

THE EFFECT OF DIFFERENT ROASTING CONDITIONS ON ANTHOCYANIN, TOTAL PHENOLIC CONTENT, AND ANTIOXIDANT CAPACITY OF POMEGRANATE PEEL

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Abstract

Pomegranate (*Punica granatum* L.) peel is a common agro-industrial waste, but high in phytochemicals such as hydrolyzable tannins, phenolics, flavonoids which includes anthocyanin, and other essential nutrients, making it a great natural source of antioxidants that may protect cells against oxidative damage caused by free radicals. Thus, it will be advantageous when the amount of extractable phenolic compounds yield can be increased by extracting using efficient extraction parameters. Therefore, this study aims to evaluate the impact of different roasting conditions which involve temperature (100°C, 150°C, 200°C) and duration (10 or 20 minutes) on the extractable anthocyanin, phenolic content, and antioxidant capacity of pomegranate peels. The phenolic compound extraction process was through the maceration method with 50% ethanol/H₂O. The Folin-Ciocalteu assay was applied to measure the phenolic content from extracts obtained while 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was utilized to measure the antioxidant activity. The result showed that roasting at 100°C for 20 minutes yields the highest phenolic content of 4.404 mg GAE/g while at 150°C for 20 minutes, the highest anthocyanin content was recorded with a percentage of 1.33%. This was also correlated with the increase of the antioxidant capacity until the roasting temperature reached 150°C. The highest antioxidant activity with a value of 33.68% was detected at 100°C for 20 minutes. Overall, the roasting activity does have a significant impact in enhancing extractable phenolic compounds in pomegranate peel which thereby led to a significant increase in the antioxidant activity. Thus, this can provide more insights for future application of the roasting procedure on other fruit peel samples in phenolic compound extraction.

Keywords: Pomegranate peel; roasting; antioxidant; phenolic content; anthocyanin

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Introduction

Free radicals are always present in human bodies. However, if free radicals exceed the human body's capacity, this can cause a condition called oxidative stress which can lead to several harmful diseases (Pizzino et al., 2017). Fortunately, these free radicals can be counteracted by antioxidants. Most of the antioxidants found in the human diet are phenolic compounds that can be found in various plant sources, such as fruits, vegetables, seeds, and more. For example, pomegranate peel is high in polyphenols like hydrolyzable ellagitannin, making it a great natural source of antioxidants (Ranjha et al., 2020). However, during the industrial processing of pomegranate, large quantities of by-products, primarily peels and seeds, are produced and are typically thrown away as waste without being valued (Gullón et al., 2020). Besides, anthocyanin which is a natural pigment possesses many beneficial properties related to health as it also has antioxidant properties and others such as anti-inflammatory qualities, making

natural pigments highly safe for human health (Le et al., 2019). Anthocyanins are found in a variety of colorful fruits across the world, including pomegranates and other fruits such as grapes, strawberries, raspberries, and more.

This study will be helpful to promote the utilization of the by-products of pomegranate fruit which is the peel to act as a source of natural antioxidants. The proper use of waste by-products will not only address environmental concerns but will also serve as a sustainable strategy for improving health through enhanced foods containing health-promoting substances. Pomegranate is widely referred to as a "superfruit" since it is a polyphenol-rich food with health benefits due to its strong antioxidative activity (El Hosry et al., 2023). In different studies, the antioxidant activity of pomegranate peel was shown to be 2.8 times greater than pomegranate seed and leaf extract (Derakhshan et al., 2018).

Several studies have shown that the volatile compound yield in extracts obtained can be increased after undergoing roasting procedure (Özcan et al., 2021; Wu et al., 2022). The impact of roasting on the nutritional and antioxidant qualities of pomegranate peels has yet to be investigated. Therefore, the objective of this research is to determine the roasting condition that yields the highest content of phenolics and anthocyanin with high antioxidant activity in pomegranate peels through DPPH assay. Phenolics and anthocyanin were extracted by maceration as these bioactive compounds contribute to antioxidant properties in pomegranate peels. Anthocyanin, phenolic, and antioxidant content at each roasting condition were also measured.

Methods

Sample collection

Fresh pomegranates were purchased at a local market in Kuala Pilah town, Negeri Sembilan, Malaysia, and were stored in a plastic container. It had been placed in a dry and cool environment before being used for the experiment.

Chemicals

Chemicals used were 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu Phenol Reagent, sodium carbonate (Na_2CO_3), ethanol, methanol, hydrochloric acid (HCl) and distilled water.

Sample preparation

Pomegranates were washed under running water to eliminate any dirt and were patted dry using paper towels. The peels were removed from the pomegranate and were finely chopped using a knife into small slices and then dried in an oven at 55°C for 24 hours (Sharifi et al., 2022). The pomegranate peels were roasted for 10 and 20 minutes at different roasting temperatures of 100, 150, and 200°C . As a control, an unroasted sample was used (Yamuangmorn et al., 2021). The roasted peels were blended into powder to prepare them for extraction.

Extraction method

Extraction was done by the maceration method. In an Erlenmeyer flask, pomegranate peel powder (20 g) was immersed in ethanol (150 mL) with a concentration of 50% (ethanol/ H_2O) and stirred using an automatic stirrer at room temperature for 48 hours without the presence of light. After 48 hours of immersion, each of the extracted products was filtered using Whatman No. 1 filter paper, and the solvents were removed by evaporation using a rotary evaporator at 40°C . The obtained extracts were kept at 4°C until further use (Marsoul et al., 2020).

Determination of Total Anthocyanin

Anthocyanin contents were determined using the procedure provided by European Pharmacopoeia 6.0. (2008) as cited by Čujić et al. (2016), with minor adjustments. Using 0.1% (v/v) solution of HCl in methanol as the compensation liquid, the absorbance of the solution was measured at 528 nm. Acidified methanol was used as a blank (Tierno et al., 2015). The percentage of anthocyanin content was expressed as cyanidin-3- O-glucoside chloride and calculated using Equation (1).

$$TA\% = A \times 5000 / 718 \times m \quad (1)$$

Where A = absorbance of the sample at 528 nm, 718 = specific absorbance of cyanidin- 3-glucoside chloride at 528 nm, and m = mass of sample in grams. Measurements were done in triplicate, and mean values were recorded as the results.

Determination of Total Phenolic content

According to Ferarsa et al. (2018), total phenolic content can be determined by using Folin- Ciocalteu assay. First, 200 μ L of extracts with 1000 μ L Folin–Ciocalteu diluted reagent (1/10, v/v) were added into a test tube. Each of the solutions was mixed by using a vortex and let the test tubes stand in a place without light for 5 minutes. Then, 800 μ L sodium carbonate solution was added with a concentration of 75 g/mL into each tube before it was mixed again by using vortex and placed in a 50°C water bath for 10 minutes. Then, the solutions were allowed to cool at room temperature. The absorbance of each solution was determined at 760 nm. Gallic acid was used as a standard antioxidant and as a positive control (So et al., 2020). Solution without extracts was used as a blank. The concentration of phenolic contents in the fruit on the dried matter was expressed using gallic acid as a standard for the calibration curve. Results were expressed in mg of Gallic acid equivalents/g dried matter (mg GAE/g DM).

Determination of antioxidant capacity

For antioxidant capacity, the DPPH assay according to Yamuangmorn et al. (2021) has been applied. Firstly, in a test tube, about 0.3 mL of the sample extracts were transferred, along with 1.6 mL methanol and 0.5 mL of 0.1 mM DPPH solution. 2.1 mL of methanol, without DPPH solution, has been used as the blank of the extracts. The solutions were mixed by shaking the test tube gently and were incubated at room temperature without the presence of light for 20 minutes and measured at 517 nm with a UV-Vis spectrophotometer. Ascorbic acid was used as a reference antioxidant (Jadid et al., 2017). The antioxidant activity (%) of samples was calculated by using Equation (2).

$$\text{Antioxidant activity (\%)} = [(AC - AS/AC) \times 100] \quad (2)$$

Where, AC = absorbance of control, and AS = absorbance of sample.

Statistical analysis

All the data were recorded in Microsoft Excel, where the results were expressed as mean value \pm standard deviation since all the tests were done in triplicate. For statistical analysis, the analysis of variance (ANOVA) test was done to indicate the significance of data for each of the tests (Park & Lee, 2021).

Result and Discussion

Through initial physical observation as displayed in Figure 1, it can be observed that the brown color of the roasted peel samples intensified as the temperature increased. This was also influenced by the duration of the roasting process, which was either 10 or 20 minutes. Other than the observable color change, the thickness of the peel was also observed to be thinner and crispier with the increase in temperature as compared to 100°C.

The increasing browning intensity of the roasted pomegranate peel samples is consistent with the browning of the dried *Citrus unshiu* peel when roasted at increasing temperatures (Ko et al., 2020). This is due to the Maillard reaction. The roasting of the samples produces heterocyclic aromatic molecules such as melatonin that absorb light while emitting a brown color appearance (Wang et al., 2021). This is to a certain extent where the higher temperature would start to initiate the carbonization process as can be observed in Figure 1 (g) which was roasted at 200°C for 20 minutes. The decrease in thickness of the pomegranate peels with the increase in temperature can be attributed to the loss of moisture through the elution of water molecules from the peel tissues due to high temperature (Bagheri et al., 2019).

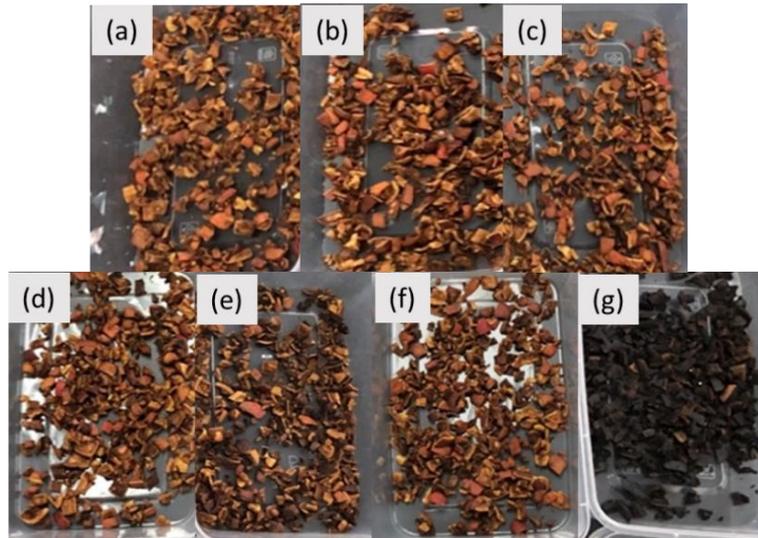


Figure 1. Physical observation of the pomegranate peel samples roasted under varying conditions. (a) unroasted dried peel, (b) and (c) were roasted at 100°C for 10 and 20 minutes respectively, (d) and (e) were roasted at 150°C for 10 and 20 minutes respectively, and (f) and (g) were roasted at 200°C for 10 and 20 minutes respectively.

Anthocyanin content

Ethanol with a concentration of 50% was used as the extraction solvent because, according to a study by Le et al. (2019), the optimal total anthocyanin content was obtained by using a solvent containing a 50/50 mixture of ethanol and water (ethanol/H₂O). According to a study by Čujić et al. (2016), the extraction of phenolics also was highest at 50% ethanol. Water aids in the swelling of plant material, but ethanol breaks down the bonds between solutes and the plant matrix, allowing more compounds to be extracted. Table 1 shows the percentage of anthocyanin extracted from the pomegranate peels treated with different roasting conditions including temperature and roasting duration. The anthocyanin percentages were calculated using Equation (1) based on the absorbance measured.

Temperature-wise, it is observed that the percentage of anthocyanin content increased with the percentages of 0.53%, 0.59%, and 0.76% when roasted for 10 minutes at 100°C, 150°C, and 200°C respectively. It can be observed that the percentage of anthocyanin increased with the increase of roasting duration for both temperatures (100°C and 150°C). The percentage doubled from 0.529% to 1.074% after roasting for an extra 10 minutes at 100°C and more than doubled when roasted at 150°C (0.590% to 1.327%). The anthocyanin value progressively increases while roasting due to the anthocyanin's resistance to heat (Juhaimi et al., 2018). According to Ko et al. (2020), roasting can cause an increase in the availability of bioactive compounds and nutrients in extracts as it modifies the lignocellulosic structure of plant tissue. This makes it easier for anthocyanin to be extracted. Roasting at 150°C for 20 minutes provided the right amount of thermal treatment to extract the most anthocyanin content from the pomegranate peel. However, roasting at 200°C for 20 minutes, the lowest anthocyanin content in dried pomegranate peels was detected, with a value of only 0.378%. This might be because anthocyanin is sensitive to extremely high temperatures with longer roasting duration. The stability and pigment intensity of anthocyanin could be significantly impacted by temperature as reported by Ursu et al. (2020). It is reported that the anthocyanin and antioxidant activity of purple maize flour extract remained stable below 180°C and started to exhibit thermal degradation when heated at higher temperatures. To add, spray-drying at 160–180°C caused the anthocyanin in glutinous rice powder to seriously degrade (Minh, 2021). Besides, the enzyme polyphenol oxidase may become inactive due to high temperatures, which causes the breakdown of anthocyanins (Romano et al., 2022). It is observed that the highest percentage of anthocyanin content obtained was when the pomegranate peel was roasted for 20 minutes at 150°C yielding 1.33% of anthocyanin content which makes this an optimum roasting temperature and duration in extracting anthocyanin from pomegranate peels.

The ANOVA analysis has shown the measurements are significant ($p < 0.005$) with the R^2 and adjusted R^2 values of 0.932 and 0.903 respectively. A two-way ANOVA revealed that there was a statistically significant interaction between the effects of roasting temperature and duration on the total anthocyanin content ($F_{2,14} = 63.36, p < 0.001$). The analysis also showed a statistically significant difference in the total anthocyanin content by roasting temperature ($F_2 = 22.47, p < 0.001$) and roasting duration ($F_1 = 10.70, p < 0.05$).

Table 1: The percentage of anthocyanin (DW%) in the samples after roasting at different temperatures and durations.

Roasting conditions		Absorbance (Abs)	Percentage content of anthocyanin (DW%)
No roasting (control)		0.996	0.578 ± 0.036
100°C	10 mins	0.912	0.529 ± 0.005
	20 mins	1.851	1.074 ± 0.001
150°C	10 mins	1.016	0.590 ± 0.033
	20 mins	2.287	1.327 ± 0.281
200°C	10 mins	1.310	0.760 ± 0.001
	20 mins	0.652	0.378 ± 0.001

Notes- The values are stated as mean \pm standard deviation. The blank used is acidified methanol and the negative control is the solution mixed with distilled water. DW% is the dry weight percentage.

Phenolic content

Table 2 portrays the total phenolic content (TPC) extracted from the pomegranate peels treated with different roasting conditions. The concentrations were measured based on the gallic acid equivalent.

The concentration of phenolic content for unroasted pomegranate peel was 3.610 mg GA/g DM while for roasted pomegranate peel, the phenolic contents ranged from 3.182 mg GA/g DM to 4.404 mg GA/g DM. There is a significant increase in the concentration of the extracted phenolic content due to roasting activities when compared with the unroasted samples. The TPC can be observed to increase gradually when roasted at 100°C with increasing duration of roasting. However, for temperatures 150°C and 200°C, increasing the roasting duration caused the TPC in pomegranate peel ethanolic extract to decrease. Even though there is a reduction in the TPC after being roasted at 150°C and 200°C, the TPC concentrations were still higher than the unroasted samples (3.610 mg GA/g) except for the roasting condition at 200°C for 20 minutes. The highest concentration of TPC was achieved after roasting at 100°C for 20 minutes (4.404 mg GA/g).

The pattern of increasing TPC due to roasting activity is consistent with other studies. According to Maghsoudlou et al. (2019), the phenolic content of roasted bitter melon fruits was increased by 5.4%, which was attributed to the dissociation of phenolic compounds from the cell matrix. In another study by Ahmed et al. (2021), the increase in phenolic compounds of sesame seeds after being roasted was due to the heat usage during roasting that caused the cellular matrix to degrade and also breaks the covalent bonds between phenolic compounds and the cell wall, which allow the formation of low-molecular-weight and extractable phenolic compounds. Other than that, pomegranate peel has a high amount of hydrolyzable tannins such as ellagic acid. According to Gumienna et al. (2016), ellagic acid has been shown to exhibit good thermal stability over a broad temperature range as its melting point is 350°C. This means that pomegranate peel extracts obtained after roasting may also contain high levels of ellagic acid. The lowest TPC obtained with a concentration of 3.182 mg GA/g was achieved at 200°C for 20 minutes. This can be due to reactions, such as degradation, oxidation, or polymerization of phenolic compounds and the generation of complexes with proteins and carbohydrates during roasting

(Maghsoudlou et al., 2019).

The ANOVA analysis has shown the measurements for total phenolic content were significant ($p < 0.005$) with high R^2 and adjusted R^2 values of 0.978 and 0.969 respectively. A two-way ANOVA revealed that there was a statistically significant interaction between the effects of roasting temperature and roasting duration on the extracted total phenolic content ($F_{2,14} = 78.56, p < 0.001$). The analysis showed that both roasting temperature ($F_2 = 136.6, p < 0.001$) and roasting duration ($F_1 = 102.0, p < 0.001$) did have a statistically significant effect on the total phenolic content.

Table 2. The concentration of phenolic content in the samples after roasting at different temperatures and durations.

Roasting condition		Absorbance (Abs)	Concentration (mg GAE/g DM)
No roasting (control)		2.140	3.610 ± 0.057
100°C	10 mins	2.485	4.225 ± 0.119
	20 mins	2.586	4.404 ± 0.144
150°C	10 mins	2.560	4.358 ± 0.057
	20 mins	2.259	3.822 ± 0.286
200°C	10 mins	2.374	4.027 ± 0.022
	20 mins	1.900	3.182 ± 0.035

Notes- Value stated as mean ± standard deviation. Blank is a solution without extracts and negative control is the solution mixed with distilled water.

Antioxidant activity

According to Table 3, the antioxidant capacity in the unroasted pomegranate peel extract in this study was 15.63% while the contents in the roasted pomegranate peel ranged from 2.60% to 33.86%. The highest antioxidant activity can be observed in the extract that was roasted at 100°C for 20 minutes, which was the same roasting condition that exhibited the highest TPC yield. This can be proven by the study by Mustafa et al. (2021), where the highest total phenolic content obtained from the shell of sweet chestnut extracts after being oven-roasted at 180°C for 25 minutes also yielded the highest antioxidant activity. It can be observed that the antioxidant capacity increased in percentage when comparing the unroasted and the samples roasted at 100°C for 10 and 20 minutes. However, a declining pattern of the antioxidant capacity percentage can be observed when the samples were roasted at 150°C to 200°C. Nonetheless, roasting activity up to 150°C for 20 minutes exhibited a higher antioxidant capacity percentage (26.56%) as compared to the unroasted sample (15.63%). This is consistent with a study by Özcan et al. (2021) where the antioxidant activity in extracts of roasted pomegranate seeds by using either a conventional or microwave oven was higher compared to raw pomegranate seeds extract. The increment could be clarified by the release of phenolic compounds resulting from heat usage and the generation of intermediate products from the Maillard reaction such as melanoidins, that possessed high antioxidant activity, which contributed to the increased antioxidant activity after oven roasting was applied (Ahmed et al., 2021). Besides, novel sugar molecules, such as 5-hydroxymethylfurfural (5-HMF) which is also one of the products obtained from the Maillard reaction, do possess antioxidant properties besides other beneficial health effects such as anti-allergenic and more (Ko et al., 2020).

The ANOVA analysis has shown the measurements for the antioxidant activity are significant ($p < 0.005$) with a very high R^2 and adjusted R^2 values of 0.999 for both. A two-way ANOVA revealed that there was a statistically significant interaction between the effects of roasting temperature and roasting duration on the antioxidant activity of the ethanolic pomegranate peel extract ($F_{2,14} = 501.6, p < 0.001$). The analysis showed that both roasting temperature ($F_2 = 6174.2, p < 0.001$) and roasting duration (F_1

= 398.1, $p < 0.001$) did have a statistically significant effect on the antioxidant activity of the extracts.

Table 3. Antioxidant capacity of unroasted and roasted samples with different temperatures and durations.

Roasting condition		Absorbance (Abs)	Antioxidant capacity (%)
No roasting (control)		0.486	15.63 ± 0.875
100°C	10 mins	0.414	28.13 ± 0.296
	20 mins	0.382	33.68 ± 0.742
150°C	10 mins	0.393	31.77 ± 0.732
	20 mins	0.423	26.56 ± 0.448
200°C	10 mins	0.561	2.6 ± 0.175
	20 mins	0.665	-15.45 ± 0.909

Notes- Value stated as mean ± standard deviation. Blank is methanol and control are DPPH solution.

Conclusion

In conclusion, roasting the pomegranate peels at 100°C for 20 minutes yielded the highest phenolic content of 4.404±0.144 mg GAE/g DM while at 150°C for 20 minutes, yielded the highest anthocyanin content (1.327±0.281%). The same pattern was observed with antioxidant capacity as the measurement increased with the increase of temperature and duration, only to decrease at 150°C for 20 minutes. The highest antioxidant capacity with a value of 33.68±0.742% was achieved when roasting the pomegranate peel sample at 100°C for 20 minutes. Through a two-way ANOVA analysis, it was found that there was a significant interaction between the effects of roasting temperature and roasting duration on the total anthocyanin content, total phenolic content, and the antioxidant activity of the ethanolic pomegranate peel extract. Overall, the application of different roasting conditions does have an impact in enhancing the extractable phenolic compounds in dried pomegranate peels which thereby increases the antioxidant capacity. Thus, this study can provide more insights for future application of roasting activity by applying the same roasting conditions to other fruit peel samples or other phenolic-rich fruit parts such as seeds.

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Author Contribution

NAM Zamrin – conceptualization, data curation, writing – original draft; SM Abu Bakar – conceptualization, data curation; MA Ibrahim – supervision, writing – review & editing.

Conflict of Interest

The authors declare there is no conflict of interest.

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