

Comparative analysis of peel extract and juice obtained from wild and cultivated pomegranate fruits

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Introduction

Pomegranate (*Punica granatum* L.) is a deciduous shrub or a small tree with perennial rootstock, belonging to the *Punicaceae* family (Holland et al., 2009). The edible part of pomegranate is about 57-85% of the whole fruit, among which juice accounts for 36-63% (Ge et al., 2021). This would mean that 25-43% of the fruit consists of peel and seeds.

Pomegranate is rich in bioactive compounds, both in the edible part and in the part that represents waste material. Previous findings have shown that pomegranate peel (PP) contains ellagic acid, ellagitannins such as punicalagin and punicalin, as well as proanthocyanidins and flavonoids (Jain et al., 2012). These secondary metabolites of pomegranate possess substantial health-promoting properties, including antidiabetic, antioxidant, immunomodulatory as well as anticarcinogenic effects (Jain et al., 2012). In addition, some compounds were found to have potential to prevent cardiovascular diseases (Hamoud et al., 2014).

In this research, the active components from pomegranate peel (wild and cultivated) were isolated using maceration as an extraction technique. Thereafter, the quantification of the most dominant compounds in the obtained extracts and juice was performed using HPLC analysis. The objective of this study was to determine and compare the content of the main bioactive compounds obtained from the peel and juice of wild and cultivated pomegranates from different locations.

Materials and methods

Plant materials

The ripe fruits of 10 wild and cultivated pomegranate plants were collected from three different localities in Montenegro (Daljam, Martinici, and Gornji Kokot) in November 2019. Peels were manually separated from the seeds and cut into small pieces. Prepared material was air-dried at room temperature for 4-6 days and grounded using a laboratory mill to obtain PP pulvis.

Preparation of samples

Maceration process was employed for the extraction (1:10, w/v) of PP pulvis (200 g) with 70% ethanol. The experiment was performed at room temperature with continuous stirring (100 rpm) during 24h. Additionally, fresh fruits were used to prepare juice using a pomegranate juicer.

HPLC analysis

The HPLC method was used for the identification and quantification of bioactive compounds in the pomegranate peel extracts and juice of both wild and cultivated pomegranates. Analysis was performed on Agilent Technologies 1200 Series (Agilent, Waldbronn, Germany), equipped with a DAD detector. Reverse phase Zorbax SB-C18 (Agilent) analytical column (150 mm ×

4.6 mm), with particle size 5 µm, was used for chromatographic analysis at 25°C. The mobile phase consisted of solvent A (1% v/v solution of orthophosphoric acid in water) and solvent B (acetonitrile). The following scheme was applied for gradient elution: 0-5 min, 98-90% A; 5-15 min, 90% A; 15-20 min, 90-85% A; 20-25 min, 85-70% A; 25-30 min, 70-40% A; 30-34 min, 40-0% A. The injection volume was 3 µL, while the flow rate was set to 1 mL/min. The UV detection was carried out at 260, 320 and 520 nm. The individual components were quantified using reference standards, and the results are presented as milligrams per gram of dry extract (mg/g) for PP powder as well as milligrams per liter (mg/L) for juice.

Results and discussion

In this study, individual phenolic compounds were quantified from PP pulvis extract and juice. Totally, seven compounds were found in the juice of fresh fruits, among which four were anthocyanins (delphinidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3,5-diglucoside and cyanidin 3-glucoside). Results obtained from HPLC analysis revealed that cyanidin 3-glycoside was dominant in both, wild (287.1-592.9 mg/L) and cultivated (124.9-220.7 mg/L) pomegranate juice samples of pomegranate. Moreover, the average value of total anthocyanins calculated by HPLC in cultivated pomegranate populations (232.7 mg/L juice) was significantly lower compared to wild populations (531.9 mg/L juice).

The ellagitannins were identified to be predominant phenolic compounds in all studied samples, including juice and peel extract. Among them, the most abundant in the juice were punicalin and punicalagin. The content of punicalin in the juice was ranging from 7.4 to 38.2 mg/L as well as 8.1-904.0 mg/L in cultivated and wild pomegranates, respectively. Similarly, higher levels of punicalagin were observed in juice of wild populations containing in average 94.6 mg/L, while the average value for cultivated populations was 31.9 mg/L. Considering the content of ellagitannins in PP pulvis extract, it could be concluded that punicalagin was the most dominant compound varying from 19.8 to 33.1 mg/g dry weight (DW) of the peel in cultivated pomegranates, while slightly higher values were observed for wild populations (17.5-49.7 mg/g DW). Gullon et al. (2016) also found that punicalagin is the most dominant in PP pulvis. Furthermore, peel extract of wild pomegranates was also

found to have higher levels of punicalin (2.3-7.3 mg/g DW) and ellagic acid (1.7-3.4 mg/g DW) compared to cultivated pomegranates (punicalin: 1.6-2.2; ellagic acid: 1.1-2.3). By analyzing the results of the quantified 7 compounds from the PP pulvis and juice, higher concentrations of all compounds were observed in the products obtained from wild species.

Conclusion

Based on the obtained results it could be concluded that wild pomegranate contains a higher content of bioactive compounds than cultivated ones, which is in accordance with previous studies (Singh et al., 2019). Considering the significant difference in the phytochemical composition of wild and cultivated pomegranate fruit, in order to cultivate the highest quality raw materials, it is necessary to determine which factors affect the synthesis of the mentioned secondary metabolites.

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