Comparative Study of Total Phenolic Content and Total Flavonoid Content Extraction from Guava Juices by Progressive Freeze Concentration and Evaporation

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Abstract
The guava fruit is rich with high valuable nutrients that have health-promoting features. It has been used over the centuries across various cultures in the world. Nowadays, people enjoy a simpler way to ensure a healthy diet by consuming supplements and juices. Guava juice was concentrated through progressive freeze concentration (PFC) or evaporation techniques. Total phenolic (TPC) content and total flavonoid content (TFC) were determined after these processes. TPC was analysed by folin-ciocalteu test and TFC was determined through Dowd method. Temperature and stirring rate were manipulated during the process. It was observed that TPC and TFC were higher for PFC than evaporation techniques, with TPC value of 8.1050 mg gallic acid equivalent (GAE)/g guava and TFC value of 0.8296 mg Quercetin/g guava, respectively. It was observed that the increase of temperature will enhance the TPC extraction due to higher mass transfer for extraction but reduce the TFC extraction due to the conversion of flavonoids into monomers.

Keywords: Progressive freeze concentration method, guava juice, total flavonoid content, total phenolic content.

1. Introduction
The guava fruit or scientifically known as Psidium guajava is categorized as a “super-fruit” due to its unique taste and health-promoting traits. This fruit originated from central America but has massive productions in the Asian countries like India, China and Thailand [1]. This tropical fruit is known for its high-profile nutrients as well as its forte in boosting immunity with high vitamin C content, promotes weight loss, able to reduce the cancer risks and high fibre that can treat constipation. Not just that, the founder of Green Universe Environmental Services Society, Osk Reddy also stated that the guava fruit has the anti-aging properties with its content of vitamin A, vitamin C, and antioxidants [2]. Generally, phenolics have an array of health promoting benefits; they are of current interest due to their important biological and pharmacological properties, especially the anti-inflammatory, antioxidant, and anti-mutagenic and anticarcinogenic activities [3,4,5]. Flavonoids are considered as important components in the human diet [6]. It is proven that the guava demonstrated various medical benefits from the pulp, leaves, seed, skin, and bark of the guava tree. The abundance supply of the guava fruit in Southern Asia is a blessing for us and can be transformed into plenty of types of processed food. The dehydration method is one of the oldest ways of food processing that uses the concept of removing water which is being applied in the current technology. Nowadays, the technology in the food industry has been branched out into various kinds of separation processes and value enriching techniques in improving the quality of the food processed. Focusing on the fruits processing industry especially for juices, there are several processes like membrane filtration, freeze-drying and thermal evaporation which are commonly used for fruit juice concentrates. However, the process requires heat in concentrating the juice which may deteriorate the quality and nutrients in the
juice probably due to the vulnerability of the valuable compounds at the operating conditions of the process [7]. Due to these consequences of heat implementation on the fruit juice, the high values of the guava fruit as mentioned earlier would be deprived along the process thus degrades the quality compared to the original fruit. Despite the downfall of the processes, the thermal methods are the conventional way in the food industry due to the availability of the technology and cost factors.

Nevertheless, Miwayaki et al. have published a paper on their study that proved a method that can retain the useful substances in the fruit juices [8]. The study compared the original content and the concentrated apple juice that underwent the progressive freeze concentration (PFC) process. It was concluded that the qualities were kept unchanged in the process after underwent the PFC technique. The team stated that the PFC method is less expensive than the suspension crystallization with a simple process of separating the water content of the juice at low temperature. The PFC method also can obtain high-quality concentrate with high sugar level that can produce higher alcohol content than the conventional way. Jusoh et al. verified that PFC system can efficiently concentrate juices at suitable operating conditions [9]. Focusing on the topic of this project, the guava fruit has a high concentration of ascorbic acid and carotenoids like lycopene, β-carotene and β-cryptoxanthin that act as antioxidant, anti-hyperglycemic and anti-neoplastic agent [10]. It was concentrated using PFC and evaporation techniques. In order to assess the retainment of organic compounds, TPC and TFC of guava juice were analysed using Folin-ciocalteu test and Dowd method, respectively.

2. Materials and methods

2.1 Materials and reagents

Guava fruits from local seller at Seri Iskandar, Perak, ethylene glycol and water were used for the coolant. Folin-ciocalteu reagent, sodium carbonate, sodium nitrite, sodium hydroxide and aluminium chloride were used for the TPF and TFC analysed. All chemicals were purchased from Avantis Laboratory Supply.

2.2 Sample Preparation

For PFC and evaporation process the sample was prepared by blending guava with distilled water using an electronic blender (LB20ES, Waring, USA). After blending, guava juice was filtered. Both PFC and evaporation samples were set at 100 g/L of concentration of guava juice. The samples were prepared by batches before each run of experiment to ensure the freshness of the guava juice.

2.3 Experimental Apparatus

The experimental set-up for the PFC system is shown in Fig. 1. About 900 ml of guava juice was placed in a cylindrical vessel (13.5 cm × 17 cm). The cylindrical vessel was placed inside a refrigerated bath (630D, PROTECH, Malaysia) for the PFC process. The initial volume of guava juice is measured before the experiment. The PFC was performed by setting the coolant temperature of the refrigerated bath of diluted ethylene glycol at -10°C. The guava juice was stirred by the automated stirrer for 20 minutes. After that, the concentrated juice was taken out for further analysis. The same procedure was repeated for different operating conditions of coolant temperature (-7, -5, -2°C) and stirring rate (150, 200, 250 and 300 rpm).
2.5 Characterization of Concentrated Guava Juice Samples

The characterization of the concentrated guava juices after PFC and evaporation process was conducted into two subsections studies.

i. Total phenolic content (TPC)

ii. Total flavonoid content (TFC)

2.5.1 Folin-ciocalteu test

The Folin-ciocalteu test was conducted to determine the TPC [7,8]. The mix of 0.5 ml of the concentrated guava juice and 2.5 ml of diluted Folin-ciocalteu reagent (1:10) was prepared and left for 5 minutes. Then, 2 ml of 10% sodium carbonate solution was added into the mixture and kept in the dark for 2 hours at room temperature. Next, a UV-Vis spectrophotometer was used in determining the TPC amount with absorbance at a wavelength of 760 nm. To obtain the TPC amount, the concentration of the sample was referred to a calibration curve of gallic acid as a standard by
comparing the absorbances. The results of TPC were measured in the unit of mg GAE per 1 g of a dry sample using the Eq. (1).

\[ TPC = \frac{cV}{m} \]  
(1)

Whereas \( c \) is the sample concentration obtained from the calibration curve (g/ml), \( V \) is the volume of guava juice used for extraction (ml) and \( m \) is the dry weight of guava fruits used.

2.5.2 Dowd method

The Dowd method was used in determining the TFC [9,10]. About 0.5 ml of the concentrated guava juice and 0.3 ml of sodium nitrite were mixed together and left for 5 minutes. Next, 0.3 ml of 10.0% aluminium chloride was added into the mixture and left for another 5 minutes. After that, 2 ml of sodium hydroxide and distilled water were added up until 10 ml before incubated in dark for 2 hours at room temperature. UV-Vis spectrophotometer was used in determining the TFC at 510 nm of absorbance wavelength. To obtain the TFC amount, the concentration of the sample was referred to as a calibration curve of quercetin solution as a standard by comparing the absorbances. The results of TFC were in the unit of mg quercetin equivalents per 1 g of a dry sample using Eq. (2) and the calibration curve of quercetin.

\[ TFC = \frac{cV}{m} \]  
(2)

Whereas \( c \) is the sample concentration obtained from the calibration curve (g/ml), \( V \) is the volume of guava juice used for extraction (ml) and \( m \) is the dry weight of guava fruits used.

3. Results and discussions

3.1 Progressive Freeze Concentration (PFC) method

The manipulated parameters considered in this study were coolant temperature and stirring rate. The initial concentration, volume and running time were fixed at 100 g/L, 900 ml and 20 minutes, respectively. In such, this study investigated the effects of coolant temperature and stirring rate towards the bioactive compounds of TPC and TFC of the concentrated guava juice.

3.1.1 Effect of Temperature Variation on TPC and TFC Extractions

In this section, the stirring rate was fixed at 250 rpm. Theoretically, the higher the temperature, the better the TPC extraction due to higher fluid viscosity that leads to higher solubility and diffusion coefficient hence improves the mass transfer [7]. Wissam, Ali et al. mentioned that flavonoid will decreases with the increment of temperature as the flavonoids will be destroyed into monomers by the hydrolysis of C-glycosides bond [16].

In this study, the result for PFC as shown in Fig. 3 indicated that the trend of TPC extraction can be seen in the range of -7 °C to -2 °C and the TFC extraction in the range of -10 °C to -7 °C. The values of TPC have been estimated using the Eq. (1) and the calibration curve of gallic acid whereas the values of TFC was predicted by Eq. (2) and the calibration curve of quercetin. Fig. 3 indicate that the TPC has a higher value than the TFC in all runs since flavonoid is one of the phenolic group. For the PFC analysis, the highest TPC extracted was 8.1050 mg GAE/g guava at the temperature of -10 °C whereas TFC has the highest value of 0.8296 mg Quercetin/g guava at -2 °C.
These findings contradicted with the study of Wissam et al. [16]. However, at the range of -7 °C to -2 °C, the TPC increased as the temperature increased [12]. Also, at the range of -10 °C to -7 °C, the TFC decreased with the rise of coolant temperature. The inconsistency of results can be influenced by the efficiency of the equipment since the refrigerated bath used for PFC study can only be performed optimally at the temperature above -10°C and below -2°C due to the ethylene glycol to water ratio used. Based on Miwayaki the recommended ethylene glycol to water ratio should be 50% (v/v) for PFC method. However, the ethylene glycol to water ratio used in this project only at the ratio of 30% (v/v) of ethylene glycol because of the chemical availability [4]. Hence, the refrigerated bath temperature could not drop lower than -10 °C.

3.1.2 Effects of Stirring Rate on TPC and TFC Extractions

Another parameter considered in this experiment was the effect of the stirring rate on the TPC and TFC extraction. The experiments were set at -5 °C and 20 minutes as constant parameters. Fig. 4 shows the relationship of the stirrer speed on the TPC and TFC extractions of concentrated guava juices by PFC respectively. Fardyanti, Istanto et al. mentioned that increasing the stirring speed may increase both TPC and TFC extractions until a certain limit [12]. It was found that both TPC and TFC have a negative parabolic curve in relation to the stirring rate based on their study.

In every PFC runs of this subsection, the TPC has a higher value than the TFC since flavonoid is one of the phenolic group. For the PFC analysis, the negative parabolic curve can be observed in the range of 200 rpm to 300 rpm. The highest TPC amount of 6.1733 mg GAE/g guava at 150 rpm whereas the highest TFC extracted was at 250 rpm with 0.3296 mg Quercetin/g guava. The values of TPC and TFC extractions decreased to 5.0100 mg GAE/g guava and 0.1630 mg Quercetin/g guava respectively with an increment of stirring rate after 250 rpm. It can be justified as higher agitation speed might create a vortex formation that caused lesser contact between the particles and solvent hence led to lesser extractions [18].
3.2 Evaporation method
The evaporation process can be defined as the vaporization of liquid into gaseous form at an elevated temperature which can occur at any temperature, unlike the boiling process [19]. The refrigerated bath temperature provided a proper surrounding of guava juice evaporation, theoretically as the higher the water bath temperature, the more evaporation takes place as it is reaching the boiling point [20]. The manipulated parameters considered in this study were bath temperature and stirring rate. However, the initial concentration, volume and running time were fixed at 100 g/L, 150 ml and 20 minutes, respectively. In such, this study investigated the effects of temperature and stirring rate towards the bioactive compounds of TPC and TFC of the concentrated guava juice.

3.2.1 Effect of Temperature Variation on TPC and TFC Extractions
This study examined the effects of water bath temperature towards the extractions of bioactive compounds of TPC and TFC by fixing the stirring rate to 50 rpm and manipulating the temperature varied between 40 and 55 °C. As for the evaporation process, the effect of temperature on TPC and TFC extractions are shown in Fig. 5. Similarly, the TPC has been estimated using the Eq. (1) and the calibration curve of gallic acid whereas the values of TFC was predicted by Eq. (2) and the calibration curve of quercetin.

Fig. 5 Effect of water bath temperature on TPC and TFC in evaporation.

Fig. 5 shows that the TPC has a higher value than the TFC in all runs since flavonoid is a type of the phenolic group. For the evaporation analysis, the highest TPC extracted was 6.2617 mg GAE/g guava at the temperature of 40 °C whereas TFC has the highest value of 0.5185 mg Quercetin/g guava.
at 55 °C. These findings contradicted with Wissam et al. but at the range of 45 to 55°C, the TPC increased as the temperature increased. Also, at the range of 50 to 55°C, the TFC decreased with the rise of refrigerated bath temperature. The figure also shows that the TFC observed at 40 °C and 45 °C have negative values due to negative absorbances which indicate that the reference absorbed more than the samples.

By comparing both PFC and evaporation approaches, the PFC method managed to show a relatively higher amount of TPC and TFC using the PFC method than the evaporation process. Even though the initial concentration of the samples was fixed, the non-thermal method was able to extract more valuable bioactive compounds specifically phenolics and flavonoids. Based on the results obtained in this study, PFC was able to extract a TPC of 29.44% more than the evaporation approach whereas 60% more TFC can be recovered by PFC compared to the evaporation method.

### 3.2.2 Effects of Stirring Rate on TPC and TFC Extractions

For this condition, the temperature was fixed at 45 °C and the stirring speed of the rotary evaporator varied from 45 to 60 rpm. Based on Fig. 6, for the evaporation analysis, the highest TPC and TFC extracted were at the stirrer speed of 50 rpm with the values of 8.3133 mg GAE/g guava and 0.9889 mg Quercetin/g guava, respectively. These findings in agreement with Fardyanti, Istanto et al. but only at the range of 45 to 55 rpm [17]. The negative parabolic curve of the correlation between stirring speed with TPC and TFC extractions shown in Fig. 6 indicates that the bioactive compounds can be well extracted at the speed of 50 rpm to reach the maximum potential before decreasing. The peak of 50 rpm can be the maximum point before excessive vortex formation was created that led to fewer extractions of TPC and TFC as the stirring speed increased. Also, Fig. 6 shows that the TFC observed at 55 rpm have negative value due to negative absorbances. This indicate that the reference which is the distilled water absorbed more UV-Visible light than the samples.

![Fig. 6 Effect of stirring rate on TPC and TFC in evaporation.](image)

By comparing both PFC and evaporation methods, the PFC method managed to show a relatively higher amount of TPC and TFC than the evaporation process. Even though the initial concentration of the samples was fixed, the non-thermal method represented by the PFC approach was able to extract more valuable bioactive compounds specifically phenolics and flavonoids. The negative parabolic trends of TPC and TFC extraction with stirring rate increments has been shown in both PFC and evaporation methods which indicate that the stirring speed should be operated at a specific value to obtain the performance of extraction required. However, specifically under the variation of stirring speed, based on the results obtained from this study, the evaporation method has extracted TPC double the amount than the PFC method whereas the TFC extracted by evaporation was about 35% more than PFC method.
4. Conclusion

PFC and evaporation techniques were tested to compare the TPC and TFC extracted from guava juice by manipulating the temperature and the stirring rate. Then, characterization for TPC and TFC was conducted by performing the Folin-Ciocalteu test and the Dowd method, respectively. The increment of TPC extraction as the temperature increases was due to the intensification of mass transfer that facilitated the extraction process. The extraction of TFC alternatively decreased as the temperature increased because of the flavonoids were destroyed into monomers. As the stirring speed increases beyond the maximum peak, the vortex formed and causing less effective contact for extraction. The TPC was found to have higher values compared to the TFC as the flavonoids are a kind of phenolics group.

To summarise, PFC has shown better performance in extracting the bioactive compounds of phenols and flavonoids with the variation of temperature by 29.44% and 60% respectively. On the other hand, the evaporation showed that by manipulating the stirring rate, the extractions of TPC and TFC were double and 35% more compared to PFC method respectively. By comparing these values, it can be concluded that stirring speed has a higher impact on the extraction of TPC and TFC than varying the temperature.

References

[1] Pariona, A., (2017). Top Guava Producing Countries in The World. World Atlas. Retrieved from https://www.worldatlas.com/articles/top-guava-producing-countries-in-the-world.html

[2] Reddy, O., (2017). Nutrition Facts & Health Benefits of Guava Fruit. Retrieved from https://www.linkedin.com/pulse/nutrition-facts-health-benefits-guava-fruit-osk-reddy/

[3] Koshihara, Y., Neichi, T., Murota, S., Lao, A., Fujimoto, Y., Tat-suno, T., Biochem. Biophys. Acta. 1984, 792, 92–97.

[4] Zhou, J., Ashoori, F., Susuki, S., Nishigaki, I., Yagi, K., J. Clin. Bio-chem. Nutr. 1993, 15, 119–125.

[5] Serrano, A., Palacios, C., Roy, G., Cespon, C., Villar, M. L., Nocito, M., Porque, P. G., Arch. Biochem. Biophys. 1998, 350, 49–54.

[6] Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of separation science, 30(18), 3268-3295.

[7] Adnan, A., Mushtaq, M. & Islam, T. ul., (2018). Fruit Juice Concentrates. Fruit Juices, page 217.

[8] Miyawaki, O., Gunathilake, M., Omote, C. & et al., (2015). Progressive freeze-concentration of apple juice and its application to produce a new type apple wine. Journal of Food Engineering, page 153 – 158. Elsevier Ltd.

[9] Jusoh, M., Nor, N. N. N & Zaria, Z. Y., (2014). Progressive Freeze Concentration of Coconut Water. Jurnal Teknologi, page 45 – 49. Faculty of Chemical Engineering, Universiti Teknologi Malaysia.

[10] Barbalho, S. M., et al., (2012). Psidium Guajava (Guava): A Plant of Multipurpose Medical Applications. Medical & Aromatic Plants. 1(4), page 3.

[11] Ramamoorthy, P. K., & Bono, A. (2007). Antioxidant activity, total phenolic and flavonoid content of Morinda citrifolia fruit extracts from various extraction processes. Journal of engineering science and technology, 2(1), 70-80.

[12] Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y., & González-Aguilar, G. A. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. LWT-Food Science and Technology, 37(7), 687-695.

[13] Ciric, A., Jeljic-Stankov, M., Cvijovic, M., & Djurdjevic, P. (2018). Statistical optimization of an RP-HPLC method for the determination of selected flavonoids in berry juices and evaluation of their antioxidant activities. Biomedical Chromatography, 32(4), e4150

[14] López-Froilán, R., Hernández-Ledesma, B., Cámar, M., & Pérez-Rodriguez, M. L. (2018). Evaluation of the antioxidant potential of mixed fruit-based beverages: A new insight on the folin-ciocalteu method. Food analytical methods, 11(10), 2897-2906.
[15] Ong, S. W. (2018). Phenolic Compound, Antioxidant and Quality of Red wine and White wine at Different Storage Period within Two Weeks (Doctoral dissertation, Tunku Abdul Rahman University College).

[16] Wissam, Z., Ali, A. et al., (2016). Optimization of extraction conditions for the recovery of phenolic compounds and antioxidants from Syrian olive leaves. Journal of Pharmacognosy and Phytochemistry, 5(5), 390—394.

[17] Fardyanti, D. S., Istanto, H., et al., (2018). Extraction of Phenol from Bio-oil Produced by Pyrolysis of Coconut Shell. Journal of Physical Science, 29(2), 195—202.

[18] Wasewar, K. L., Heesink, A. B. M., Versteeg, G. F., & Pangarkar, V. G. (2002). Equilibria and kinetics for reactive extraction of lactic acid using Alamine 336 in decanol. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 77(9), 1068-1075.

[19] Chen, X., & Buchberger, S. G. (2018). Exploring the relationships between warm-season precipitation, potential evaporation, and “apparent” potential evaporation at site scale. Hydrology and Earth System Sciences, 22(8), 4535-4545.

[20] Sossi, P. A., Klemme, S., O'Neil, H. S. C., Berndt, J., & Moynier, F. (2019). Evaporation of moderately volatile elements from silicate melts: Experiments and theory. Geochimica et Cosmochimica Acta.

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