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# Optimization of cold plasma and pulsed ultrasound conditions in the extraction of phenolic compounds from pomegranate seed meal

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**ABSTRACT**

The waste generated during the processing and production of food products is one of the major problems in the food industry. Disposing of waste leads to the loss of valuable bioactive compounds, which, if identified and extracted, can have many applications in food products. Pomegranate is one of the fruits whose processing generates a significant amount of waste, and this waste is usually discarded without any reuse. In this study, the preparation of phenolic extract from pomegranate seed meal obtained by cold pressing was investigated. For this purpose, the microwave-roasted meal was treated with cold plasma for 10 min as a pretreatment for extracting phenolic compounds, and the conditions of voltage (16, 18, and 20 kV) and time (5, 10, and 15 min) were optimized in terms of achieving an appropriate amount of total phenolic content and antioxidant activity. Optimization of power (100, 200, and 300 W) and time (5, 10, and 15 min) was also conducted for the extraction of compounds using pulsed ultrasound. In the end, the type and amount of phenolic compounds were analyzed using HPLC. Based on the results, applying a cold plasma pretreatment with a voltage of 20 kV for 15 min and extraction with ultrasonic waves at 200 W for 15 min were the best conditions for extracting phenolic compounds. HPLC results also indicated a high amount of gallic acid compared to other compounds in the extract.

## 1. Introduction

The pomegranate fruit (*Punica granatum L.*) is rich in bioactive compounds exhibiting antioxidant, anti-inflammatory, anticancer, and antimicrobial properties [1]. This fruit has a strong antioxidant potential due to its high levels of phenolic compounds, flavonoids, anthocyanins, tannins, ascorbic acid, and gallic acid. The health benefits of pomegranate are attributed to its diverse range of bioactive constituents. These compounds act synergistically, enhancing overall bioactivity [2]. Of the total pomegranate fruit, juice accounts for 38–50%, peel for 39–53%, and seeds for 8–12% of the fruit's weight [3]. Since pomegranate seeds are a rich source of oil, they are used for oil extraction and further processing or commercialization. In this context, cold pressing is one of the oldest methods of oil extraction, without thermal treatment, refining, or solvent use. An advantage of this method is that it minimizes nutrient degradation in the oil.

One of the by-products of pomegranate seed oil production is the meal generated during processing, constituting 10–30% of the raw input and potentially contributing to environmental issues if not properly managed. Therefore, for the food industry, developing waste disposal systems and managing by-products properly are crucial to minimizing environmental risks. The potential to recover by-products depends on their chemical, functional, and antimicrobial properties. Food waste can be used to enrich various food products or as a source of food components such as colorants, antimicrobial agents, and fiber. Nutrient recovery from food waste is a promising strategy for utilizing by-products rich in bioactive compounds, including antioxidants and antimicrobials [4]. Studies show that the amount of phenolic compounds in plant extracts can be increased before extraction by applying processes to the raw material, such as roasting and using cold plasma.

Roasting is a traditional processing method that enhances the nutritional and sensory qualities of food by releasing aromatic compounds. Additionally, Roasting influences the antioxidant activity of products [5]. The effects of roasting depend on temperature and time, which induce physical and chemical changes. Roasting breaks down cellular components, releases phenolic and flavonoid compounds, and forms heat-induced compounds [6]. Roasting before extraction can increase the amount of extractable phenolic compounds in the obtained extract. This process can be performed using different methods such as oven or microwave roasting, each producing different effects on the properties of the material depending on time and temperature. Bolek (2022) studied microwave roasting (600 W) of tamarind seeds and found that increases in phenolic compounds were accompanied by significant enhancements in antioxidant activity. Roasting for 8, 16, and 24 min increased antioxidant activity from 32.22% in the unroasted sample to 35.36%, 39.73%, and 40.48%, respectively [7]. Plasma, the fourth state of matter, behaves differently from solids, liquids, and gases. It is a neutral gas consisting of a substantial proportion of ionized particles. In this state, the gas becomes electrically conductive under the influence of electric and magnetic fields. Plasma is typically generated by energizing a gas using electrical discharge, radio frequency, or microwave radiation [8]. Studies have shown that cold plasma has applications in the food industry, particularly for preventing microbial contamination in fruits and vegetables and inactivating enzymes [9]. Acidic or alkaline hydrolysis is commonly used to enhance the extraction efficiency of phenolic compounds. However, this method is not environmentally friendly. The use of strong acidic or alkaline chemicals, combined with high temperatures (80–95 °C during acidic hydrolysis), can degrade phenolic compounds and decrease extraction efficiency [10]. Cold plasma pretreatment

has been shown to enhance the extraction of various bioactive compounds, such as diosmetin from *Valerianella locusta* leaves and phenolic compounds from blueberry juice, highlighting its effectiveness in improving phenolic extraction [9]. One study reported that the antioxidant activity of prickly pear cactus fruit extract increased by 1.8% after cold plasma treatment at 750 W for 40 min and by 1.7% at 856 W for 36 min [11].

Extraction methods for phenolic compounds are generally categorized as either simple or complex.

Solid-liquid extraction, a simple and widely used method, does not require advanced technology and can be performed using basic equipment. This method typically employs polar solvents such as methanol, ethanol, acetone, chloroform, and ethyl acetate for phenolic compound extraction. Ethanol is often preferred over other solvents due to its lower toxicity and its ability to promote faster water evaporation [12]. Ultrasound-assisted pulsed extraction is an efficient and cost-effective method that does not require expensive equipment. Ultrasound induces cavitation, leading to bubble formation that disrupts plant cell walls and releases intracellular contents [12].

Compared to conventional extraction methods, pulsed ultrasound-assisted extraction reduces extraction time, energy consumption, solvent usage, and chemical requirements. Additionally, pulsed ultrasound extraction causes less equipment wear compared to continuous ultrasound. Due to its intermittent on/off cycles, it generates less heat, making it more suitable for extracting heat-sensitive compounds such as polyphenols [13]. A study comparing ultrasonic bath, ultrasonic probe, and shaking methods found that the ultrasonic probe achieved the highest extraction efficiency of phenolic compounds from walnut shells, yielding 2.51 mg GAE/g DW—twice that of the other methods [14]. The enhanced extraction efficiency observed with ultrasound is attributed to its mechanical effects. Cavitation bubbles collapse on the surface of the solid matrix, disrupting cell walls and increasing mass transfer, which facilitates deeper solvent penetration into the sample [15]. Pomegranate seed meal, a by-product of oil extraction, contains valuable nutritional compounds but is often discarded as food waste or used as animal feed. This research aims to add value to this food industry by-product by improving the extraction efficiency of phenolic compounds from pomegranate seed meal. Different treatments were applied to determine the optimal extraction conditions for these valuable compounds.

## 2. Materials and Methods

### 2.1. Materials

Pomegranate seed meal was obtained from Destchin Company (Isfahan, Iran). Ethanol (96%) was purchased from Nasr Isfahan Company. Other chemicals, including methanol, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate, were obtained from Merck, Germany**.**

### 2.2. Investigation of pomegranate seed meal composition

The composition of pomegranate seed meal was determined using the following analyses:

#### 2.2.1. Moisture measurement

Moisture content was determined according to the method described by Özcan et al. (2021). First, an aluminum container was dried in an oven at 105 °C until constant weight was achieved. Then, 10 g of pomegranate seed meal was weighed into the container and evenly spread. The sample-containing container was dried again at 105 °C until a constant weight was reached. Moisture content (%) was calculated using the following formula [16];

$$\text{Moisture } \% = \left(\frac{\text{Sample weight before drying} - \text{Sample weight after drying}}{\text{Sample weight before drying}}\right) \times 100$$

#### 2.2.2 Determination of Oil Content

The oil content was measured according to the AOAC (1990) standard. Five grams of the sample were weighed and placed into a paper thimble. The thimble was then transferred to the extraction chamber of a Soxhlet apparatus, and petroleum ether was added as the solvent. The solvent was heated to reflux, causing it to evaporate, condense in the condenser, and drip onto the sample, facilitating oil extraction. The extraction was continued for six hours to ensure complete oil recovery. After extraction, the flask containing the solvent and oil was placed in a rotary evaporator under vacuum at 50 °C to remove the solvent. The oil content (%) was calculated by weighing the flask before and after evaporation, using the following formula [17];

$$\%\ \text{Oil content} = \left(\frac{\text{weight of the flask containing oil} - \text{weight of empty flask}}{\text{sample weight}}\right) \times 100$$

### 2.2.3 Determination of Protein Content

One gram of pomegranate seed meal sample was poured into the test tube of the Kjeldahl apparatus; then five grams of catalyst (including 4.5 g of potassium sulfate and 0.5 g of copper sulfate) along with 15 mL of 98% sulfuric acid were added to the tube and the digestion tube was heated for 3 h at 300 °C until the digestion was complete and the mixture became completely transparent. When the mixture was completely cooled, 75 ml of distilled water was added and placed in the apparatus for distillation and titration. 40% sodium hydroxide was used for the distillation stage and 0.1 normal hydrochloric acid and boric acid were used for titration. The apparatus was zeroed with a control that included all the items mentioned except pomegranate seed meal. The total nitrogen in the sample was calculated using the following equation, and the protein percentage was obtained by multiplying the total nitrogen by the coefficient 6.25 [18];

$$\%\ \text{Total nitrogen} = \left(\frac{14 \times \text{Acid normality} \times \text{Volume of acid consumed}}{\text{Sample weight (g)} \times 1000}\right) \times 100$$

### 2.3. Roasting the meal

For this purpose, the pomegranate seed meal was spread in a single uniform layer and roasted in a microwave at 640 W for 10 min [16]. After the heat treatment, the roasted meal was ground and then sieved through a 40-mesh sieve.

### 2.4. Extraction of Phenolic Compounds from Roasted Meal

To evaluate the effect of roasting on the properties of pomegranate seed meal, phenolic compounds were extracted using a 50:50 (v/v) mixture of water and ethanol from both the microwave-roasted sample and the control sample (raw pomegranate seed meal).

To prepare the extract from the samples, 5 g of each treatment was weighed and transferred to a 250 mL screw-capped Erlenmeyer flask. Then, 50 mL of a water–ethanol mixture (50:50, v/v) was added, maintaining a sample-to-solvent ratio of 1:10. The flask was placed in a shaking incubator at 100 rpm and 40 °C for 24 h. After incubation, the contents were transferred to a Falcon tube to separate the extract from the solid phase. and centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was subsequently filtered using filter paper and stored at 4 °C in glass tubes covered with aluminum foil to prevent light-induced degradation until analysis was performed [19].

### 2.5. Color analysis of meal extract

To assess the suitability of the extract for use in food products, color changes in the extract derived from raw and roasted meal were measured. A colorimeter (ZE 6000,

Nippon Denshoku, Japan) was used for the analysis. The color parameters $L^*$ (lightness, from black to white), $a^*$ (from green to red), and $b^*$ (from blue to yellow) were evaluated to determine the effect of roasting on the extract color, by comparing roasted and raw meal extracts.

### 2.6. Cold plasma pretreatment

Roasted meal, prepared by heating in a 640 W microwave for 10 min, was subjected to cold plasma as a pretreatment for the extraction process. The cold plasma device used in this study was a dielectric barrier discharge (DBD) system operating at atmospheric pressure. For plasma pretreatment, 2 g of the roasted sample powder were evenly spread as a thin layer in the device's container. To prevent the powder from dispersing, the surface of the Petri dish was covered with a layer of cellophane. The treatment was conducted at three voltages (16, 18, and 20 kV) and for three exposure durations (5, 10, and 15 min). Following the cold plasma treatments, extracts were prepared using a 50:50 (v/v) water–ethanol mixture in a shaking incubator (100 rpm, 40 °C) for 24 h. The total phenolic content and antioxidant activity of the extracts were evaluated, and the optimal voltage and treatment duration for plasma pretreatment were determined.

### 2.7. Ultrasound-Assisted Extraction

The ultrasound-assisted extraction conditions were first optimized by evaluating different power levels and extraction durations. To conduct the extraction, 5 g of roasted seed meal powder (prepared by microwave heating at 640 W for 10 min) were placed in a beaker. Then, 50 mL of a water–ethanol mixture (50:50, v/v) was added. The extraction was carried out using ultrasound at three power levels (100, 200, and 300 W) and three extraction times (5, 10, and 15 min). The resulting extracts were analyzed for total phenolic content and antioxidant activity.

### 2.8. Determination of Total Phenolic Content

The total phenolic content was measured during both the optimization of cold plasma pretreatment and ultrasound-assisted extraction. The analysis was performed based on the method described by Rajha et al. (2020). In this method, 50 µL of extract was mixed with 1 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water) and 0.8 mL of sodium carbonate solution (7.5%). The mixture was thoroughly vortexed and incubated in the dark at room temperature for 90 min. Absorbance was then measured at 765 nm using a UV–Vis spectrophotometer. For the standard curve, gallic acid was dissolved in a 50:50 (v/v) water–ethanol mixture to prepare solutions of various concentrations. For each concentration, 50 µL of gallic acid solution was mixed with 1 mL of diluted Folin–Ciocalteu reagent and 0.8 mL of sodium carbonate. The mixtures were incubated under the same conditions. Absorbance was measured at 765 nm, and a standard calibration curve was generated by plotting absorbance against gallic acid concentration [20].

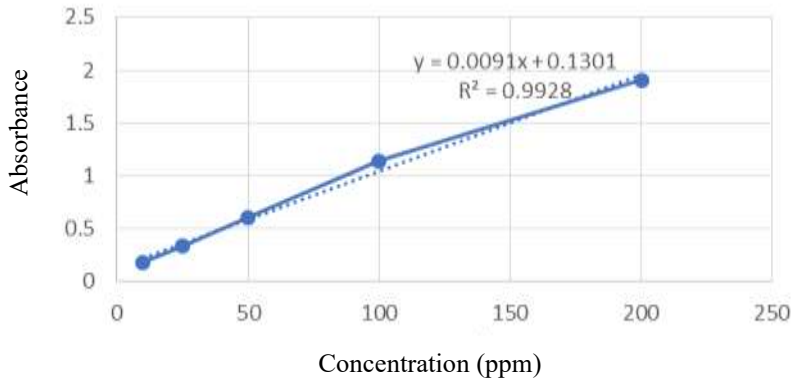


**Figure 1-** Absorption curve against different concentrations of gallic acid as standar.

### 2.9. Determination of Antioxidant Activity

The antioxidant activity of the extracts was evaluated in both the cold plasma pretreatment and ultrasound-assisted extraction experiments. First, a 0.15 mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared in methanol. Then, 50 µL of the extract was added to 3 mL of the DPPH methanol solution and thoroughly vortexed. For the control sample, 50 µL of a 50:50 (v/v) water–ethanol mixture was added to 3 mL of the DPPH solution instead of the extract. All samples were covered with aluminum foil to protect them from light exposure and were incubated at room temperature for 30 min. After incubation, the absorbance was measured at 517 nm using a UV–Vis spectrophotometer [21].

The antioxidant activity was calculated using the following formula:

$$\%\text{Inhibition} = \left(1 - \frac{\text{sample absorbance}}{\text{blank absorbance}}\right) \times 100$$

### 2.10. Qualitative and Quantitative Identification of Phenolic Compounds by HPLC

To identify and quantify the phenolic compounds in the pomegranate seed meal extract, an HPLC system (Agilent HP 1090) equipped with a C18 column was used. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% acetonitrile in water), delivered at a flow rate of 1.0 mL/min at 25 ˚C [22]. Extracts obtained from ultrasound-assisted extraction (with and without cold plasma pretreatment) were analyzed to determine the qualitative and quantitative profiles of phenolic compounds. Prior to injection, the extracts were filtered through a 0.22 µm syringe filter, transferred to the sample vial, and injected into the HPLC system via the injection port. The concentration of each identified compound was calculated using calibration curves obtained from external standards.

### 2.11. Statistical Analysis

The collected data were statistically analyzed using Statistix software (version 8). A completely randomized design (CRD) was employed in this study. Analysis of variance (ANOVA) was performed to determine significant differences among treatments. When significant differences were detected ($p < 0.05$), the least significant difference (LSD) test was used for mean comparison at a 95% confidence level.

## 3. Results and Discussion

### 3.1. Composition of pomegranate seed meal

Protein, oil, and moisture contents of pomegranate seed meal are reported in Table 1.

Table 1- Chemical compounds in pomegranate seed meal

| Chemical composition | Content (%) |
|---|---|
| Protein | 0.08 |
| Moisture | 11.8± |
| Oil | 3.9±0.14<br>9.44±0.06 |

-Data express mean ± standard deviation (SD).

Based on the data reported in Table 1, the levels of protein, moisture, and oil were 11.8%, 3.9%, and 9.44%, respectively. Teh et al. (2019) reported protein, moisture, and oil contents in pomegranate seed meal obtained through the pressing process as 11.7%, 1.4%, and 6.8%, respectively [23]. It is worth noting that the percentages of these components in pomegranate seeds vary significantly among different varieties; therefore, differences in composition are also observed in pomegranate seed meal derived from various varieties.

### 3.2. Examining the Effect of Roasting on the Color of the Extract

Roasting pomegranate seed meal can affect its composition and properties. Factors such as temperature, method, and roasting time can influence the extent of these changes and cause alterations in characteristics such as color. Therefore, in this section, we measured the color of extracts obtained from both roasted and raw pomegranate seed meal. As shown in Figure 2, roasting resulted in visible changes in the color of the extract compared to the raw meal extract. According to the results shown in Figure 3, roasting significantly decreased the $L^*$ parameter and increased the $a^*$ and $b^*$ parameters compared to the control sample ($p < 0.05$). The $L^*$ parameter, which indicates lightness, was 58.25 in the raw meal extract. After microwave roasting for 10 min, it decreased to 44.44. Thermal processes like roasting mainly cause color changes through reactions such as degradation and browning [24]. Therefore, the decrease in $L^*$ can be attributed to the formation of brown pigments resulting from caramelization and Maillard reactions during thermal roasting [25].

The $a^*$ parameter (which indicates greenness to redness) showed a significant increase ($p < 0.05$) compared to the control sample. The level of $a^*$ was 11.36 in the raw sesame meal extract and increased to 16.48 after microwave roasting for 10 min. A study investigating sesame roasting with hot air attributed the redder color caused by heat application to the formation of brown pigments from non-enzymatic browning and phospholipid decomposition [26]. The $b^*$ parameter (which indicates blueness to yellowness) showed a significant increase compared to the control sample, rising from 38.72 to 43.21 in the roasted sample. This increase may be attributed to the presence of brown substances such as melanoidin formed during the Maillard reaction [24].

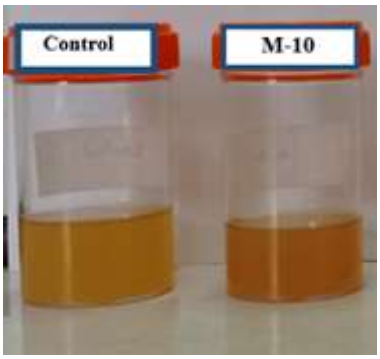


**Figure 2-** Visual appearance of the meal extract in the microwave for 10 min compared to the raw extract. Abbreviations: M: Microwave roasted. The

number next to the letter M indicates the duration of the process in minutes.

In general, the differences observed between the roasted and raw meal extracts are influenced by chemical reactions and changes in pigment solubility after extraction, which affect both the visual perception of color and measurable color parameters.

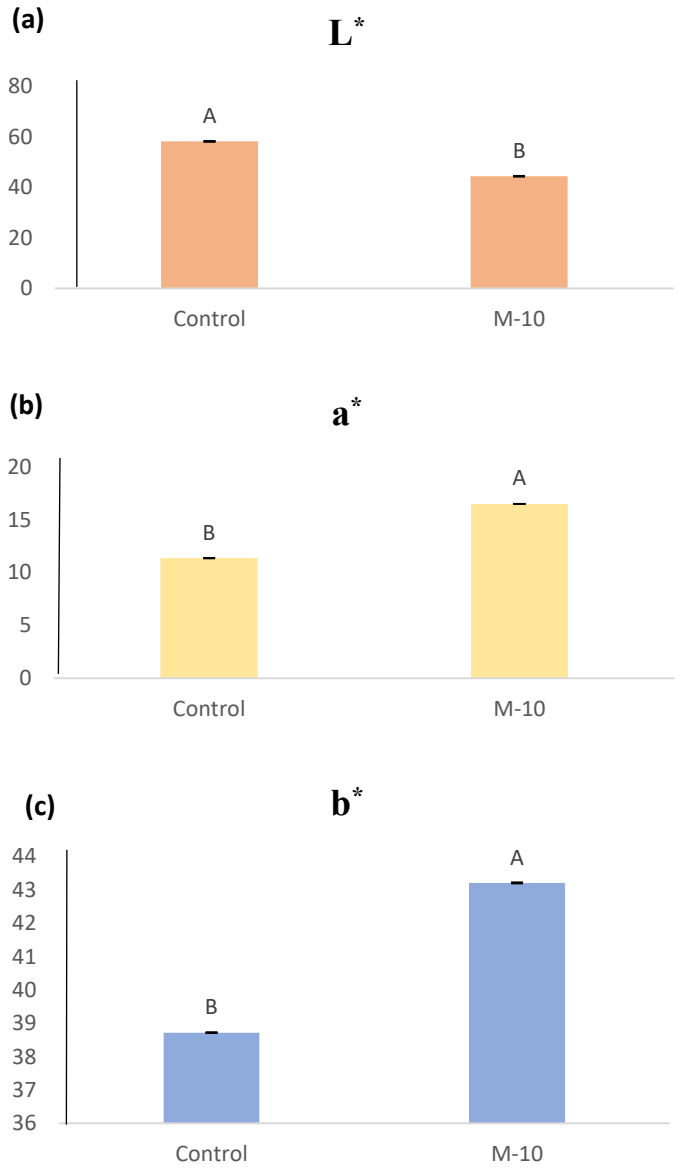


**Figure 3**- Color values of the roasted extract compared to the raw extract. Abbreviation: M: microwave roasted. The number next to the letter M indicates the processing time in minutes. (a): $L^*$, (b): $a^*$, (c): $b^*$.

### 3.3. Optimization of cold plasma conditions

The roasted meal was microwaved for 10 min prior to treatment with cold plasma applied at three different durations (5, 10, and 15 min) and three voltages (16, 18, and 20 kV) to determine the optimal pretreatment conditions for obtaining an extract with the highest phenolic content and antioxidant properties.

The results indicate a significant effect ($p < 0.05$) of plasma pretreatment on total phenolic content and antioxidant activity in pomegranate seed meal. According to the results in Table 2, the total phenolic content ranged from 146.47 to 197.03 mg gallic

acid equivalents (GAE) per liter of extract. At voltages of 16 and 18 kV, across all three treatment times, the total phenolic content did not differ significantly from the control sample. However, when the voltage increased to 20 kV, the difference became significant, with the highest phenolic content observed at 15 min of treatment.

At a constant voltage of 20 kV, increasing the exposure time from 5 to 15 min led to a progressive increase in phenolic content. Similarly, antioxidant activity also increased with longer treatment times at each voltage. The observed enhancements are likely due to significant disruption of the cell wall structure at higher voltages, which facilitates the release of phenolic compounds. For example, cold plasma treatment of grape pomace at high voltage resulted in a 10.9–22.8% increase in phenolic content compared to the untreated control, attributed to cellular structure breakdown [27]. With increasing exposure time to cold plasma, disruption of the cellular structure increases, resulting in the release of more intracellular compounds, potentially enhancing antioxidant activity. On the other hand, it is important to optimize both the voltage and duration of cold plasma exposure. In a study investigating the effect of cold plasma exposure time on freshly cut apples, the phenolic compound profile was affected both quantitatively and qualitatively after 10 min of treatment, showing an increase of approximately 20%. However, with longer exposure times, a gradual decrease in all polyphenols was observed, with antioxidant activity following a similar trend [28].

Bioactive compounds, especially polyphenols, exist in two forms: free and bound. Some of these compounds are bound to the cell wall. Plasma primarily affects the surface, particularly the cell wall, inducing structural changes that lead to the release of bound phenolic compounds [29]. Therefore, pomegranate seed meal likely contains phenolic compounds bound to the cell wall, and plasma treatment facilitates their release, resulting in increased total phenolic content and antioxidant activity.

In this study, cold plasma pretreatment at 20 kV for 15 min, which yielded the highest phenolic content and antioxidant activity, was selected as the optimal treatment.

**Table 2** - Total phenolic content and antioxidant activity under different cold plasma conditions

| Treatment (Voltage-Time) | Parameter | |
|---|---|---|
| | Total phenolic content (mg GAE/L) | Antioxidant activity (%) |
| **Control (without cold plasma)** | $162.08\pm0.26^{B}$ | $85.91\pm0.70^{C}$ |
| **16-5** | $159.29\pm16.52^{B}$ | $86.53\pm0.07^{BC}$ |
| **16-10** | $155.99\pm13.66^{B}$ | $90.77\pm4.91^{A}$ |
| **16-15** | $148.71\pm13.13^{B}$ | $88.29\pm0.39^{ABC}$ |
| **18-5** | $146.47\pm16.80^{B}$ | $78.71\pm1.81^{D}$ |
| **18-10** | $171.18\pm20.41^{B}$ | $86.97\pm0.60^{ABC}$ |
| **18-15** | $165.80\pm15.01^{B}$ | $89.42\pm0.99^{ABC}$ |
| **20-5** | $173.56\pm19.54^{AB}$ | $89.75\pm0.44^{ABC}$ |
| **20-10** | $173.13\pm1.49^{AB}$ | $85.99\pm0.66^{C}$ |
| **20-15** | $197.03\pm1.72^{A}$ | $90.31\pm0.81^{AB}$ |

-Data express mean ± standard deviation (SD). Means with different superscripts in each column are significantly different at $p< 0.05$. The control refers to the seed meal sample microwaved for 10 min that has not been subjected to plasma pre-treatment. The numbers 16, 18, and 20 represent voltage (kV), and the numbers 5, 10, and 15 represent the plasma process time (min).

### 3.4. Optimization of Ultrasound Extraction Conditions

After optimizing the plasma pretreatment conditions, further studies were conducted to optimize ultrasound extraction conditions. Ultrasound extraction was performed at three power levels (100, 200, and 300 W) and three durations (5, 10, and 15 min), and the resulting extracts were evaluated for total phenolic content and antioxidant activity. Statistical analysis showed that ultrasound extraction parameters significantly affected the total phenolic content and antioxidant activity of pomegranate seed meal.

**Table 3 -** Total phenolic content and antioxidant activity in different treatments extracted with ultrasonic waves

| Treatment (Power-Time) | Parameter | |
|---|---|---|
| | Total phenolic content (mg GAE/L) | Antioxidant activity (%) |
| **100-5** | $72.47\pm1.43^{G}$ | $41.16\pm1.04^{D}$ |
| **100-10** | $67.18\pm0.12^{H}$ | $39.83\pm1.07^{D}$ |
| **100-15** | $84.32\pm0.40^{F}$ | $51.10\pm3.66^{C}$ |
| **200-5** | $133.87\pm0.89^{E}$ | $65.26\pm6.37^{B}$ |
| **200-10** | $147.06\pm0.17^{C}$ | $73.74\pm2.27^{AB}$ |
| **200-15** | $158.19\pm0.37^{A}$ | $78.06\pm3.96^{A}$ |
| **300-5** | $145.41\pm2.23^{CD}$ | $71.87\pm6.17^{AB}$ |
| **300-10** | $142.69\pm0.85^{D}$ | $77.36\pm3.45^{A}$ |
| **300-15** | $152.81\pm2.32^{B}$ | $80.32\pm0.03^{A}$ |

-Data express mean ± standard deviation (SD). Means with different superscripts in each column are significantly different at $p< 0.05$. The numbers 5, 10, and 15 represent the duration under consideration in minutes, and the numbers 100, 200, and 300 represent the power (W) under consideration.

According to the results in Table 3, total phenolic content increased with rising power and longer extraction times. However, interestingly, the phenolic content at 300 W was lower than at 200 W, which likely caused degradation of phenolic compounds. Therefore, the optimal conditions for maximum phenolic content and antioxidant activity were 200 W for 15 min.

Ahmed et al. (2022) investigated the optimization of phenolic content extraction and antioxidant activity in plum pulp using ultrasound waves. Their results showed that increasing extraction time led to higher phenolic content, suggesting that longer extraction likely facilitates the release of polyphenols from the tissue [30]. Altemimi et al. (2016) found that ultrasound at 37 kHz was more effective than at 80 kHz in extracting phenolic compounds from pumpkin and peach. In ultrasound-assisted extraction, the reduction in phenolic compounds at higher power levels can be attributed to cavitation. Cavitation refers to the formation and collapse of bubbles in the solvent and near food tissues induced by ultrasound waves. This collapse generates intense local heat and pressure, breaking down cell walls and releasing compounds. However, excessive power causes more intense cavitation, which may degrade sensitive compounds like phenolics,

thereby reducing overall extraction efficiency [31].

As shown in Table 3, antioxidant activity also increased with increasing power and time. The extract obtained at 200 W for 15 min, which had the highest phenolic content, also showed high antioxidant activity. However, unlike phenolic content, which decreased at 300 W, antioxidant activity remained high at this power across different treatment times. Ghasemi et al. (2009) stated that there is not always a direct linear correlation between phenolic content and antioxidant activity [32]. Furthermore, the difference between phenolic content and antioxidant activity may be attributed to the presence of compounds other than polyphenols that react with the DPPH assay (used to measure antioxidant activity) but do not react with the Folin-Ciocalteu reagent (used to determine total phenolic content) [33].

### 3.5. Identification of phenolic compounds in the extract by HPLC

In this section, the type and amount of phenolic compounds in the extracts obtained from pulsed ultrasound extraction with and without cold plasma pretreatment were investigated. According to the information in Table 4, among the compounds identified in both extracts extracted with ultrasound (with and without plasma pretreatment), gallic acid has the highest amount, which is very significant in terms of quantity compared to other compounds. The amount of gallic acid in the plasma-pretreated extract (40.69 mg/L extract) was higher than that in the extract without plasma pretreatment (35.19 mg/L extract), indicating the positive effect of this pretreatment on increasing the amount of this compound. On the other hand, syringic acid and chlorogenic acid were present in the plasma-pretreated extract, which were not observed in the extract without plasma pretreatment. In a study, the effect of cold plasma pretreatment on the antioxidant activity of apple slices was investigated, and the results indicated an increase in antioxidant activity. This change was attributed to the oxidation of catechins, which results in the formation of procyanidins that have greater antioxidant activity than catechins [11]. Therefore, we can point to the effective role of newly created compounds as a result of plasma pretreatment, which increased antioxidant activity.

Özcan et al. (2020) also identified phenolic compounds in pomegranate seed extract roasted in a microwave at 720 W for 10 min; among the reported compounds, gallic acid, with a content of 192.77 mg/100 g of sample, accounted for the highest amount compared to other phenolic compounds [16]. Alsataf et al. (2021) investigated the phenolic compounds in pomegranate seed powder extract using HPLC. They reported that gallic acid, caffeic acid, and 4-hydroxybenzoic acid were among the important phenolic compounds in pomegranate seed extract at 14.5, 2.5, and 0.6 µg/g, respectively [34].

**Table 4**- The type and amount of phenolic compounds identified in the pomegranate seed meal extract

| | Phenolic compound | Retention Time (min) | Phenolic compound content (mg/L of extract) |
|---|---|---|---|
| **C-Ul** | **Gallic acid** | 5.5 | $40.69\pm0.72^{A}$ |
| | **Syringic acid** | 16.1 | $12.13\pm0.25^{C}$ |
| | **Ferulic acid** | 29.7 | $6.07\pm0.23^{D}$ |

| | | | |
|---|---|---|---|
| | **Chlorogenic acid** | 13.7 | 1.63±0.13$^{E}$ |
| **N-Ul** | **Gallic acid** | 5.5 | 35.19±0.59$^{B}$ |
| | **Ferulic acid** | 29.7 | 5.91±1.1$^{D}$ |
| | **Syringic acid** | 16.1 | - |
| | **Chlorogenic acid** | 13.7 | - |

-Data express mean ± standard deviation (SD). Means with different superscripts in each column are significantly different at p< 0.05. Abbreviations: Ul: Ultrasonic extraction method, C: Cold plasma pre-treatment processes, N: Non-applying cold plasma pre–treatment.

## 4. Conclusion

Pomegranate seed meal is one of the food wastes containing phenolic compounds. Since these valuable wastes are usually discarded without proper use, by identifying and applying appropriate extraction methods, in addition to preventing the wastage of these compounds, they can be used in many fields. The results of this study showed that by roasting with microwave (640 W for 10 min), using cold plasma pretreatment (20 kV for 15 min), and extraction with ultrasound waves (200 W for 15 min), appropriate amounts of phenolic compounds can be obtained from pomegranate seed meal and this extract can be used more effectively in various industries, including the food industry.

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# بهینه سازی شرایط پلاسمای سرد و فراصوت پالسی در استخراج ترکیبات فنولیک از کنجاله هسته انار

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**چکیده**

ضایعات ایجاد شده طی فراوری و تولید محصولات غذایی، یکی از مشکلات عمده در صنعت غذا هستند. دفع ضایعات، سبب از بین رفتن ترکیبات زیست فعال با ارزش می‌شود که در صورت شناسایی و استخراج، می‌توانند کاربردهای زیادی در محصولات غذایی داشته باشند. انار، از جمله میوه‌هایی است که فراوری آن موجب تولید مقدار قابل توجهی ضایعات می‌شود و این ضایعات، معمولا بدون استفاده‌ی مجدد، دور ریخته می‌شوند. در این پژوهش، تهیه‌ی عصاره‌ی فنولیک از کنجاله هسته انار به دست آمده با پرس سرد، بررسی شد. بدین منظور، کنجاله‌ی برشته شده با مایکروویو به مدت ۱۰ دقیقه، تحت پلاسمای سرد به عنوان پیش تیماری برای استخراج ترکیبات فنولیک قرار گرفت و شرایط ولتاژ (۱۶، ۱۸ و ۲۰ کیلوولت) و زمان (۵، ۱۰ و ۱۵ دقیقه) از لحاظ دستیابی به میزان مناسب کل ترکیبات فنولیک و خاصیت آنتی اکسیدانی بهینه سازی شد. بهینه سازی توان (۱۰۰، ۲۰۰ و ۳۰۰ وات) و زمان (۵، ۱۰ و ۱۵ دقیقه) نیز برای استخراج ترکیبات با امواج فراصوت پالسی انجام شد. در انتها، نوع و میزان ترکیبات فنولیک با استفاده از HPLC بررسی شد. بر اساس نتایج، اعمال پیش تیمار پلاسمای سرد با ولتاژ ۲۰ کیلو ولت با مدت زمان ۱۵ دقیقه و استخراج با امواج فراصوت با توان ۲۰۰ وات به مدت ۱۵ دقیقه، بهترین شرایط برای استخراج ترکیبات فنولیک بودند. نتایج HPLC نیز حاکی از وجود مقدار زیاد گالیک اسید نسبت به سایر ترکیبات در عصاره بود. بنابراین می توان گفت که استفاده از پیش‌تیمار پلاسمای سرد به همراه استخراج با امواج فراصوت، روشی مؤثر و بهینه برای استخراج ترکیبات فنولیک ارزشمند از کنجاله هسته انار است.