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Investigation of the Effects of Microwave, Ultrasound, and Combined Microwave–Ultrasound Extraction on Antioxidant Properties and Mineral Content (Calcium, Potassium, and Iron) of Pomegranate Pomace Extract

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ABSTRACT

Considering the significant economic and social impacts of food waste, this study aimed to investigate the effects of different extraction methods on the antioxidant properties and mineral content (calcium, potassium, and iron) of pomegranate pomace. Four extraction methods were applied, including ultrasound (30 min), microwave (90 s), a combined microwave–ultrasound method (90 s microwave followed by 30 min ultrasound), and conventional maceration as a control. The obtained extracts were analyzed for extraction yield, total phenolic content, DPPH radical scavenging activity, and mineral concentrations. The results demonstrated that ultrasound and microwave methods, particularly the combined method, achieved the highest extraction yield (6.59%), DPPH radical scavenging activity (64.5%), and mineral contents of calcium (0.71 ppm), iron (0.23 ppm), and potassium (205 ppm). Moreover, the combined microwave–ultrasound, ultrasound, and microwave methods increased the total phenolic content by 256.35%, 88.58%, and 205.15%, respectively, compared to conventional maceration. These findings indicate that modern extraction techniques such as ultrasound and microwave can serve as effective alternatives to traditional methods for recovering bioactive compounds and mineral elements from fruit residues.

1- INTRODUCTION

Oxidation of fats in food products significantly reduces their shelf life, leading to the delivery of unacceptable quality foods to consumers. Various methods exist to prevent oil oxidation in foods, one of which is the addition of substances known as antioxidants. These compounds delay the development of off-flavors and rancidity by extending the product's stability. Antioxidants inhibit lipid oxidation through diverse mechanisms, such as controlling oxidation substrates, managing pro-oxidants, and scavenging free radicals. Conversely, the use of synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ), has come under scrutiny due to their adverse effects on human health. Consequently, in recent years, efforts to identify new natural antioxidant sources have expanded to mitigate the risks associated with synthetic antioxidant consumption [1, 2].

Pomegranate, scientifically known as *Punica granatum* L. (family Punicaceae), is widely cultivated in Iran and other tropical countries. The antioxidant activity of pomegranate is attributed to the presence of phenolic compounds, flavonoids, anthocyanins, tannins, ascorbic acid, and gallic acid. In recent years, the use of agricultural by-products has gained significant attention in various studies due to their cost-effectiveness. Pomegranate peel, as a non-edible by-product of the juice industry, exhibits high antioxidant activity and accounts for more than 50% of the fruit's weight [3]. Food loss and waste lead to the misuse of resources, such as water, land, energy, and fertilizers, as well as an increase in methane and carbon dioxide emissions resulting from the natural decomposition of food. Furthermore, food loss and waste have substantial economic and social impacts on governments; therefore, reducing these wastes is one of the United Nations' Sustainable Development Goals. Although fruit by-products are often considered low-value food waste, pomegranate by-products, as a source of bioactive compounds, possess high added value and demonstrate the potential for developing new food additives and reducing waste in the agricultural and food industries [4].

Various methods are employed for the extraction of phenolic compounds from plant sources. Conventional methods, such as maceration and Soxhlet extraction, are often time-consuming and inefficient, leading to increased production costs; these methods typically utilize hot water or low-molecular-weight alcohols as solvents. Other extraction techniques include microwave-assisted extraction, ultrasound-assisted extraction, pulsed electric field, and subcritical water extraction. These are considered novel methods, which are more efficient, faster, and require minimal solvent consumption compared to conventional approaches [1, 5, 6]. Microwaves are non-ionizing electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz, positioned between radio waves and infrared radiation in the electromagnetic spectrum; they are composed of two oscillating perpendicular fields, namely the electric and magnetic fields. Conventional heating is dependent on conduction and convection phenomena, which ultimately results in significant thermal energy loss to the surroundings, whereas with these waves, heating occurs along a specific and selective path without thermal loss to the environment. These waves cause the evaporation of water from the plant material structure and an increase in internal pressure, which can lead to material degradation, membrane disruption, and an enhancement in the efficiency of extracting extracts from the cell membrane [7]. Researchers, in a study regarding the extraction of phenolic compounds from eucalyptus leaves using microwave radiation, reported that novel extraction methods, such as microwave-assisted extraction, are rapid and effective techniques for extracting bioactive compounds from plant tissues; furthermore, it is possible to extract the maximum concentration of phenolic compounds with high purity from the target material compared to conventional methods [8]. One of the green technologies that has become widespread in various industries today is ultrasound-assisted extraction (UAE). This process operates based on the phenomenon of cavitation, which is generated by either an ultrasonic probe or an ultrasonic bath, functioning at frequencies of 20 and 40 kHz, respectively. During the ultrasonic process in a liquid medium, the sound waves produced by the device propagate into the medium, creating alternating cycles of compression (high pressure) and rarefaction (low pressure); consequently, the cavitation phenomenon is observed, which facilitates the intense mixing of solvent and solid particles. The microbubbles resulting from these implosions cause intense particle collisions, leading to increased internal diffusion as well as eddy diffusion, which subsequently ruptures the plant cell walls and enhances mass transfer and extraction rates [9]. Mineral elements, as essential micronutrients, play vital roles in the biochemical, structural, and regulatory functions of the human, animal, and plant bodies. Deficiencies or excessive consumption of these elements can lead to health disorders such as cardiovascular diseases, osteoporosis, immune disorders, and metabolic diseases. Food sources rich in these elements, such as dairy products, cereals, and vegetables, are essential for maintaining homeostatic balance. For instance, approximately 99% of calcium and phosphorus are stored in bones and teeth, and they are critical for bone mineralization and neuromuscular function [10, 11]. On the other hand, the presence of iron in processed foods or supplements can compromise the oxidative stability of oils. For instance, free iron or iron complexed with proteins can react with dissolved oxygen, leading to lipid degradation, which poses a significant challenge in the food industry. Studies indicate that even low concentrations of iron (at the micromolar level) are sufficient to increase oxidation rates by up to three times, particularly in the presence of heat or irradiation [12]. Researchers have demonstrated that the application of ultrasound and microwaves leads to an increase in the extraction of metals from plants [13, 14]. Given that the implementation of novel extraction methods, including ultrasound and microwave, can enhance the extraction yield of phenolic compounds and improve the antioxidant capacity and micronutrient content of extract while simultaneously potentially increasing the extraction of pro-oxidant substances this study aimed to investigate and compare the efficiency of three extraction methods, namely ultrasound, microwave, and the combination of these two processes (ultrasound-assisted microwave extraction), for obtaining antioxidant extracts and evaluating the mineral content present in the resulting extracts.

2- Materials and Methods

2-1- Materials

For this research, pomegranate pomace was obtained from a juice store in Hamadan County, Iran; Folin-Ciocalteu reagent, sodium carbonate, sodium phosphate buffer, DPPH solution, and calcium, iron, and potassium standards were procured from reputable companies, Merck (Germany) and Sigma-Aldrich (USA).

2-2- Extraction of Extract from Pomegranate Pomace

In this study, pomegranate pomaces were first dried in the shade and ground (TS1300, Iran) to obtain a powder with a particle size of 0.3 mm, and the resulting samples were extracted using four different methods. In the ultrasound method, the samples were subjected to an ultrasonic bath (vCLEAN-L4, Iran) made of stainless steel with a frequency of 50 Hz at a temperature of 20 °C (Agilent, USA) and an ultrasonic wave intensity of 75% for a duration of 30 minutes. For microwave-assisted extraction, the samples were placed in a microwave (LG, South Korea) at a power of 540 W for 90 seconds. For the microwave-ultrasound combined method, the samples were first subjected to microwave for a duration of 90 seconds and subsequently exposed to ultrasonic waves for a duration of 30 minutes; finally, for the conventional extraction method (control method), 24 hours of soaking in water was utilized. It should be mentioned that in all samples, the sample-to-solvent (water) ratio was 1:10. The extracts obtained from the four aforementioned methods were filtered using Whatman No. 1 filter paper, and the following assays were performed on these extracts [2, 15, and 16].

2-3-Extraction Efficiency

The extraction efficiency (EE) was determined by pouring 10 mL of the extracts (m_1) into the bottoms of plates that were completely clean, dry, and accurately weighed, and placing them at a temperature of 80 °C in a laboratory oven (Memmert, Germany) until a constant weight (m_2) was achieved; the extraction efficiency was then calculated using Equation (1) [17].

Equation (1)……………………………………………………………… $EE = \frac{m_2}{m_1} \times 100$

4- Total Phenolic Content (TPC) Determination

To quantify the phenolic compounds within the obtained extracts, the method described by Dolatabadi et al. (2025) was employed with slight modifications. In this procedure, a fixed concentration of the extracts was transferred into test tubes, followed by the addition of 2.5 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water). Subsequently, 2 mL of 7.5% (w/v) sodium carbonate was added, and the mixture was incubated at room temperature for 30 minutes. Upon completion of the reaction, the absorbance was measured at a wavelength of 765 nm using a spectrophotometer (Biochrom, UK) in 1 cm quartz cuvettes. A standard calibration curve was constructed using gallic acid concentrations ranging from 10 to 200 ppm. The total phenolic content of the extracts was calculated and expressed as milligrams of gallic acid equivalent per 100 grams of dry matter (mg GAE/100 g DM) [5].

2-5- DPPH Free Radical Scavenging Activity Assay

In this reductive method (assessing hydrogen-donating ability or radical scavenging capacity), the ability of the extracted samples to scavenge the stable DPPH radical evidenced by a color change from purple to yellow was investigated at a wavelength of 517 nm. Specifically, 5 mL of the extracted sample solution was mixed with 1 mL of 1 mM methanolic DPPH solution, followed by incubation for 30 minutes at room temperature in the dark. Subsequently, the absorbance of the samples was measured at 517 nm, and finally, the DPPH free radical scavenging activity was calculated using Equation (2) [17].

Equation (2)………………………………………………………….. $DPPH(\%) = \frac{AC-AS}{AC} \times 100$

In equation 2, AC and AS represent the absorption number of the control and sample, respectively.

2-6- Determination of Calcium, Potassium, and Iron Content

To prepare the samples and quantify heavy metals, a modified version of the method described by Dogajak and Merchik (2024) was employed. The concentrations of the elements were determined using Flame Atomic Absorption Spectrometry (Shimadzu, Japan). For the analysis, the instrument was first calibrated using a series of standard solutions, followed by the injection of the respective samples. Finally, the concentrations of calcium, potassium, and iron in each sample were quantified and presented as graphical data via the manufacturer's specialized software [18].

2-7- Statistical Analysis

Statistical evaluation of the data was conducted via a Completely Randomized Design in triplicate using SAS software (version 9.4). Treatment means were compared using Duncan's Multiple Range Test at a 5% significance level ($P<0.05$). Graphs were constructed using Microsoft Excel (2016).

3- Results and Discussion

3.1. Effect of extraction method on extraction yield

Duncan's Multiple Range Test revealed that the highest extraction yield (6.59%) was achieved using the combined microwave-ultrasonic method, followed by the ultrasonic-assisted method (Figure 1). Notably, all studied methods significantly increased the extraction yield compared to the control group. The primary mechanism of ultrasonic waves is the disruption and implosion of cavitation bubbles; the energy released from these bubble implosions enhances cell wall degradation, thereby facilitating increased mass transfer from the plant matrix to the solvent [9]. Ultrasound also causes a reduction in particle size, which increases the contact surface and consequently increases the solvent diffusion into the tissue, resulting in more extracted compounds [19]. Microwave waves also cause the evaporation of water from the plant material structure and an increase in internal pressure, which can cause degradation of materials, membrane disruption, and increased extraction efficiency [20]. Tan Rueon et al. (2025) showed that the use of microwave leads to an increase in the extraction yield of cinnamon plant, which was consistent with the results of this section [21]. On the other hand, the results of this section were consistent with the results of Karimi et al. (2020), who stated that the use of the combined microwave-ultrasonic method leads to an increase in the extraction yield of Thymus syriacus [22].

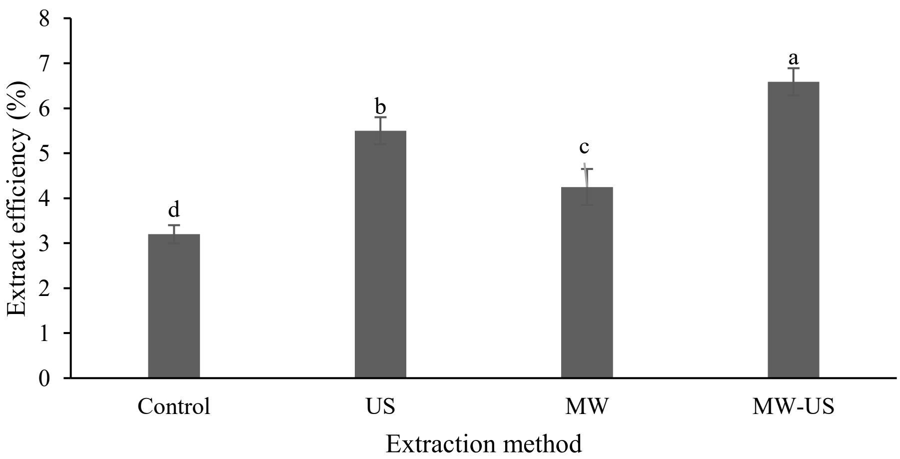

Figure 1- Effect of extraction method on extraction efficiency

3.2. Effect of extraction method on total phenolic content of extract

Phenolic compounds are defined as a wide group of natural compounds with diverse structures, including phenolic acids, flavonoids, tannins, and lignans. These compounds are not only important in plant extraction but are also used as active ingredients in natural products for treating diseases and synthesizing drugs [23]. The results shown in Figure 2 showed that, respectively, the combined microwave-ultrasonic, ultrasonic, and microwave methods led to an increase of 256.35%, 88.58%, and 205.16% in phenolic compounds compared to the conventional extraction method. Ultrasound-assisted extraction method extracts bioactive compounds relying on the mechanical action of ultrasound on plant cell walls [24]. The mechanical action of ultrasound increases the surface contact between solvent molecules and the plant sample matrix. Therefore, these waves alter and disrupt the physical and chemical properties of plant materials, accelerate the release of plant chemicals, and further enhance the mass transfer of the solvent system into the plant cells [25]. The use of this method reduces energy consumption and increases the recovery of plant chemicals from plants [26]. During the extraction process, microwaves heat the solvent and the plant tissue and increase the extraction kinetics. Microwaves heat the sample by directly acting on polar molecules. The conversion of microwave energy to heat involves dipole rotations. Heating is directly proportional to the dielectric constant of the solvents [27]. Solvent viscosity significantly affects the extraction process because lower viscosity facilitates ion dispersion and, consequently, solubility [28]. The extraction process involves the diffusion of solvents into the sample, the subsequent separation of the solute from the functional site, and finally, the release of solutes into the solvents. This technique is good in preserving the biological activities of extracts. For example, microwave optimization in green tea extraction confirmed the improvement of the antioxidant activity of plant compounds and improved the total phenolic content and quality of the extracts [29].

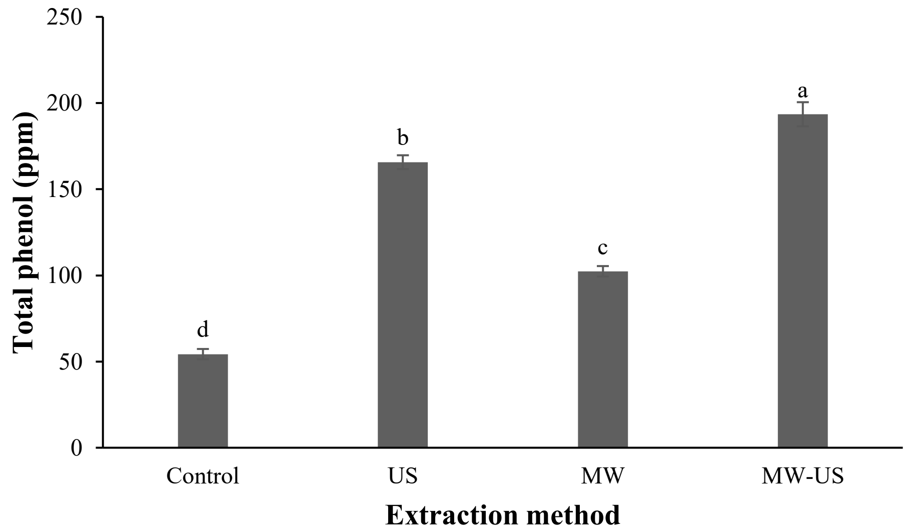

Figure 2- Effect of extraction method on total phenol

3.3. Effect of extraction method on DPPH free radical scavenging ability

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that is widely used as a reagent in antioxidant assays to measure the radical scavenging activity of bioactive compounds, such as phenolics. This method is based on the reduction of the purple color to a pale yellow color by antioxidants [23]. Figure 3 showed that the extract obtained from the combined microwave-ultrasonic method had the highest DPPH free radical scavenging ability (5.64%), followed by the extract obtained from the ultrasonic method. On the other hand, it was found that the results of this section had a high correlation with the results obtained from the total phenolic content of the samples, such that the sample with more phenolics had a higher DPPH free radical scavenging ability. Researchers have stated that antioxidant capacity is mainly determined by low molecular weight peptides, and ultrasonic cavitation leads to the release of low molecular weight peptides, which are more likely to interact with free radicals. The increase in antioxidant capacity may also be due to protein degradation by ultrasound, where the amino acid residues and side chains present in the extract increase the antioxidant capacity [30]. Razaghandi et al. (2024) attributed the increase in the DPPH free radical scavenging ability of yarrow plant extract during ultrasonication to the increased release of antioxidant compounds, such as tocopherols, into the extract [1]. The increase in the DPPH free radical scavenging ability in the extract obtained by the microwave method compared to the conventional method can also be attributed to the direct effects of microwave energy on phytomolecules through ionic conduction and dipole rotation, which leads to the disruption of the plant material cell walls and consequently increases phenolic and antioxidant compounds [31]. Juric et al. (2025) also showed that the use of microwaves leads to an increase in the DPPH free radical scavenging ability of orange peel extract, which is in line with the results of this section [32].

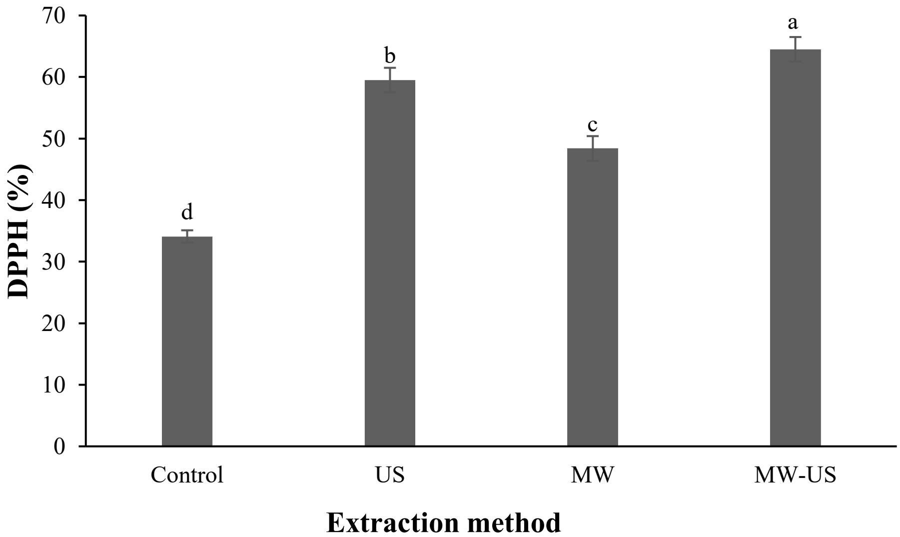

Figure 3- Effect of extraction method on DPPH radical scavenging activity

3.4. Effect of extraction method on the amount of mineral elements (calcium, potassium, and iron) in the extract

The results showed that the maximum amounts of calcium (0.71 ppm), iron (0.23 ppm), and potassium (205 ppm) were obtained when the combined microwave-ultrasonic method was used for extraction. Furthermore, it was found that all three methods used for extraction (ultrasonic, microwave, and their combination) led to an increase in mineral elements in the extract compared to the conventional method

(Figure 4). The increase in mineral elements in the extract during the use of microwave and ultrasound can be attributed to the disruption of complexes containing these compounds by ultrasonic and microwave waves [33]. Perhaps the increase in these compounds can also be attributed to the increased extraction efficiency of ultrasonic and microwave methods, which was fully explained in Section 3.1. Nababi et al. (2025) also stated that the use of ultrasound leads to an increase in mineral elements in extracts obtained from plants [34]. The results of this section were consistent with those of Olorada et al. (2003), which showed that the use of microwave and ultrasound leads to increased extraction of mineral elements from plants [35].

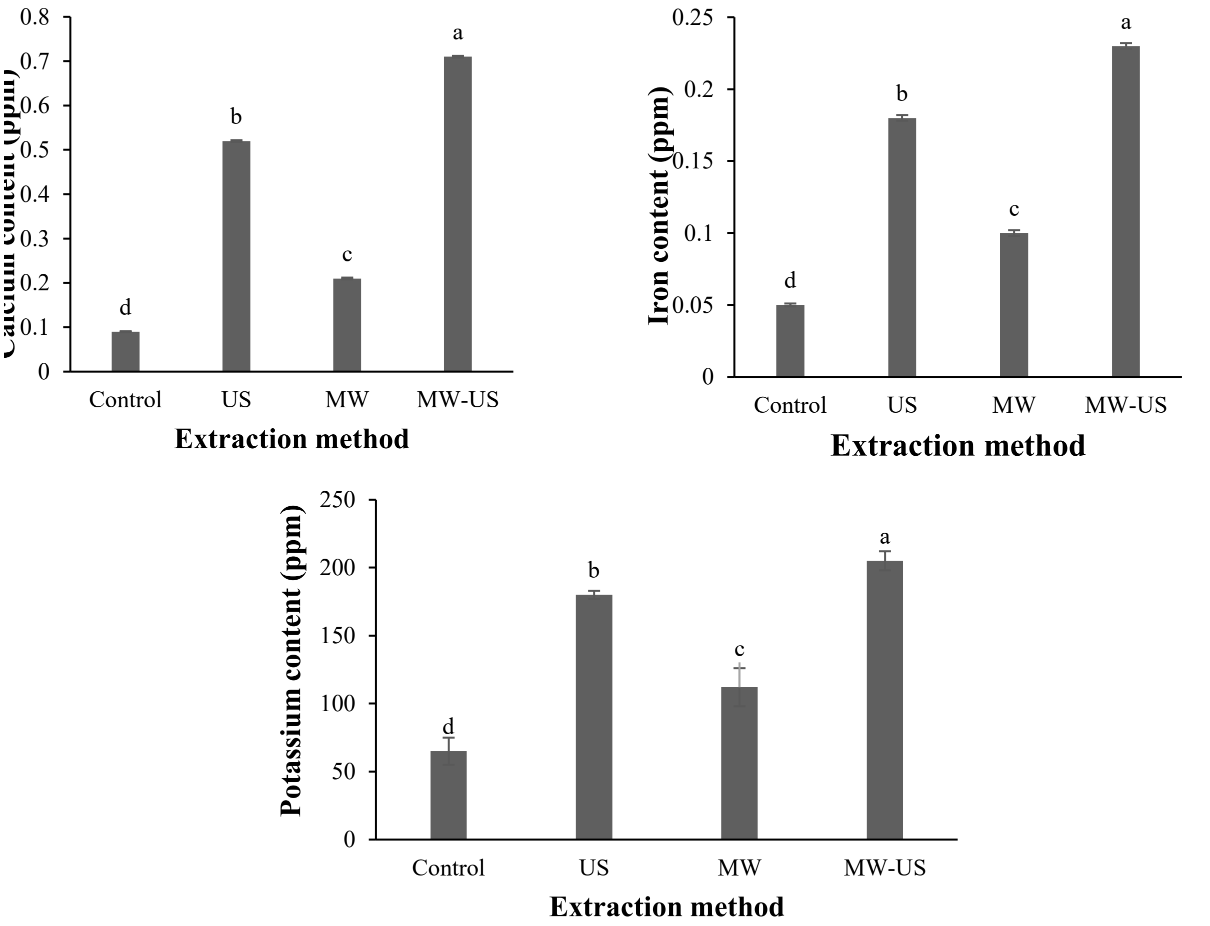

Figure 4- The effect of extraction method on the content of a) calcium, b) iron, and c) potassium in pomegranate pulp extract

4- Conclusion

The results of this study demonstrated that the extraction method plays a crucial role in determining the antioxidant properties and the content of mineral elements, including calcium, potassium, and iron. Modern extraction techniques, such as ultrasound and microwave, particularly the combined microwave-ultrasonic method, exhibited superior performance compared to the conventional maceration method in releasing these compounds. Ultrasound facilitates the release of mineral elements and phenolic compounds through the generation of cavitation and the disruption of cellular structures, whereas microwave-assisted extraction enhances this process by increasing solvent penetration and rapidly increasing the solution temperature. By leveraging the synergistic advantages of both techniques, the combined method provided the highest extraction yields for calcium, potassium, and iron, as well as the highest antioxidant activity. These findings indicate that the application of modern extraction methods can serve as an efficient and rapid solution for recovering bioactive compounds and mineral elements from fruit wastes, such as pomegranate pulp. Consequently, these techniques represent suitable alternatives to traditional methods in the food and fruit processing industries.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The author declares that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

Maryam Ebrahimi: Conceptualization; Methodology; Investigation; Formal analysis; Writing – original draft.

5-Resources

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چکیده

از آنجا که اتلاف و ضایعات مواد غذایی تاثیرات زیادی بر جنبه‌های اقتصادی و اجتماعی دولت‌ها دارد، این مطالعه با هدف بررسی تاثیر روش‌های مختلف استخراج بر خواص آنتی‌اکسیدانی و عناصر معدنی شامل کلسیم، پتاسیم و آهن از تفاله انار صورت پذیرفت. در این مطالعه از ٤ روش استخراج شامل فراصوت به مدت ۳۰ دقیقه، مایکروویو به مدت ۹۰ ثانیه، روش ترکیبی مایکروویو-فراصوت (۹۰ ثانیه مایکروویو و سپس ۳۰ دقیقه فراصوت) و شاهد (روش خیساندن) استفاده گردید. عصاره‌های به‌دست‌آمده از هر روش از نظر راندمان استخراج، فنول کل، توانایی مهار رادیکال‌های آزاد DPPH آنتی‌اکسیدانی و غلظت عناصر معدنی آنالیز شدند. نتایج نشان داد که روش‌های فراصوت و مایکروویو، به‌ویژه روش ترکیبی، بیشترین راندمان استخراج عصاره (٦/٥٩ درصد)، میزان توانایی مهار رادیکال‌های آزاد DPPH (٦٤/٥ درصد)، میزان کلسیم (۰/۷۱ ppm)، آهن (۰/۲۳ ppm) و پتاسیم (۲۰۵ ppm) را داشت. از طرفی مشخص گردید که روش ترکیبی مایکروویو- فراصوت، فراصوت و مایکروویو به‌ترتیب منجر به افزایش ۲٥٦/۳٥، ۸۸/٥۸ و ۲۰٥/۱٦ درصدی فنول کل عصاره نسبت به روش مرسوم استخراج گردید. در پایان نتایج این مطالعه نشان داد که استفاده از روش‌های نوین استخراج مانند فراصوت و مایکروویو می‌تواند به عنوان جایگزینی مؤثر برای روش‌های سنتی در بازیابی ترکیبات مفید و عناصر معدنی از ضایعات میوه‌ها مورد استفاده قرار گیرد.