



EFFECT OF DRYING TEMPERATURE ON ANTIOXIDANT ACTIVITY, TOTAL PHE-NOLIC CONTENT, AND TOTAL FLAVONOID CONTENT OF POMEGRANATE (*Punica granatum L.*) ARILS

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Abstract: Pomegranate (*Punica granatum L.*) arils contain thermolabile bioactive compounds, whose stability during thermal processing requires optimization for industrial applications. This study evaluated the effects of convective drying at 50 °C, 60 °C, and 70°C on the antioxidant capacity, total phenolic content (TPC), and total flavonoid content (TFC). The highest antioxidant capacity, by DPPH radical scavenging assay, was in fresh arils (874.34 ± 15.23 µg TE/g FW). The antioxidant capacity of arils decreased with increasing temperature. The retention of antioxidant capacity was 95.1% at 50°C, 92.3% at 60°C, and 55.5% at 70°C (p 0.0001). Both total phenolic content (TPC) and total flavonoid content (TFC) increased with drying, with the peak values recorded at 50°C of 716.19 ± 18.34 µg GAE/g FW and 252.96 ± 9.87 µg QE/g FW, respectively, showing 3.9-fold and 4.9-fold increment, respectively, over the fresh sample. The apparent enrichment due to the membrane breakdown of cells and release of bound phenolics from the plant matrix oscillated after a certain temperature range with increasing temperature. The grade of thermoxidative damage was reported at a high temperature and 30 min retention time. The study showed that the retention of antioxidant activity and maximum extractability of phenolic compounds was observed at 50°C, which established that 50°C is the ideal temperature for processing pomegranate aril. This study can serve as a basis for industrial processing to maintain bioactive compounds while offsetting processing efficiency and retention of nutritional quality.

Keywords: *Punica granatum*, thermal processing, bioactive compounds, DPPH assay, polyphenols, food preservation

1. Introduction

Pomegranate (*Punica granatum L.*) has emerged as an interesting functional food owing to its exceptional polyphenolic profile, which contains anthocyanins, ellagitannins, flavonoids, and hydrolyzable tannins [1]. These bioactive compounds exert beneficial effects on cardiovascular disease, inflammation, and chemical prevention by performing a strong antioxidant action [2]. Unlike other anthocyanin-rich fruits, pomegranates contain unique ellagitannins. These compounds undergo gut microbiota-mediated conversion to urolithins. This produces sustained systemic antioxidant effects [1].

Industrial processing of pomegranate arils, particularly drying, is a major hurdle for the preservation of bioactive compounds. The physicochemical transformations that occur during thermal processing are complex.

Moderate heating enhances extractability, causing cell walls to break down and the release of bound phenolics. However, excessive heating leads to oxidative degradation and structural decomposition of thermolabile compounds [3]. According to the previous studies, it was found that the temperature higher than 60°C began the rapid breakdown of anthocyanins and other heat sensitive polyphenols [4].

The effect of temperature on the drying process and the retention of bioactive compounds varies widely between plant matrices. According to [5], despite freeze-drying usually maximizing retention, this process is too costly for industrial applications. In contrast, hot-air drying is the most widely used industrial method owing to its lower cost and ease of scaling. Thermal parameter optimization is required for efficient processing while preserving the nutritional quality [6].

Even though there have been many studies on pomegranate peel and whole fruit processing, there have been very few systematic studies on the effects of temperature on aril bioactivity. This knowledge gap is vital primarily because arils are the main edible part (better known as a seed) and differ substantially from other components of the fruit [7]. As a result, the study sought to: (i) determine the effect of three different industrially recognised drying temperatures (i.e. 50, 60 and 70 °C) on the antioxidant capacity, total phenolic content and total flavonoid content of pomegranate arils; (ii) explain how or why these changes occur; and (iii) establishing evidence-based processing conditions for the maximisation of health-benefiting compounds.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

Fresh pomegranate (cv. Salakhani) at commercial maturity (total soluble solids: 16.5 ± 0.3 °Brix, titratable acidity: 0.42 ± 0.02 g citric acid/100 mL) was sourced from a certified organic producer located in Halabja, Kurdistan Region, Iraq ($35^{\circ}10'N$, $45^{\circ}59'E$) in October 2023 [8]. Fruits were selected based on their similar size (300 ± 20 g) and lack of physical defects. The arils that were disinfected with 200 ppm sodium hypochlorite and washed with distilled water from a surface point of view were manually extracted under hygienic conditions and randomized for testing.

2.2. Drying Treatments

Convective drying was carried out in a laboratory-scale hot air dehydrator (MEYKEY AMB005330_8_EU_2, Germany), with forced air circulation (1.5 m/s) and temperature control ($\pm 0.5^{\circ}C$). Arils (100 ± 0.5 g) were placed uniformly in a single layer in perforated stainless-steel trays (loading density: 2.5 kg/m²) and subjected to treatment at 50, 60, and 70°C. The drying was continued until a constant weight was achieved (moisture content <5% w.b.), which was checked every 30 min [9]. All experiments were performed in triplicate.

2.3. Extract Preparation

Dried and fresh arils were ground to a fine powder using liquid nitrogen to prevent thermal degradation. Extracts were prepared by macerating 0.1 g of powder with 1 mL of 80% (v/v) methanol, followed by vortexing (40 min) and overnight incubation at 4°C. After centrifugation ($12,000 \times g$, 10 min, 4°C), supernatants were collected and stored at -20°C until analysis within 48 h [10].

2.4. Antioxidant Capacity (DPPH Assay)

The DPPH radical scavenging assay was used to evaluate antioxidant capacity. The extract (50 µL) was layered with 2 mL of DPPH solution (0.038 mg/mL in 95% methanol). Absorbance was recorded at 517 nm after incubation in the dark for 30 min. Trolox was used as the standard (0-3.4 µg/mL) ($R^2=0.996$). The results are expressed in µg Trolox equivalents per gram fresh weight (µg TE/g FW). The protocol was followed according to [11] with some modifications.

2.5. Total Phenolic Content (TPC)

TPC was ascertained with the Folin-Ciocalteu method with some modifications [12]. Briefly, 50 μL of the extract was mixed with 1,050 μL of diluted reagent. After 7 min, 850 μL of 10% (w/v) Na_2CO_3 was added. Absorbance was measured at 750 nm (UV-6100, MAANLAB, Sweden) following a 30-min incubation in the dark. Gallic acid (0-300 $\mu\text{g}/\text{mL}$) was used as a reference standard ($R^2 = 0.998$), and the results were expressed in μg of GAE per gram of fresh weight (μg GAE/g FW).

2.6 The total flavonoid content (TFC)

TFC was quantified using the aluminum chloride colorimetric assay, adapted from the method described by [12]. Sample extracts were prepared as previously described for TPC analysis. The reaction mixture was combined by taking 50 μL of the extract and adding to it 0.90 mL of 80% (v/v) methanol, 0.30 mL of 2% (w/v) aluminum chloride, 0.08 mL of 1 M potassium acetate and 1.72 mL of deionized water. The solution was maintained at room temperature for 32 min before measuring the absorbance at 415 nm. Measurements were performed using a UV-visible spectrophotometer (UV-365, SHIMADZU, Japan). Quantification was based on a standard calibration curve generated using quercetin standards (0–80 $\mu\text{g}/\text{mL}$) with $R^2 > 0.99$. The final TFC was expressed as micrograms of quercetin equivalents per gram of fresh weight (μg QE/g FW).

2.7 Statistical Analysis

Data are expressed as mean \pm standard deviation ($n = 3$). One-way ANOVA followed by Duncan's multiple range test ($p < 0.05$) was performed using XLSTAT 2023.1 (Addinsoft, France). Pearson correlation analysis was used to examine the relationships between parameters [13].

3. Results and Discussion

The antioxidant capacity of pomegranate arils showed a significant temperature-dependent variation ($p < 0.0001$). Fresh arils exhibited the highest DPPH radical scavenging activity at 874.339 ± 15.23 μg TE/g FW, establishing the baseline for comparison with dried samples (Figure 1).

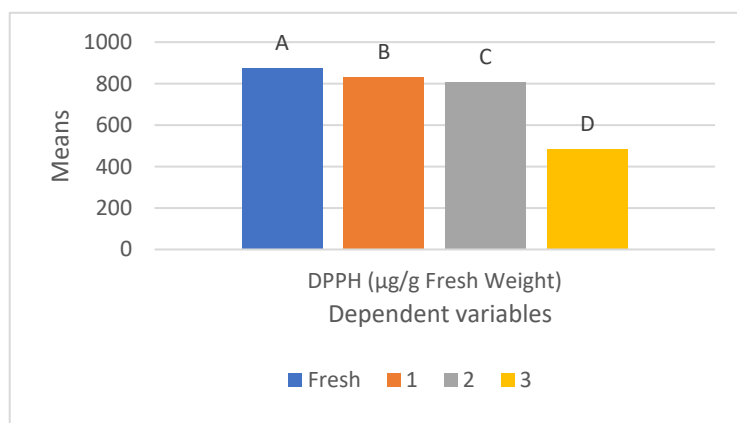


Fig 1. Antioxidant capacity of pomegranate arils subjected to different drying temperatures

Mean DPPH values ($\mu\text{g}/\text{g}$ Fresh Weight) for samples A (fresh, blue), B (dried at 50 °C, orange), C (dried at 60 °C, gray), and D (dried at 70 °C, yellow).

Values represent the mean \pm SD ($n=3$). Different superscript letters indicate significant differences ($p < 0.05$) according to Duncan's multiple range test

Pomegranate arils displayed a significant degradative tendency in antioxidant capacity as a function of drying temperature ($p < 0.0001$). The antioxidant capacity of fresh arils exhibited the maximum activity (874.339

$\pm 15.23 \mu\text{g TE/g FW}$). Thermal processing at 50°C and 60°C produced decreases in antioxidant capacity of 4.9% and 7.7%, respectively. The loss of 44.5% at 70°C indicates that a significant thermal stability threshold was exceeded. This sudden change in degradation behavior between 60°C and 70°C confirms the thermolabile nature of some bioactive compounds in pomegranate. The results obtained were in agreement with those of [14] which showed significant losses when processed above 60°C for pomegranate peel. Significant heat treatment may cause a significant drop owing to the rapid thermal decomposition of heat-sensitive compounds, particularly anthocyanins [15].

Figure 2 shows the impact of thermal treatment on the total phenolic content (TPC), which increased notably in contrast to fresh samples ($p < 0.0001$). Fresh arils had the lowest TPC value ($181.798 \pm 6.54 \mu\text{g GAE/g FW}$), whereas all dried samples had much higher values. This TPC pattern is rather unusual compared to that of the antioxidant capacity.

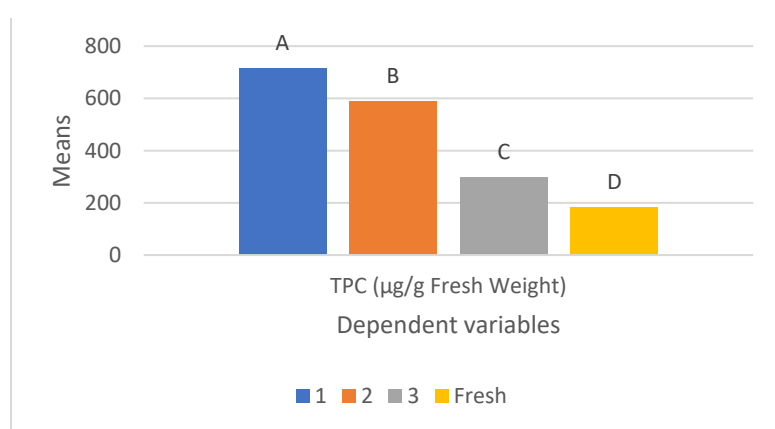


Fig 2. Total phenolic content of pomegranate arils subjected to different drying temperatures

Mean Total Phenolic Content (TPC) values ($\mu\text{g/g Fresh Weight}$) for samples A (fresh-blue), B (dried at 50°C - orange), C (dried at 60°C - gray), and D (dried at 70°C - yellow). Values are presented as the mean \pm SD ($n=3$). Different superscript letters indicate significant differences ($p < 0.05$) according to Duncan's test.

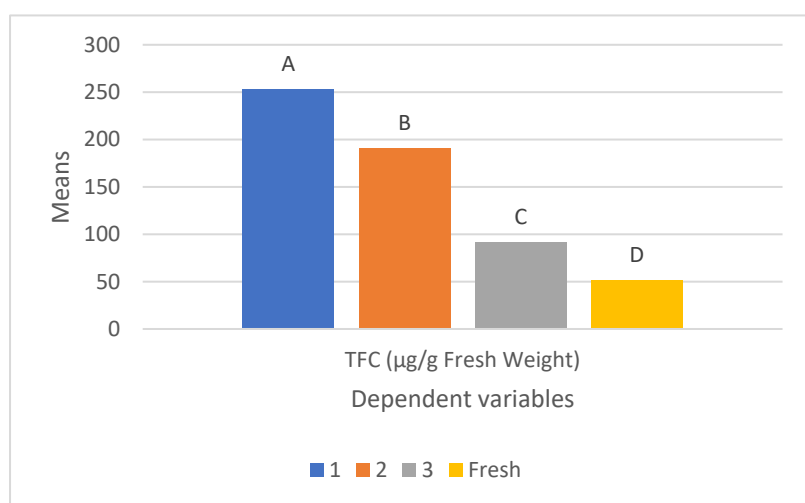
TPC showed a nonlinear response to temperature treatment. All dried samples exhibited a significantly higher TPC than that of fresh arils ($181.798 \pm 6.54 \mu\text{g GAE/g FW}$). A maximum TPC of $716.186 \pm 18.32 \mu\text{g GAE/g FW}$ was obtained at 50°C , which is nearly four times the fresh control. The TPC was gradually reduced as a result of the increased temperature (60°C and 70°C) treatment, yet the values remained high compared with the fresh value. The peak TPC at moderate temperatures was possibly due to multiple mechanisms acting simultaneously. Through heat treatment, cellular disruption occurs, including protein denaturation and lipid phase transition. Moreover, it enhances the extractability of sequestered phenolic compounds from the plant matrix [16]. Second, moderate heat probably promotes the partial hydrolysis of high-molecular-weight polymers, such as ellagitannins, into smaller and more accessible phenolic units that react more efficiently with the Folin-Ciocalteu reagent [17]. In conclusion, the formation of Maillard reaction products and other neo-formed compounds can positively interfere with the TPC assay, increasing the measured value, which does not correspond to a real increase in antioxidant capacity [18]. The subsequent decrease in TPC at temperatures above 50°C indicates that oxidative cleavage and polymerization pathways of thermal degradation begin to dominate their liberation and formation [5].

Drying temperature had a strong negative relationship with antioxidant capacity ($r = -0.892$, $p < 0.01$), while TPC was curvilinear, based on Pearson correlation analysis. This indicates that TPC is not an adequate representation of the functional bioactivity of thermally processed pomegranate arils. Notably, at 50°C , the TPC to antioxidant capacity ratio increased four times to 0.861 compared to the fresh control (0.208). The shift shows

that although a larger pool of Folin-reactive compounds was freed after thermal treatment, the average specific radical-scavenging efficiency was markedly decreased.

In terms of the industrial and processing aspects, 50 °C is the optimal drying temperature. This condition is an attractive compromise for the maximization of phenolic recovery and can maintain almost 95% of a reasonable time and energy cost of the original antioxidant capacity [5]. On the other hand, temperatures above 60°C help in the rapid removal of moisture. However, there is almost a 50% loss in the antioxidant potential, which is quite a loss in quality. Hence, it is not favorable for manufacturers looking for quality functional food and nutraceutical applications.

Figure 3 shows a complex and highly significant effect of hot-air drying on the TFC of pomegranate aril total flavonoid content ($p < 0.0001$). The data revealed that all thermal treatments resulted in a TFC much higher than that of the fresh arils (51.62 $\mu\text{g/g}$ FW) in a non-linear manner. The highest value of TFC which was 252.96 $\mu\text{g/g}$ FW) was obtained at 50 °C, which is almost five times higher than that of the fresh control. When the temperature was raised to 60 °C and then further increased to 70 °C, the TFC decreased to 190.38 $\mu\text{g/g}$ FW and 91.78 $\mu\text{g/g}$ FW, respectively. Interestingly, the sample dried at the highest temperature was still significantly richer in flavonoids than the raw material.



Mean Total Flavonoid Content (TFC) values ($\mu\text{g/g}$ Fresh Weight) for samples A (fresh-blue), B (dried at 50 °C - orange), C (dried at 60 °C - gray), and D (dried at 70 °C - yellow).

Values are presented as the mean \pm SD ($n=3$). Different superscript letters indicate significant differences ($p < 0.05$) according to Duncan's test.

The first increase in measurable flavonoids due to mild heating is a well-known phenomenon observed during the analysis of plant material. This is usually not related to flavonoid synthesis, but rather to increased extractability. The main mechanism that allows for higher extractable flavonoid concentrations is the heat-mediated degradation of cell structures, such as cell walls and membranes, which releases flavonoids that are not normally accessible in fresh tissue due to being bound or sequestered in cell compartments [19]. In addition, the application of thermal energy can lead to the hydrolysis of larger, more complex phenolic polymers such as tannins into simpler monomeric flavonoid units that are more easily detected by the assay [20].

On the other hand, the next decrease in TFC at 60 °C and 70 °C indicates the thermolabile nature of the compound. When the temperature exceeds a certain level, the binding of flavonoids is broken. Their liberation from the food matrix occurs faster than their decay through oxidation and structural cleavage. The degree of temperature degradation of the flavonoids of Madagascar and Namwah bananas showed concurrence with other fruits that scientists reported above 60 °C, flavonoid degradation takes off, particularly that of anthocyanins [19, 21]. Based on these data, 50 °C is the ideal temperature for pomegranate arils, as it is suitable for enhancing extractability while limiting thermal damage. These results indicate that, from a processing point of

view, 50 °C is better for maximizing flavonoid recovery; however, any drying process, even at less-than-optimal temperatures, will produce a material with higher bioaccessible flavonoids than fresh arils [21, 22].

4. Conclusion

According to the current study, drying temperature is an important factor affecting the retention of bioactive compounds in the arils of pomegranate. The fresh arils had maximum antioxidant capacity, but by drying at 50°C, 95% of the antioxidant capacity of the dry arils was retained with an apparent enrichment of total phenolic compounds and total flavonoid content. The marked degradation observed at 70°C proves that high temperatures should be avoided in pomegranate processing. The differences in antioxidant capacity and total phenolic content reveal the complexity of the thermal effects on bioactive compounds and point to the necessity of quality assessments. Our researchers recommend that 50°C is the best temperature to dry the pomegranate arils because of the balance achieved between processing efficiency and the quality of nutrition. This will be helpful for the food industry to produce pomegranate products with optimum retention of health-beneficial components. The nutritional value of dried pomegranate juice powder can help in formulating fine quality recipes. Furthermore, it can also meet the growing functional food market. Future research should examine the mechanism of phenolic change during drying as well as the bioavailability of these compounds in processed products.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

R. D. Mustafa1 and N. A. Mirzan*2; methodology, writing original draft preparation, N. A. Mirzan; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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No Informed Consent Statement.

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No Data Availability Statement.

Conflicts of Interest:

no conflicts of interest

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