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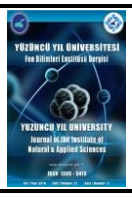

Research Article

Effect of Different Drying Processes on Antioxidant and Antidiabetic Properties of Pomegranate Press Wastes

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Abstract: Today, with the understanding of the favourable effects of fruit consumption on health, the consumption of pomegranate has increased gradually, and solution methods were sought to evaluate the peel and seed parts that emerge after the fruit is sorted. In this study, the effects of different drying processes (microwave, drying oven, and freeze-drying) and different extraction solvents (ethanol and methanol) on the antioxidant and antidiabetic activities of pomegranate press wastes were investigated. While the highest total phenolic content (TPC) was in freeze-dried methanolic extracts (5758.70 mg GAE 100 g^{-1}), the highest total flavonoid content (TFC) and total monomeric anthocyanin (MA) contents were determined in microwave-dried ethanolic extracts with values of 1068.75 mg QE 100 g^{-1} dw and 215.44 mg cyn-3-glu 100 g^{-1}, respectively. All samples had higher α-glucosidase inhibitory activity compared to acarbose. The results showed that pomegranate press waste, which is thought to have no use in the food industry, is a potential resource that can be used in the pharmacology and cosmetics industries as well as its use as a food supplement; thanks to its high antioxidant and antidiabetic properties.

Farklı Kurutma İşlemlerinin Nar Pres Atıklarının Antioksidan ve Antidiyabetik Özellikleri Üzerine Etkisi

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Anahtar Kelimeler
Antidiyabetik aktivite,
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Nar,
Pres atığı

Öz: Günümüzde meyve tüketiminin sağlığa faydalı etkilerinin anlaşılmasıyla birlikte nar tüketimi giderek artmış ve meyvenin ayıklanması sonrasında ortaya çıkan kabuk ve çekirdek kısımlarının değerlendirilmesi amacıyla çözüm yöntemleri aranmaya başlanmıştır. Bu çalışmada, farklı kurutma yöntemlerinin (mikrodalga, etüv ve dondurarak kurutma) ve farklı ekstraksiyon solventlerinin (etanol ve metanol) nar pres atıklarının antioksidan ve antidiyabetik aktivitesi üzerine etkileri incelenmiştir. En yüksek toplam fenolik madde içeriği (TPC) dondurarak kurutulmuş metanolik ekstraktlarda (5758.70 mg GAE/100 g), en yüksek toplam flavonoid madde içeriği (TFC) ve toplam monomerik antosiyanin (MA) içerikleri mikrodalgada kurutulmuş etanolik ekstraktlarda sırasıyla 1068.75 mg QE/100 g dw ve 215.44 mg cyn-3-glu/100 g olarak belirlenmiştir. Tüm örnekler akarboza kıyasla daha yüksek α-glukozidaz inhibitör aktiviteye sahipti. Sonuçlar, gıda endüstrisinde kullanım imkânı bulunmadığı düşünülen nar pres atıklarının yüksek antioksidan ve antidiyabetik özellikleri sayesinde gıda takviyesi olarak kullanımının yanı sıra farmakoloji ve kozmetik endüstrilerinde de kullanılabilecek potansiyel bir kaynak olduğunu göstermiştir.

1. Introduction

Pomegranate (*Punica granatum* L.), one of the earliest known edible fruits, is a type of fruit rich in polyphenols that can be grown in droughty and semi-arid climates (Wetzstein et al., 2011). Pomegranate press waste is a by-product that occurs in large quantities during the production phase, especially in fruit juice factories (Kalaycıoğlu & Erim, 2017). Waste pomegranate seeds obtained after pressing the fruit constitute 10% of the fruit used in production (Abbasi et al., 2008). In some studies, it was determined that apart from the edible parts of the fruit, the bark, roots and leaf extracts of the tree also have therapeutic properties (Naqvi et al., 1991). These beneficial therapeutic effects of pomegranate are due to bioactive components such as ellagitannins, ellagic acid, flavonoids, and anthocyanins in their structure (Jurenka, 2008). Today, the peel and seeds of the pomegranate are generally used as animal feed, and no economic gain can be achieved by processing them into products with high commercial value. However, by-products that emerge during the production phase of the food industry have the potential to be used in important fields such as medicine and pharmacy, thanks to their high antioxidant activity and bioactive components (Amyrgialaki et al., 2014; Okumuş & Bakkalbaşı, 2021).

The polarities of the hydroxyl groups in the structure of phenolic compounds are quite variable due to their ability to bind with sugars, acids, or alkyl groups. Therefore, it is tough to develop a single solvent and process for the ideal extraction of whole phenolic components (Mokrani & Madani, 2016). The drying process removes moisture from the product by evaporation to a specific value. Although drying techniques are very diverse, conventional air and freeze-drying are the most widely applied methods in the food industry (Tsami et al., 1998). Conventional air drying is one of the most common methods of drying food products. In this process, high-temperature value and long-time performance are required. In addition, the microwave drying method is also widely used to minimize the decrease in the quality of foods and to provide fast and effective heat distribution in the product (Díaz et al., 2003). It is known that drying processes applied to foods cause changes in the number of bioactive components in the structure of the products and their antioxidant activity (Leong & Oey, 2012). However, more scientific studies are needed to prove the changes in the bioactive compositions of the products applied in different drying processes.

Diabetes (Diabetes Mellitus) is one of the most critical and common chronic illnesses of our age, which shortens life expectancy, causes disability, and can result in death. In recent years, the beneficial effects of phytochemicals on health have been revealed in scientific studies, and the use of plants as nutraceuticals has become increasingly widespread (Andlauer & Furst, 2003; Arshadi et al., 2015).

This study aims to determine the effects of different drying processes (microwave, dry-oven, and freeze-drying) and solvents (ethanol and methanol) on the bioactive components, antioxidant, and antidiabetic properties of pomegranate press wastes. It is thought that the results will show guiding features in using and evaluating pomegranate waste.

2. Material and Methods

2.1. Materials

Hicaz variety pomegranate press waste used in the study belongs to the 2021 harvest year, and it was obtained from Aroma Gıda Ind. Trade Co. Ltd., Bursa, Turkey. 50% of the press waste was the seeds, and 50% was the endocarp parts of the pomegranate. Pomegranate press wastes dried in the microwave oven (Arçelik, MD 554), dry oven (Şimşek Laboratory), and lyophilizer (Labconco freeze dryer 117) were ground and kept at -24 °C for further analysis.

2.2. Standards and chemicals

Gallic acid (purity ≥99%), aluminium chloride (purity ≥99.99%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothia-zoline-6-sulfonic acid) (ABTS), α-glucosidase, α-amylase, dinitrosalicylic acid and 4-nitrophenyl α-D-glucopyranoside were obtained from Sigma (St. Louis, MO, USA). Ethanol, methanol, and sodium carbonate (Na_2CO_3) were procured from Merck (Darmstadt, Germany). Acarbose (tablets Glucobay®, Turkey) was purchased from a local pharmacy.

2.3. Methods

2.3.1. Drying procedure

The drying process of pomegranate press waste in a microwave oven (MW) was applied at 600 Watt for 5 minutes. The drying process in the oven (DO) was carried out at 70 °C for 48 hours, and a lyophilizer (FD) for drying was used at -54 °C and 0.045 mbar pressure for 72 hours. It was found that the moisture of the press wastes from all drying processes was below 2%.

2.3.2. Extraction

Approximately 2.5 g of pomegranate press waste powder was suspended in 10 mL of solvent (ethanol or methanol) and stirred for 2 hours at 250 rpm at room temperature. Then, it was centrifuged at 10 000 rpm for 10 minutes (Hettich Universal 320r, Germany). The same extraction procedure was applied a second time to the insoluble residue. At the end of the process, the combined supernatants were brought to a total volume of 25 mL with the appropriate solvent and filtered with filter paper (Whatman No. 1) (Köse, 2018). The filtrate was stored at -24°C and in amber containers until further analysis.

2.3.3. Colour values

The samples' L*, a*, and b* values were determined using the Konica Minolta CR 400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) (AOAC, 2006).

2.3.4. Total phenolic and total flavonoid content

Total phenolic content (TPC) analysis was performed according to Singleton & Rossi (1965). Results were expressed as gallic acid equivalent (mg GAE 100 g^{-1}). Total flavonoid content (TFC) was determined by the $AlCI_3$ method (Zhishen et al.,1999) and results were specified as quercetin equivalents (mg QE 100 g^{-1}).

2.3.5. Total monomeric anthocyanin

Total monomeric anthocyanin (MA) was determined at 528 nm and 700 nm according to the method developed by Giusti & Wrolstad (2001). Results were calculated as mg cyn-3-glu 100 g^{-1}.

2.3.6. Antioxidant capacity-DPPH and ABTS

DPPH analysis was performed using the method Pyo et al. (2004) described, and the results were expressed as % inhibition. The ABTS assay was carried out according to Re et al. (1999).

2.3.7. Antidiabetic activity

The α-amylase and α-glucosidase inhibition activity of the samples were analyzed as described previously by Okumuş & Bakkalbaşı (2021). The analysis method was also repeated for acarbose used as a standard. The results were calculated graphically and expressed as IC_{50} in mg mL^{-1}.

2.3.8. Statistical analysis

Results were given as mean and standard deviation ($\bar{X}\pm SD$). Significant differences between groups were determined one-way analysis of variance and Duncan's multiple range test. $p<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Colour values

Colour is an essential parameter in the consumer's appreciation and demand for the products. It is necessary to closely follow the colour changes that occur in the product after the applied process to obtain a good product. The measured L*, a*, and b* values of various dried pomegranate press wastes are given in Table 1. It was determined that the press wastes dried in the lyophilizer had the highest L* and a* values. The difference between the L*, a*, and b* values of the samples dried in the microwave and the drying oven was insignificant ($p>0.05$). The least change in the colour of the samples compared to the initial values (mean values for L*, a*, and b* are 59.45, 14.27, and 10.33, respectively, not given in the table) were detected in the lyophilized samples. Mphahlele et al. (2016) determined the L* and a* value in freeze-dried pomegranate peels as 61.46 and 23.33, respectively. In the same study, the L* and a* values of the pomegranate peels dried in an oven at 60 °C were found to be 42.04 and 25.24, respectively. Differences in pomegranate variety, harvest year, maturity level (Okumuş & Bakkalbaşı, 2021), storage conditions, and drying methods can affect colour results.

Table 1. Colour values of pomegranate press wastes after different drying methods

	Colour values		
	*L**	*a**	*b**
MW	55.94±0.70^{a}	9.40±0.46^{a}	11.56±0.08^{b}
DO	55.76±0.36^{a}	9.28±0.39^{a}	11.96±0.36^{b}
FD	61.15±0.42^{b}	13.68±0.05^{b}	9.55±0.19^{a}

^{a,b} indicate the differences ($p<0.05$) between all applications.

3.2. Antioxidant activity

Results of TPC, TFC, monomeric anthocyanin, and antioxidant activity values after drying in pomegranate press wastes are given in Table 2. The highest TPC in the samples was measured as 5758.70 mg GAE 100 g^{-1} in samples dried by freeze dryer and using methanol as solvent. It was concluded that the type of solvent used and the drying method significantly affected the results ($p<0.05$). On the other hand, the highest TFC in the samples was determined in the press wastes subjected to ethanolic extraction in the microwave drying process ($p<0.05$). Press wastes dried in a drying oven in methanolic extraction had higher TFC content than samples dried with microwave and lyophilizer ($p>0.05$). In terms of TFC results, it was determined that while the difference between samples was significant in ethanolic extraction, it was not significant in methanolic extraction.

Table 2. TPC, TFC, MA contents (dw) and antioxidant activity of pomegranate press wastes after different drying methods

		TPC (mg GAE 100 g^{-1})	**TFC (mg QE 100 g^{-1})**	**MA (mg cyn-3-glu 100 g^{-1})**	**DPPH (%)**	**ABTS (mmol Trolox g^{-1})**
Ethanol	MW	4780.43±15.37^{dC}	1068.75±8.84^{eC}	215.44±2.63^{dB}	92.79±0.24^{dB}	4.82±0.32^{aA}
	DO	3432.61±30.74^{aA}	854.38 ±11.49^{cA}	5.24±2.27^{aA}	90.39±0.00^{cA}	8.72±0.27^{bC}
	FD	4073.91±0.00^{bB}	941.25±10.61 ^{dB}	3.42±2.73^{aA}	99.14±0.00^{eC}	6.66±0.11^{abB}
Methanol	MW	4361.96±23.06^{cA}	265.00±1.77^{abA}	72.72±3.18^{cB}	92.80±0.00^{dC}	15.97±1.38^{dAB}
	DO	5296.74±7.69^{eB}	282.50±12.37^{bA}	15.22 ±1.62^{bA}	86.62±0.00^{bB}	13.27±0.05^{cA}
	FD	5758.70±0.00^{fC}	258.13±7.95^{aA}	17.21±0.93^{bA}	78.64±0.36^{aA}	18.20±2.27^{dB}

^{a,b,c,c,d,e,f} indicate the differences ($p<0.05$) between all applications.

$^{A, B, C}$ indicate the differences ($p<0.05$) between application groups.

The highest monomeric anthocyanin content in the samples was detected in the microwave-dried samples in both solvents. In addition, samples dried in a microwave in ethanolic extraction had the highest MA value among all treatments with 215.44 mg cyn-3-glu 100 g^{-1} ($p<0.05$). The difference between the MA contents of the samples dried in a drying oven and lyophilizer in both solvents was not statistically significant ($p>0.05$).

The highest DPPH value of 99.14% was determined in the ethanolic extracts from press wastes dried in a lyophilizer ($p<0.05$). However, the lowest % inhibition value was found in the samples dried in a lyophilizer and applied methanolic extraction. It was determined that the applied drying process and the solvent used in the extraction significantly affected the % inhibition results. Contrary to the DPPH antioxidant activity results, the highest ABTS antioxidant activity value was 18.20 mmol Trolox g^{-1} in lyophilized and methanolic extract applied samples. In addition, methanolic extracts obtained from all drying processes had higher ABTS antioxidant activity values than ethanol ($p<0.05$).

Thermal treatments applied to foods generally cause losses in polyphenols (Kaur & Kapoor, 2001). Asami et al. (2003) reported that freeze-drying preserves phenolic components better than hot air drying. However, it was reported that free radicals formed during the oxidation reaction might be associated with antioxidant capacity (López et al., 2013). Karaman et al. (2014) found higher TPC in freeze-dried samples due to limited chemical and thermal degradation as it was carried out at low temperatures. Moreover, some studies showed that the high drying temperature in the oven drying process increases the radical scavenging activity (Lee Mei Ling et al., 2013; Rodriguez et al., 2014). Similar to the literature results, in the current study determined the highest TFC and MA values in ethanolic press wastes dried with a microwave oven. In addition, high antioxidant activity values were detected in the samples dried with a lyophilizer. The difference between the results and the literature may be due to differences in the picking time of the samples, drying method, and extraction process (Tian et al., 2020). Also, the different total phenolic content from different solvents may be due to the polarity of the solvents and the varying solubility of phenolic components (Gao et al., 2014).

3.3. Antidiabetic activity of pomegranate wastes

In diabetes treatments, reducing the blood glucose level after meals is generally preferred in combination with other treatment methods. For this purpose, commercial medicine such as acarbose inhibits enzymes and slows the hydrolysis of complex carbohydrates (starch, glycogen, etc.) in the intestinal tract. Nevertheless, patients often suffer from various side effects of such drugs. Because of these complaints and considering that it is a traditional, complementary treatment, they turn to medicinal plant-based natural pharmaceuticals (Temiz, 2021). Phenolic compounds, with their natural α-amylase and α-glucosidase inhibitor properties, cause a decrease in glucose absorption rate by delaying/prolonging the carbohydrate digestion time, and as a result, they reduce the postprandial blood glucose increase. The IC_{50} values of the antidiabetic activities are given in Table 3.

Table 3. α-amylase and α-glucosidase inhibitory activities of pomegranate press wastes

		IC_{50}(α-amylase) (mg mL^{-1})	**IC_{50}(α-glucosidase) (mg mL^{-1})**
Ethanol	MW	22.85 ± 1.74^{dB}	24.17 ± 1.88^{cB}
	DO	16.04 ± 0.21^{cA}	16.82 ± 0.21^{bA}
	FD	17.65 ± 0.10^{cA}	18.43 ± 0.06^{bA}
Methanol	MW	8.87 ± 0.18^{bA}	9.57 ± 0.20^{aA}
	DO	17.03 ± 0.37^{cB}	17.85 ± 0.39^{bB}
	FD	28.56 ± 2.90^{eC}	29.88 ± 2.85^{dC}
	Acarbose	2.37 ± 0.12^{a}	35.05 ± 3.98^{e}

^{a,b,c,d,e} indicate the differences ($p<0.05$) between all applications.

$^{A, B, C}$ indicate the differences ($p<0.05$) between application groups.

The highest α-amylase enzyme inhibition in the samples was determined with an IC_{50} value of 8.87 mg mL^{-1} in the samples that were dried in the microwave and applied methanolic extract ($p<0.05$). The sample showing the lowest α-amylase inhibition was found in the lyophilized and methanolic extract applied sample. Ethanolic extracts from oven-dried and lyophilized samples showed similar α-amylase enzyme inhibition ($p>0.05$). All samples had lower α-amylase enzyme inhibition compared to acarbose. Similar to the results of α-amylase inhibition, the highest α-glucosidase enzyme inhibition was found in microwave dried and methanolic extract applied samples (IC_{50} value of 9.57 mg mL^{-1}). The lowest α-glucosidase inhibition belonged to the lyophilized and methanolic extracted samples. In addition, all samples showed higher α-glucosidase enzyme inhibition compared to acarbose. This is due to the phenolic components of pomegranate press waste and its high antioxidant activity (Tadera et al., 2006; Adisakwattana & Chanathong, 2011; Okumuş & Bakkalbaşı, 2021).

4. Conclusion

The study's results showed that the applied drying processes and the extraction solvent were effective on the antioxidant activity of pomegranate press wastes. It is also revealed that microwave drying can be investigated as an effective method in addition to oven and freeze-drying to preserve the antioxidant activity in drying pomegranate waste. As a result, it was concluded that pomegranate press waste is a by-product with a high potential for use in the pharmacology and food industries, rather than being a waste, thanks to its antidiabetic activity and antioxidant activity. Future studies should continue in the form of carrying out studies to improve the use of pomegranate press wastes in animal nutrition, as well as its use in medicine, pharmacology, and food sectors.

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