together and coalescing (Nakamura *et al*., 2006; Kuan and Liong, 2008). The SDF content in PGP residues from R, S, and D were low. The protein fraction also plays an important role in anchoring the moiety of fibre to the oil or water interface. This is probably

due to low content of protein and SDF in the samples of PGP residues. The ES capacity was also reported to correlate well with the protein and lipid content (Yadav *et al*., 2007; Kuan and Liong, 2008), which enables them to act as good oil absorbers. Our results showed that the samples exhibited lower protein, lipid, and SDF content (Table 1), which led to low EA and ES. Other factor that will affect the EA and ES properties are related to the size of particles and pH value of the sample. The sample used in this study is raw, without any physical treatment on reducing the size of particles or pH adjusting. Commercial corn gluten meal at pH 4.0 had poor emulsifying properties. Even for small particle sizes, good emulsifying activity and emulsion stability could be achieved by both increasing the pH to 6.6 and decreasing the particle size to < 15 μm (Wu, 2001).

Carbon-to-nitrogen ratio

Carbon to nitrogen (C/N) ratio was done to evaluate the fermentation ability of the PGP residue. Carbon is used as energy source to form new cells. Nitrogen is used to synthesize new cells, including the synthesis of protein, enzymes, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) (Parkin and Owen, 1986). C//N ratio plays an important role in fermentation activity. C/N ratios ranging from 15 to 70 (on a mass basis) have been used for aerobic composting and anaerobic digestion (Kayhanian and Tchobanoglous, 1992). If C/N ratio is above or below the ideal composition, it may lead to several problems (Rughoonundun *et al*., 2012). High C/N ratio indicates the lack of nitrogen while low C/N ratio indicates high nitrogen content. Lacking of nitrogen will slow the digestion rate in the fermentation due to the insufficient cells to maintain active microbial biomass. But, if the nitrogen content is too high in the C/N ratio, it can evolve ammonia, which is toxic to microorganisms and can completely stop the fermentation. Based on the results obtained in Table 3, residues from R (46:1) is the most suitable ratio for fermentation activity. The ratio for the residues from S (84:1) and D (115:1) were considered too high and need modification on the nitrogen content.

Sugar analysis

Table 1 shows the presence of fructose, glucose and sucrose in PGP residues. Maltose was not found in the PGP residues with the main sugar content is fructose. The highest fructose content with 2.18% was in PGP residue from D source followed by 1.89% in S and 0.78% in R. It shows the same trend for the other sugar content, glucose and sucrose. The composition of the sugar content for PGP residues have the same trend as in apple pomace (13.60%) reported by Joshi and Attri (2006) where the fructose is the highest component compared to other sugars. Sugar identification is important in selecting suitable microbes that can consume the sugar as their carbon source in biosurfactant production.

Conclusion

This study showed the possibility on utilizing the PGP residues as bioresource. Based on C/N ratio, the carbon content in PGP residues is high; thus, the PGP residues is potential to be carbon source in fermentation. However, the fat content obtained is considered low for biosurfactant production which involves the fermentation activity. The high content of TDF also contributes to the dietary fiber source. The low pH value of the residues leads to the low EA value. Pretreatment of the residues is needed in order to release other trapped component from intact fiber; hence it will improve the ability as an emulsifier and enhance the composition of the residues in order to be a good bioresource.

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