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Optimization of Parameters for Anthocyanin Extraction from Pomegranate (*Punica granatum* L.) Peel

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ABSTRACT

Background: Anthocyanin is a natural non-toxic, water soluble and heat sensitive colouring pigment which is abundantly found in nature. Standardization of simple and viable technology for anthocyanin extraction from processing waste can replace the synthetic food colourants. Study on standardisation of dehydration method and solvent for anthocyanin extraction from pomegranate (*Punica granatum* L.) peel was carried out using maceration.

Methods: Ripe pomegranate fruits (var. kabuli) of good quality and uniform maturity were cleaned, surface sanitized using 2 ppm ozonised water for 10 minutes and outer peel was separated from the arils and mesocarp. The peels were cut into uniform pieces of approximate 2cm^3 and subjected to different dehydration treatments. Best dehydration treatment was selected based on highest anthocyanin content. The peel after dehydrating with the best method was macerated using three different solvents for selection of best solvent for extraction based on highest anthocyanin content.

Result: Dehydration resulted in enhanced anthocyanin content. Cabinet drying of peel pieces at 50±5°C for 24 hrs followed by maceration using acidified ethanol with 1% HCl at 2:1 solvent to solid ratio can yield 25.5% colour extract with high anthocyanin content (84.57 mg 100 g^{-1}) and antioxidant activity (82.83%).

Key words: Anthocyanin, Colour extraction, Maceration *Punica granatum,* Waste-utilization.

INTRODUCTION

Colour is an important appealing attribute of fresh or processed products. However, during food processing often colour degradation occurs and hence the colourants are added to replace the lost colour in food. Today, natural colourant is consumer's selection because it can be used to replace synthetic colourants which have many side effects. One of the natural colourants is anthocyanin. Anthocyanin can display a great diversity of colours from orange to blue depending on their chemical structure and environment (Santos-Buelga *et al*., 2014). Besides the colour attributes, interest in anthocyanins has intensified because of their possible health benefits and in recent years, anthocyanins have emerged out as one the most promising ingredients for food industry because of their colour and other functional properties. Anthocyanins are widely distributed in nature and present in flowers, fruits, vegetables, cereals and leaves (Khoo *et al.,* 2017). Anthocyanins recovered from food waste have high potential in being used in different food and biotechnological applications, *e.g.*, as food supplements, nutraceuticals and as food additives. In this study, peel of pomegranate (*Punica granatum* L.) comprised of about 50% of fruit weight, which is usually discarded as waste from processing industries and create environmental pollution (Nair *et al*., 2018) is used as source of anthocyanin.

The extraction method often used for heat sensitive pigment like anthocyanin is maceration, which is the easiest method, where the samples are soaked in a solvent at room temperature for a period of 2-7 days with frequent agitation until the soluble matter is dissolved. Since anthocyanins are not stable in neutral or alkaline solutions, acidic aqueous solvents are used as extraction solvents in order to disrupt cell membranes and to dissolve the water-soluble pigments. Naresh (2016) reported highest anthocyanin content (61.07 mg 100 g^{-1}) for acidified solvent extraction method (20% ethanol + 0.5% citric acid) of anthocyanin pigment from Jamun. Flesh and peels of pomegranate and grape were extracted independently using 100% ethyl alcohol using cold solvent extraction method adopting 1:1 and 1:2 w/v solid to solvent ratio for 24 and 48 hrs. Per cent yield and pigment content increased with increased duration of extraction and solid to solvent ratio (Mini *et al.,* 2021).

Achieving sustainable processing methods depends mostly on the incorporation of relevant processes before, during and after extraction, that may allow recovery of the desired biomaterials within an environmentally friendly processing system. The appropriate integration of pre-processing with processing potentially increases the overall extraction efficiency in a sustainable manner. Drying is a pre-processing method which is ideally incorporated into

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the processing flow, at the preliminary stage prior to the actual extraction process and helps in the overall extraction efficiency and quality of the final product.

It is necessary to have efficient and fast extraction procedures that provide higher yield, preserving the nutritive and bioactive compounds for heat sensitive pigments like anthocyanin. The present study was conducted during 2022-2023 to find out the best dehydration treatment and solvent to extract anthocyanin from pomegranate (*Punica granatum* L.) peels, which are usually discarded as waste in processing industry, through the maceration process.

MATERIALS AND METHODS

Good quality pomegranate fruits (cv. Kabuli) of uniform maturity were purchased from Swasraya Karshaka Vipani of VFPCK, Trivandrum, cleaned, sanitized using 2 ppm ozonized water for 10 minutes. Peel was removed from the mesocarp and arils and cut into uniform pieces of approximately 2 cm^3 size.

The experiment was carried out in two continuous parts. In the first part of the study, the peel pieces were subjected to the four different dehydration treatments *viz.*, cabinet drying at 50±5°C for 24 hrs (D_1), shade drying for 24 hrs (D_2), cabinet drying at 50± 5°C for 1 hr followed by shade drying for 24 hrs (D_3), drying in blancher-cum drier at 50± 5°C for 24 hrs (D_4) and compared with fresh peel pieces without drying as control (D_5). Dried pomegranate peels were analysed with 4 replication for percent yield, moisture content (%), total anthocyanin content (mg100 g^{-1}) and total anti-oxidant activity (%) along with fresh control sample to select the best dehydration treatment. Weight of the peel was recorded before and after drying on an electronic balance and yield was calculated as percentage. Moisture content of the dehydrated peel was determined using moisture analyser which dries the sample using a halogen lamp and gives the moisture content based on the principle of thermo-gravimetric analysis.

In the second part of the study, the peel pieces were dried using the best dehydration treatment selected from first part of the experiment, crushed and macerated using three different solvents *viz.*, acidified ethanol (1% HCl) (S_1), acidified methanol (1% HCl) (S_2) and 50% ethanol + 0.2% citric acid (S_3) in 2:1 liquid to solid ratio for 48 hours under ambient temperature (30-35°C and 75-80% RH). The infusion mixture was filtered after specified time and evaporated under water bath at 60°C for complete removal of solvent. The collected extracts were analysed in 6 replications and compared for yield, time taken for extraction (hr), total anthocyanin content and total anti-oxidant activity to select the best solvent for anthocyanin extraction.

Total anthocyanin content (mg 100 g^{-1}) of dehydrated peel and the extracts was estimated by the method described by Abdel-Aal *et al.* (2006). The total antioxidant activity (%) of dehydrated peels and extracts was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as per the procedure described by Sharma and Bhat (2009). The data generated were analysed statistically using completely randomized design (CRD) and significance was tested using analysis of variance.

RESULTS AND DISCUSSION

Effect of dehydration in anthocyanin content

The use of suitable drying technique helps in designing a competing extraction system. When different dehydration methods were compared for various physico-chemical parameters, there was significant difference between percent yield of pomegranate peel (Table 1). The yield of 24 hr shade dried peel (D2) was highest (76.15%) due to retention of highest moisture content (35.98%) (Table 1). When the peels were cabinet dried at 50±5°C for 24 hrs followed by shade drying, the moisture content was reduced (31.98%), with a reduced yield (56.15%). When the peels were dehydrated using cabinet drier (D1) and blancher-cum-drier (D4), the moisture content was further reduced (13.3% and 16.88% respectively) and as a result the yields were reduced to 26.15% and 19.98% respectively. Moisture content of all the dried or dehydrated peels were lower compared to fresh peels with 59.16% moisture. Drying comprises heat movement from the energy source to the interior of food and mass transfer of moisture from inside to the outer surface of the food, which is subsequently evaporated in the air to facilitate the reduction in the food moisture content to a certain proportion (Ndukwu *et al.*, 2017). Hence, there exists a negative correlation between moisture content and yield.

All the dehydration techniques resulted in enhanced anthocyanin content of peel (Fig 1). This is in accordance with the findings of Safarzadeh-Markhali (2021) who had

Table 1: Effect of dehydration methods on peel yield (%) and moisture content (%).

Dehydration methods	Yield (%)	Moisture content (%)
D_1 - Cabinet drying at 50± 5°C for 24 hrs	26.15	13.30
D_2 - Shade drying for 24 hrs	76.15	35.98
D_3 - Cabinet drying at 50± 5°C for 1 hr followed by shade drying for 24 hrs	56.15	31.98
D_4 -Drying in blancher-cum drier at 50± 5°C for 24 hrs	19.98	16.88
D_5 - Control (Fresh sample without drying)	-	59.16
SEm±	0.56	0.39
CD (0.05)	1.72	1.20

reported that pre-processing methods including drying influenced the overall extraction of phenolics from olive (*Olea europaea*). Fresh peels had lowest anthocyanin content (6.70 mg 100 g^{-1}) only, due to the high moisture content. Charmongkolpradit *et al.* (2021) reported highest anthocyanin content in purple corn kernel at the reduced moisture content of 13% and stated that total anthocyanin content decreased by increasing moisture content. Even with simple traditional shade drying, anthocyanin content was increased to 10.10 mg $100g^{-1}$. When the peels were cabinet dried before shade drying, anthocyanin content was further increased to 17.37 mg $100g^{-1}$. When the samples were dehydrated completely using specially designed machines, anthocyanin content was very high compared to that in fresh samples and the highest anthocyanin content (55.91 mg $100g^{-1}$) was recorded in cabinet dried peel sample. Compared to other drying modes, cabinet drying removes moisture quickly and effectively due to continual drying under controlled temperature conditions resulting in highest total anthocyanin concentration and absence of intermittent drying cycles and changing temperatures. The total antioxidant content was highest (63.70%) in fresh peel (Fig 2). Hamid (2019) reported maximum antioxidant content in fresh peel due to the comparative advantage of low temperature treatment leading to the better stability. Availability and feasibility of drying equipment, drying circumstances and drying efficiency affect the choice of the best drying technique (Sagar and Kumar, 2010). The proper and successful drying can result in product with good flavor, texture and nutritional properties, especially antioxidant compounds and thus preserve the bioactive compounds (Charmongkolpradit *et al*., 2021). Cabinet drying at 50± 5°C for 24 hours was chosen as the best dehydration method considering the yield, retention of anthocyanin content and antioxidant activity.

Standardisation of solvent for anthocyanin extraction

The major factors affecting the anthocyanin extraction are

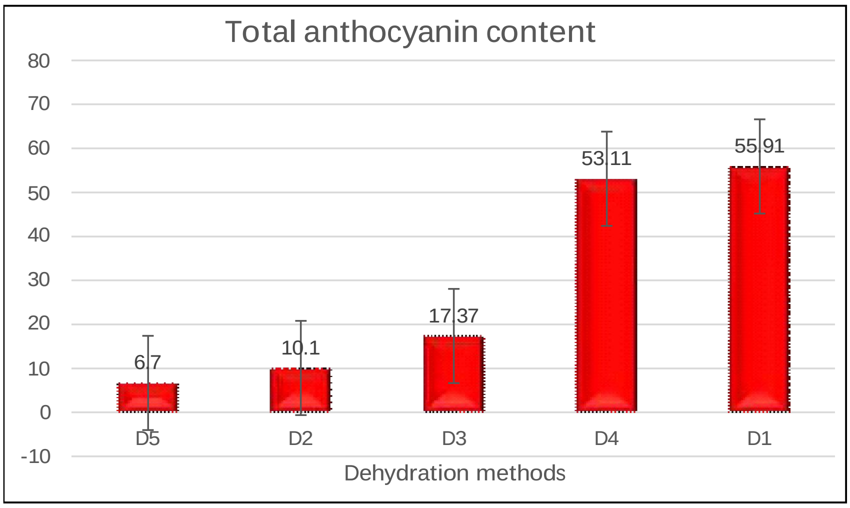
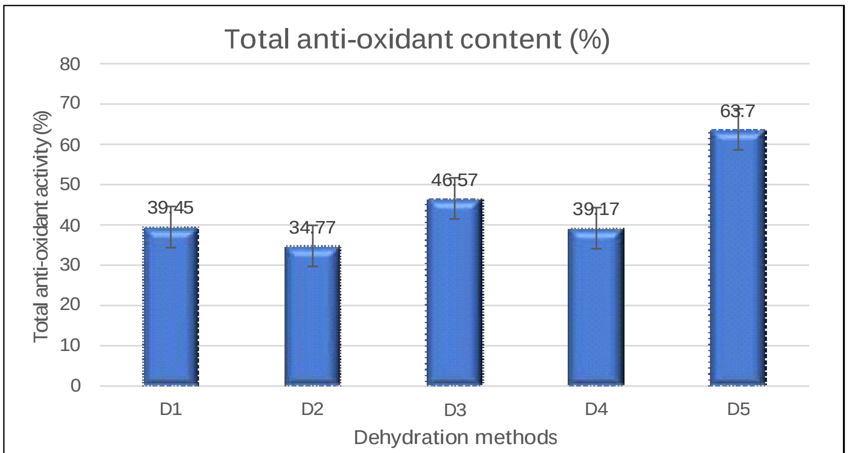


Fig 1: Total anthocyanin content as influenced by various dehydration methods.



D_1- Cabinet drying (50± 5°C) for 24 hrs. D_2- Shade drying for 24 hrs. D3- Cabinet drying (50± 5°C) for 1 hr followed by shade drying for 24 hrs. D_4- Drying in blancher-cum drier (50± 5°C) for 24 hrs. D_5- Control (Fresh sample without drying).

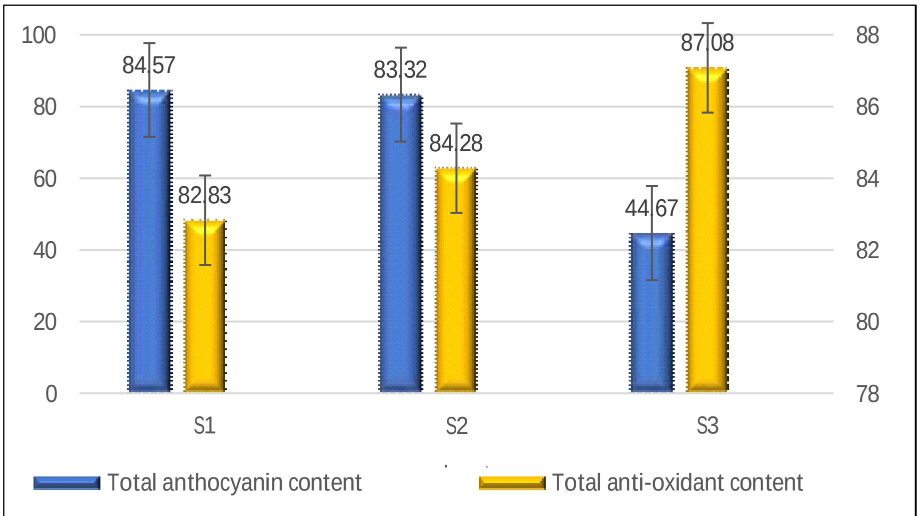
Fig 2: Effect of dehydration methods on total anti-oxidant activity of peels.

characteristics of the sample matrix *viz.*, water activity, rigidity of plant cell wall and extraction process parameters *viz.*, pH, solvent, temperature, time *etc.* Hence it is very important to select an appropriate solvent for anthocyanin extraction. The pomegranate peel pieces were dried using cabinet drier at 50±5°C for 24 hrs, the best dehydration treatment selected from the first part of the experiment, crushed and macerated with different solvents. The collected anthocyanin extracts were analysed and compared for chemical parameters to select the best solvent. Table (2) shows the effect of different solvents on extract yield and time taken for extraction. The highest yield (25.5%) was observed when acidified ethanol (S_2) with 1% HCl was used as the solvent and the least extract yield (19.08 %) and time taken for extraction (0.805) were observed when 50% ethanol + 0.2% citric acid was used as solvent (S_3) (Table 2.).

Anti-oxidant activity of peel extract was highest (87.08%) when 50% ethanol + 0.2% citric acid was used as solvent (Fig 3). Hosseini *et al*. (2016) reported that citric acid solution has the greatest DPPH radical scavenging activity and is one of the finest solvents for phenolic and anthocyanin extraction. Citric acid is a weak organic acid that is nutritionally safe. Organic acids work to prevent the pigment from decomposing during extraction by not removing the acyl group linked to sugar Shetty *et al*. (2017). Hikmawanti *et al*. (2021) studied the effect of ethanol concentration of 50%, 70% and 96% as the extraction solvent on antioxidant activity of star gooseberry (*Sauropus androgynus)* leaf extracts and reported that the best antioxidant was extracted using 50% ethanol assolvent. But in the present experiment, the anthocyanin content of the extract was least (44.67 mg $100g^{-1}$) when the solvent used was 50% ethanol + 0.2% citric acid. As the prime objective of the experiment was to select the best solvent for anthocyanin extraction, this was not selected as the best solvent. The anthocyanin content (84.57 mg 100 g^{-1}) was highest in extract obtained using HCl acidified ethanol (Fig 3). This is in accordance with the findings of Manjula *et al.* (2022) who had reported highest acidity and anthocyanin content in treatment of 1.5 N ethanol in large scale extraction of bicolour from roselle calyces (*Hibiscus sabdariffa)*. As ethanol is a polar solvent with a high dielectric constant and dipole moment, it is useful in extracting other polar chemicals. HCl acidified ethanol was selected as the solvent for anthocyanin extraction as it also fulfils food safety requirements as well as allows for good anthocyanin stability when subjected to pH, heat and light (Sipahli *et al.,* 2017). By adding acid to ethanol, anthocyanins are extracted more effectively. Silva *et al*. (2017) reported that there was no significant difference in anthocyanin extraction from

Table 2: Extract yield and time taken for extraction as influenced by different solvents.

Solvents	Extract yield (%)	Time taken for extraction (hr)
S_1-Acidified ethanol (1% HCl)	25.50	1.50
S_2-Acidified methanol (1% HCl)	22.17	1.68
S_3-50% ethanol + 0.2% citric acid	19.08	0.80
SEm±	0.43	0.07
C.D (0.05)	1.30	0.23



S_1-Acidified ethanol (1% HCl), S_2-Acidified ethano (1% HCl), S_3-50% ethanol + 0.2% citric acid.

Fig 3: Effect of solvents on anthocyanin and anti-oxidant activity.

blueberry (*Vaccinium corymbosum*) using methanol and ethanol as solvents, but ethanol was chosen as the best solvent taking into account both the toxicity of methanol and the goal of producing food grade extracts.

CONCLUSION

The study was conducted with the objective to standardize dehydration method and solvent for anthocyanin extraction from pomegranate fruit peel using maceration. All the dehydration techniques had resulted in enhanced anthocyanin content. The yield of the dehydrated pomegranate peel was highest (76.15%) when the peel was shade dried for 24 hrs. The moisture content was least (13.30%) and total anthocyanin content (55.91 mg $100g^{-1}$) was highest in cabinet dried sample at 50± 5°C for 24 hrs and hence selected as the best dehydration treatment. Extraction using acidified ethanol with 1% HCl had recorded highest yield (25.5%) and anthocyanin content (84.57 mg $100g^{-1}$) along with comparatively lesser time for extraction (1.50 hr) and antioxidant activity (82.83%). The achievement of sustainable extraction depends on the integration of associated factors before, during and after extraction processing that may enable recovery of target biomaterials within an eco-friendly processing system.

Conflict of interest

All authors declare that they have no conflicts of interest.

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