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Aayushi Pandey
Department of Food Safety and Quality Assurance, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

Dr. JK Momin
Department of Food Safety and Quality Assurance, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

Dr. RB Modi
Department of Food Processing Technology, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

KS Damle
Department of Food Safety and Quality Assurance, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

Dr. RM Dhingani
Department of Food Safety and Quality Assurance, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

Dr. BH Joshi
Department of Food Safety and Quality Assurance, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

Corresponding Author:
Aayushi Pandey
Department of Food Safety and Quality Assurance, College of Food Processing technology and Bioenergy, Anand Agricultural University, Anand, India

Standardization of Vacuum Drying Conditions Based on Biochemical Composition of Pomegranate Peel for Polyphenol Extraction

Aayushi Pandey, Dr. JK Momin, Dr. RB Modi, KS Damle, Dr. RM Dhingani and Dr. BH Joshi

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Abstract
Pomegranate (*Punica granatum* L.) peel is a nutrient-rich agro-industrial by-product, valued for its high concentration of polyphenols and antioxidant compounds. However, these bioactive constituents are sensitive to thermal degradation, necessitating the standardization of drying techniques. This study was conducted to optimize vacuum tray drying conditions for the Mridula variety of pomegranate peel to maximize the retention of total phenolic content (TPC). Pomegranate peels were subjected to vacuum drying at -600 mmHg pressure across three temperature levels (40 °C, 50 °C, and 60 °C). The influence of these temperatures on moisture reduction, TPC, and overall antioxidant activity was systematically evaluated using a Completely Randomized Design (CRD). Results indicated that drying temperature significantly influenced phenolic retention ($p < 0.05$), with TPC decreasing as the temperature increased from 40 °C to 60 °C. The highest TPC yield of 242.15 ± 3.56 mg GAE g^{-1} was achieved at 40 °C, identifying this as the optimal processing condition. These findings provide a scalable, effective method for preserving the functional quality of pomegranate peel powder for nutraceutical and food industry applications.

Keywords: Pomegranate peel, Vacuum tray drying, Polyphenols, Antioxidant activity, Bioactive compounds

Introduction
Pomegranate (*Punica granatum* L.) peel is an abundant agro-industrial by-product, recognized for its rich profile of bioactive compounds, including polyphenols, flavonoids, and potent antioxidants. Compared to the edible arils, the peel possesses significantly higher concentrations of these health-promoting agents (Venkataramanamma *et al.*, 2016) [16]. Furthermore, pomegranate peel extracts exhibit significant anti-inflammatory and oxidative stress modulation potential, and their valorization is considered a key strategy for sustainable waste management.

However, effective recovery of these compounds is constrained by thermal sensitivity. Post-harvest processing, specifically drying, is a critical step in preserving the bioactive profile; however, inconsistent parameters can severely undermine phytochemical quality (Magangana *et al.*, 2020) [8]. Vacuum drying has emerged as a superior alternative, enabling moisture removal at lower temperatures and reduced oxygen exposure, which minimizes oxidative and thermal damage to bioactive constituents (Tran *et al.*, 2024) [15].

Despite the theoretical advantages of vacuum drying, there remains a need for precise optimization of processing parameters such as temperature and pressure to balance drying kinetics with the retention of bioactive quality. Furthermore, the literature reveals significant variability in outcomes due to differing, unstandardized operational parameters, which complicates the identification of optimal processing conditions for specific fruit varieties (Al-Rawahi *et al.*, 2013) [1]. Consequently, this study aims to standardize vacuum tray drying conditions specifically for the *Mridula* variety of pomegranate peel. By systematically evaluating the influence of varying drying temperatures at a constant vacuum pressure of -600 mmHg, this research seeks to establish an optimal processing protocol that maximizes the retention of polyphenols and overall antioxidant capacity in pomegranate peel powder.

Materials and Methods

Materials

Pomegranate (*Punica granatum* L.) fruits of the Mridula variety were procured from a local market at commercial maturity. All analytical grade chemicals including sodium carbonate, sodium hydroxide, potassium acetate, copper sulphate, gallic acid, DPPH (High Media Laboratories Pvt. Ltd.), Folin & Ciocalteu phenol reagent (Research Lab Pvt. Ltd.), anhydrous aluminum chloride, methanol (Loba Chemie Pvt. Ltd.), quercetin, sulphuric acid, hydrochloric acid, boric acid, and methyl red indicator (Sisco Research Laboratories Pvt. Ltd.) were used as received. All reagents were prepared following standard laboratory protocols. For packaging, 50 mL black metallized vacuum stand-up pouches (without zipper) were sourced from Swiss Packaging Pvt. Ltd.

Vacuum Tray Drying of Pomegranate Peels

Pomegranate fruits were washed thoroughly under running tap water to remove surface contaminants. Arils were manually separated from the peels. The collected peels were washed with potable water, drained of excess surface moisture, and cut into uniform pieces (3–4 mm) to facilitate consistent heat and mass transfer. Drying was performed using a laboratory-scale vacuum tray dryer equipped with a condenser (0.37 kW) and a rotary vacuum pump (0.75 kW). The drying chamber contained five tray shelves. Prior to loading, the dryer was pre-equilibrated for 20 min to reach the target temperature. Samples were subjected to vacuum drying at -600 mmHg pressure at three temperature levels (40, 50, and 60 °C). The process continued until the moisture content was reduced to <10% (w.b.). Following drying, samples were cooled to room temperature, ground using a laboratory mill, and passed through a 40-mesh sieve to ensure uniform particle size. The resultant powder was vacuum packaged in 50 mL black metallized stand-up pouches (vacuum pressure: 60 mbar; sealing time: 2.3 s; cooling time: 0.5 s). The use of light-impermeable packaging minimized light-induced degradation and moisture absorption. Sealed pouches were stored at -18 °C until further analysis.

Proximate Analysis

The proximate composition of the pomegranate peel powder including moisture, crude protein, crude fat, total ash, and crude fiber, was determined according to the standard analytical procedures of the Association of Official Analytical Chemists (AOAC, 2019). Moisture content was determined by the oven drying method (Method 925.10) until a constant weight was achieved. Crude protein was estimated using the Kjeldahl method (Method 920.87), employing a conversion factor of 6.25 to convert total nitrogen to crude protein. Crude fat was analyzed via solvent extraction using a Soxhlet apparatus (Method 920.39). Total ash content was measured by dry ashing the sample in a muffle furnace at 550 °C (Method 923.03). Crude fiber was determined using the acid-base digestion method (Method 962.09). Total carbohydrate content was calculated by the difference method, subtracting the sum of moisture, protein, fat, ash, and fiber contents from 100%.

Extraction of Phenolic Compounds

Extraction was performed according to the method of Shiban *et al.* (2012) [14] with minor modifications. Briefly, 10 mg of the dried, powdered pomegranate peel sample was macerated with 10 mL of methanol to facilitate the extraction of bioactive compounds. The mixture was stored at 5 °C for 24 h to ensure complete extraction, followed by centrifugation at 3000 rpm for 15 min. The resulting supernatant was collected, filtered, and used as the crude extract for further analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content was estimated using the Folin-Ciocalteu (FC) colorimetric assay, following the protocol of Redha *et al.* (2018) with slight modifications. In a reaction vessel, 1 mL of the crude extract was mixed with 10 mL of distilled water and 2 mL of Folin-Ciocalteu reagent. The mixture was allowed to react for 2 min. Subsequently, 4 mL of 0.7 M Na_2CO_3 solution was added, and the solution was vortexed thoroughly. Finally, 8 mL of distilled water was added to the mixture. The reaction mixture was incubated at room temperature in a dark environment for 30 min to ensure color development. The absorbance of the resulting blue-colored complex was measured at 760 nm using a UV-VIS spectrophotometer against a blank prepared with distilled water and identical reagents. Gallic acid was utilized as the standard, and the total phenolic content was calculated from the standard calibration curve and expressed as mg GAE g^{-1}.

Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminium chloride colorimetric assay, as described by Zhishen *et al.* (1999) with minor modifications. Briefly, 1 mL of the crude extract was mixed with 5 mL of 60% methanol. After an equilibration period of 5 min, 0.2 mL of 10% aluminium chloride ($AlCl_3$) and 0.2 mL of 1 M potassium acetate were added to the mixture. The final volume of the reaction mixture was adjusted to 10 mL using distilled water. The solution was vortexed thoroughly and incubated in the dark at room temperature for 15 min. The absorbance was measured at 510 nm using a UV-VIS spectrophotometer against a blank prepared with distilled water and identical reagents. The TFC was calculated from a quercetin standard calibration curve and expressed as mg QE g^{-1}.

Antioxidant Activity

The antioxidant potential of the pomegranate peel extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the protocol of Brand-Williams *et al.* (1995) [3] with minor modifications. Briefly, an aliquot of the methanolic extract (equivalent to 100 mg of sample) was transferred to a reaction vessel and mixed with 2.9 mL of 0.1 mM DPPH solution. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 30 min to ensure complete reaction. Absorbance was subsequently measured at 517 nm using a UV-VIS spectrophotometer, with methanol serving as the blank. The percentage of DPPH radical scavenging activity was calculated as follows:

$$\% \text{ Scavenging Activity} = [(\text{blank} - \text{sample}) / \text{blank}] \times 100$$

where blank is the absorbance of the DPPH solution without the extract, and sample is the absorbance of the reaction mixture containing the extract.

Statistical Analysis

To ensure the reliability and reproducibility of the findings, all experiments were conducted in triplicate (n=3), and data were expressed as mean ± standard deviation. The data obtained from the Completely Randomized Design (CRD) were subjected to one-way analysis of variance (ANOVA) to determine the significance of the effects of vacuum drying temperature on the biochemical composition and antioxidant activity of pomegranate peel. Duncan's Multiple Range Test (DMRT) was employed to determine the significance of differences between treatment means at a significance level of $p < 0.05$. All statistical computations and graphical representations were performed using [Insert your software, e.g., SPSS Version 20.0 / OPSTAT / Minitab].

Results and Discussions

Biochemical Composition of Pomegranate Peel

The initial moisture content of the fresh pomegranate peel was determined to be 69.98 ± 0.13% (w.b.). Following vacuum tray drying, the moisture content was reduced to 7.21 ± 0.50% (d.b.), ensuring the stability and shelf-life of the resultant powder. The proximate composition and bioactive potential of the processed pomegranate peel powder are presented in Table 1.

The proximate analysis revealed that the powder is predominantly comprised of carbohydrates (73.31 ± 0.24%) and dietary fiber (16.26 ± 0.17%), which highlights its suitability as a functional ingredient for food fortification. The protein (2.62 ± 0.31%) and ash (4.54 ± 0.25%) contents align with the nutritional profiles reported for similar pomegranate varieties. Furthermore, the high content of total phenolics (264.80 ± 0.43 mg GAE g^{-1}) and the significant antioxidant activity (94.18 ± 0.21%) confirm the potential of this agro-industrial by-product as a source of natural bioactive compounds. These findings are consistent with the observations of Muhammad *et al*. (2023), Hossain *et al*. (2023), and Ibrahim *et al*. (2026) [10, 6, 7], reinforcing that the processing conditions employed successfully preserved the functional properties of the peel. Observed variations in composition compared to earlier studies (Fawole *et al*., 2012) [4] may be attributed to differences in genetic variety, fruit maturity at harvest, and the specific vacuum drying parameters applied.

Vacuum Tray Drying of Pomegranate Peels

Lower drying temperatures for pomegranate peel extended drying time as shown in Table 2, whereas higher drying temperatures resulted in a loss of total phenolic content negatively impacting the responses. These findings align with those reported by Sarkar *et al*. (2024) [13] regarding the thermal degradation of bioactive compounds, where elevated drying temperatures resulted in reduced antioxidant capacity and phenolic stability.

Instantaneous drying rates (g h^{-1}) were calculated as weight loss per hour. Initial (0-1 h) drying rates increased with temperature: 32.94 g h^{-1} (40 °C), 62.06 g h^{-1} (50 °C) and 79.18 g h^{-1} (60 °C). Across all temperatures, drying rate declined over time, indicating a transition from a short constant-rate due to surface moisture removal period to a prolonged falling-rate period due internal diffusion controlled. By 6–8 h, rates at different temperatures converged to low values less than 2 g h^{-1}. The experimental data obtained was employed to generate a drying curve depicting the relationship between moisture content and drying time as presented in Fig. 3. The vacuum drying treatment conducted at 40 °C required the longest drying duration of 10 h to achieve the desired moisture content, whereas the shortest drying time of 6 h was observed at 60 °C. Drying at 50 °C required an intermediate duration of 8 h to attain comparable moisture reduction. At lower temperatures prolonged drying period was required to achieve moisture reduction in pomegranate peels whereas 60 °C accelerated the process. These observations confirm the influence of drying temperature on the moisture removal of pomegranate peels under vacuum conditions. The drying profile showed two distinct phases, an initial phase with a notable decrease in moisture content over time followed by a slower drying rate in the final stage due to reduced mass transfer driving force. This behaviour likely resulted from the faster evaporation of surface moisture initially while internal moisture required longer diffusion pathways to reach the surface. Similar findings were reported by Hameed *et al*. (2022) [5].

Standardization of Vacuum Drying Process Parameters

The standardization of vacuum drying parameters was directed toward optimizing the recovery of bioactive compounds, with Total Phenolic Content (TPC) serving as the primary response variable. The experimental data (Table 3) illustrates a significant temperature-dependent impact on phenolic retention, with visual trends depicted in Fig. 4.

The highest TPC yield was recorded at 40 °C (242.15 ± 3.56 mg GAE g^{-1}), followed by 50 °C (222.00 ± 5.21 mg GAE g^{-1}), and 60 °C (194.73 ± 3.75 mg GAE g^{-1}). These results reveal a clear inverse correlation between drying temperature and TPC retention, suggesting that elevated processing temperatures promote the thermal degradation of heat-sensitive compounds. This trend aligns with the findings of Mphahlele *et al*. (2016) [9], who reported that extended exposure to higher temperatures during drying leads to the thermolabile breakdown of bioactive constituents.

Statistical analysis via one-way ANOVA (Table 4) confirmed that drying temperature exerted a significant influence on the TPC of the pomegranate peel powder ($p < 0.05$). The high F-value (31.52) indicates that temperature is the dominant factor driving the variance in phenolic retention. The application of Duncan's Multiple Range Test (DMRT) further validated distinct performance gaps between the 40 °C, 50 °C, and 60 °C treatments (Table 3), reinforcing the suitability of 40 °C as the optimal processing condition for maximizing the functional quality of the peel powder.

These findings align with studies on the vacuum drying of various vegetables, which demonstrate that elevating drying temperatures or extending processing durations consistently leads to a reduction in phenolic compounds. The reduction in TPC observed in pomegranate peels as temperatures rose from 40 °C to 60 °C can be attributed to the thermal degradation of heat sensitive phenolic compounds during extended thermal exposure (Mphahlele *et al*., 2016) [9].

The analysis of variance (ANOVA) presented in Table 4 confirms a statistically significant relationship ($p < 0.05$) between drying temperature and the TPC of pomegranate peel. The high F-value indicates that the differences in mean values across the tested temperatures are robust and not attributable to random chance. Furthermore, the sum of squares analysis reveals that drying temperature is the

primary driver of variance in phenolic retention, accounting for the majority of the total observed variation. The low coefficient of variation underscores the precision and reproducibility of the experimental measurements, validating the suitability of the proposed vacuum drying protocol for high-quality product output. These bioactive-rich extracts are highly promising for the food industry; recent research highlights their efficacy not only as functional ingredients but also as natural preservatives capable of enhancing shelf life in food systems.

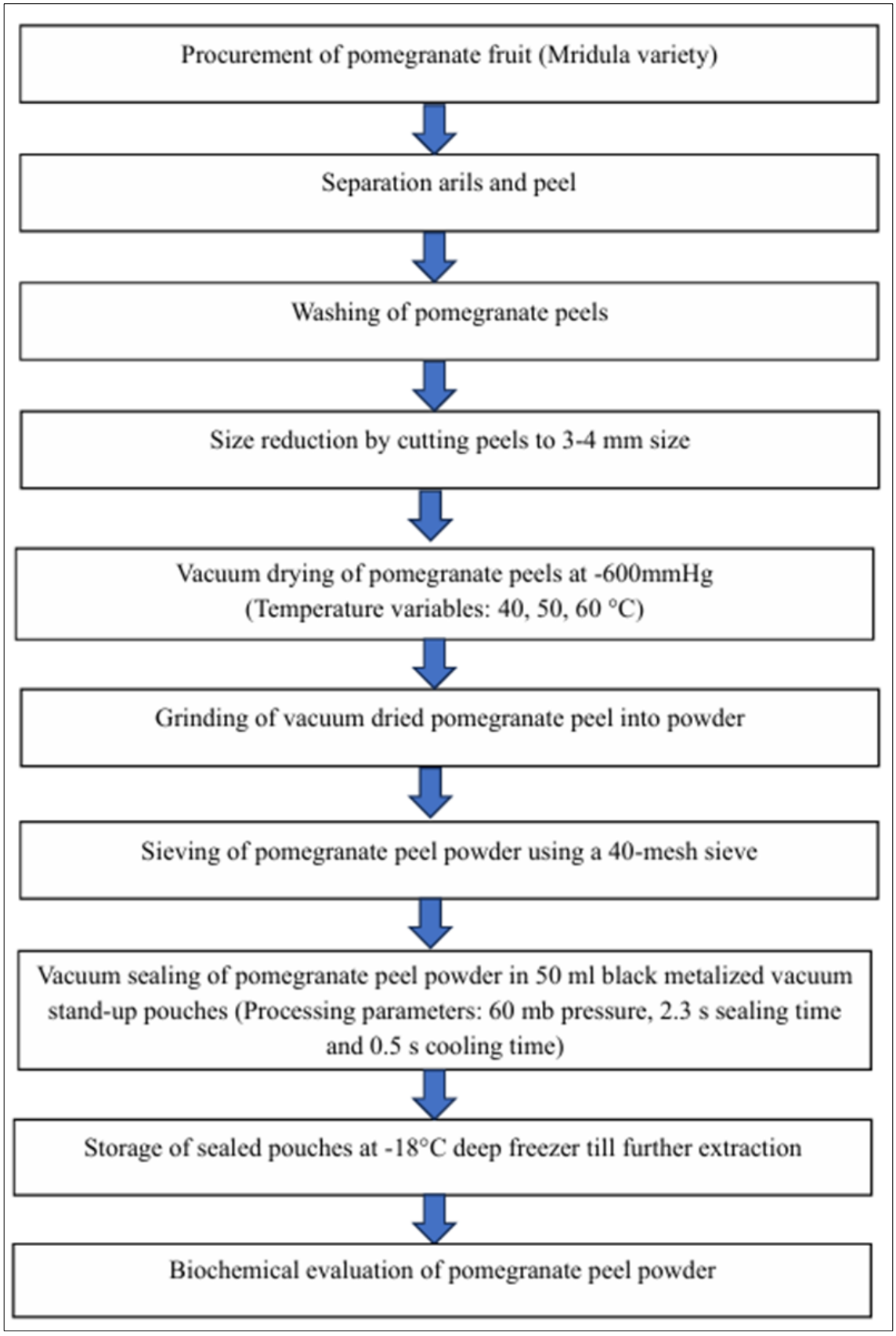

Fig 1: Schematic flow diagram of the vacuum drying process for pomegranate peel

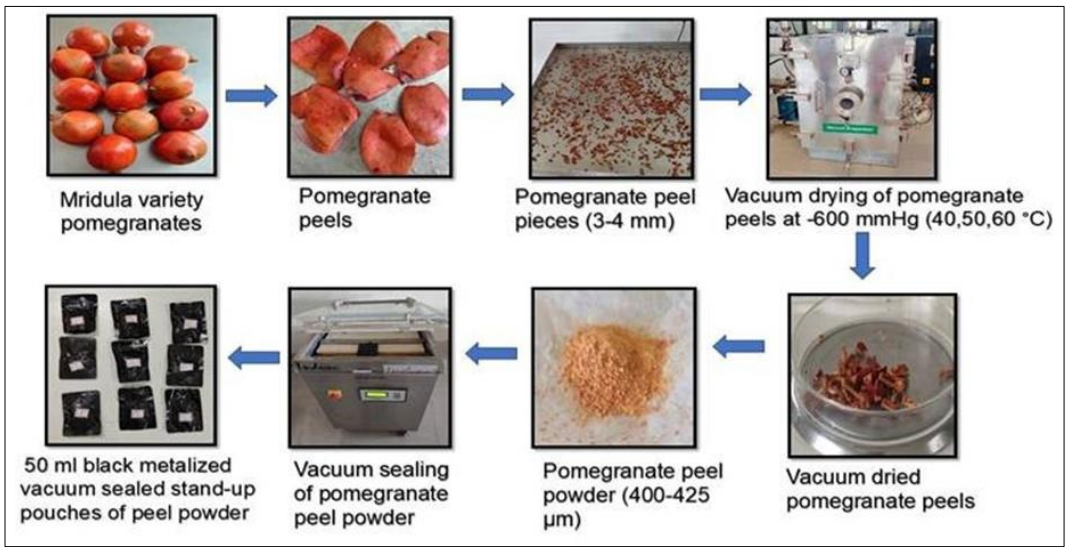

Fig 2: Pictorial overview of the experimental procedure for pomegranate peel processing

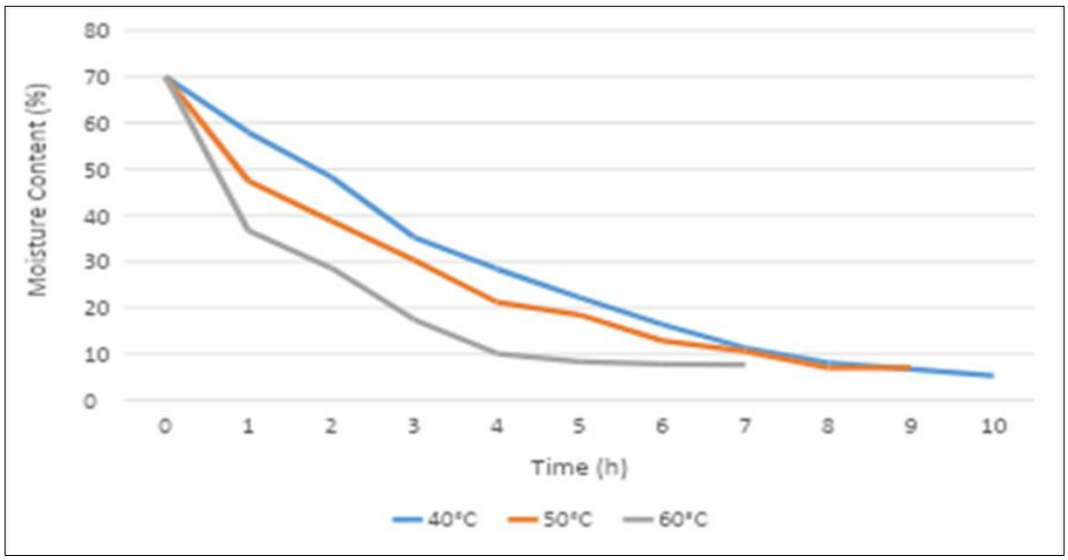

Fig 3: Influence of vacuum drying temperature on the moisture content reduction of pomegranate peel over time

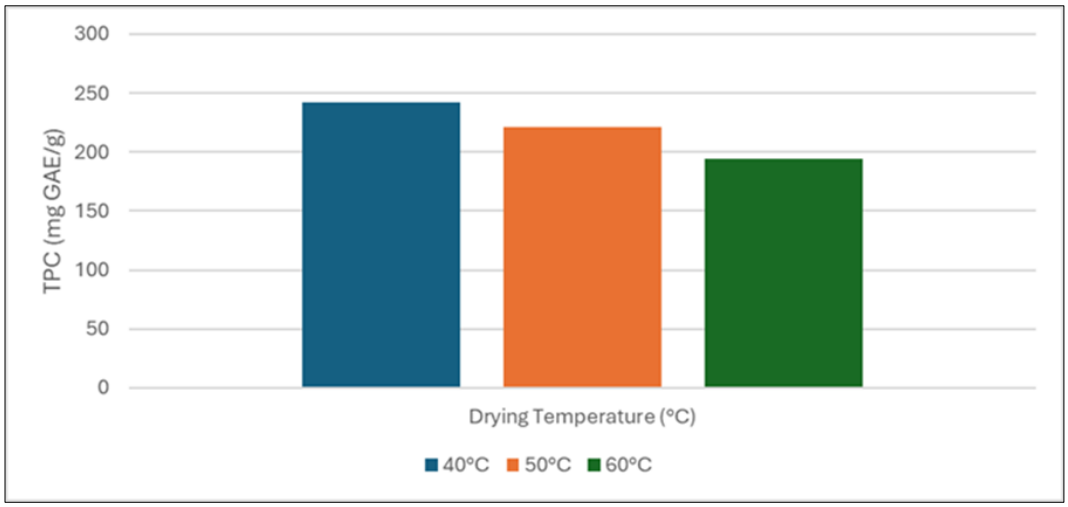

Fig 4: Influence of vacuum drying temperature on the total phenolic content (TPC) of pomegranate peel

Table 1: Proximate composition and bioactive potential of pomegranate peel powder

Parameters	Value (d.b.)*
Proximate Composition (%)	
Moisture	7.21 ± 0.50
Crude Fat	0.60 ± 0.15
Crude Protein	2.62 ± 0.31
Total Ash	4.54 ± 0.25
Crude Fibre	16.26 ± 0.17
Carbohydrate (by difference)	73.31 ± 0.24
Bioactive Compounds	
Total Phenolic Content (mg GAE g^{-1})	264.80 ± 0.43
Total Flavonoid Content (mg QE g^{-1})	35.26 ± 0.08
Antioxidant Activity (%)	94.18 ± 0.21

*Note: All values are expressed as mean ± SD (n=3) on a dry basis (d.b.). The initial moisture content of fresh peel was 69.98 ± 0.13% (w.b.).

Table 2: Influence of vacuum drying temperature on the weight reduction of pomegranate peel

Drying Time (h)	Weight at 40°C (g)	Weight at 50°C (g)	Weight at 60°C (g)
0	150.00 ± 0.00	150.00 ± 0.00	150.00 ± 0.00
1	117.06 ± 0.50	87.94 ± 0.40	70.82 ± 0.35
2	87.72 ± 0.45	73.34 ± 0.35	62.92 ± 0.30
3	69.58 ± 0.40	64.58 ± 0.30	54.51 ± 0.25
4	62.94 ± 0.35	57.82 ± 0.25	50.08 ± 0.20
5	57.89 ± 0.30	55.22 ± 0.25	49.12 ± 0.15
6	53.72 ± 0.25	51.63 ± 0.20	48.86 ± 0.15
7	50.39 ± 0.20	50.32 ± 0.20	48.49 ± 0.15
8	49.14 ± 0.20	48.65 ± 0.15	—
9	48.77 ± 0.15	48.33 ± 0.15	—
10	48.28 ± 0.15	—	—

*Note: Initial sample weight was 150 g. Values represent the mean ± standard deviation (n=3). "—" indicates the point at which the final moisture content (<10%) was achieved.

Table 3: Effect of vacuum tray drying temperature on Total Phenolic Content (TPC) of pomegranate peels

Vacuum Drying Temperature (°C)	TPC (mg GAE g^{-1})
40	242.15 ± 3.56^{a}
50	222.00 ± 5.21^{b}
60	194.73 ± 3.75^{c}
C.D. ($p < 0.05$)	14.95
S.E.(m)	4.24

Values are expressed as Mean ± S.E. (n=3). Different superscript letters (a, b, c) within the same column indicate significant differences ($p < 0.05$) as determined by Duncan's Multiple Range Test.

Table 4: ANOVA table for total phenolic content of pomegranate peels

Source	df	Sum of squares	Mean squares	F-Value	P-Value
Temperature (°C)	2	3397.93	1698.96	31.52	0.001
Error	6	323.44	53.91	—	—
Total	8	3721.37	—	—	—

df: degrees of freedom. Significance is determined at $p < 0.05$.

Conclusion

This study successfully standardized vacuum tray drying conditions for the *Mridula* variety of pomegranate peel, identifying 40 °C at -600 mmHg as the optimal protocol for maximizing the retention of bioactive compounds. Proximate analysis confirmed that the resulting powder is a nutrient-dense functional ingredient, rich in dietary fiber (16.26%) and carbohydrates (73.31%), with a significant total phenolic content (264.80 mg GAE g^{-1}) and robust antioxidant activity (94.18%). Experimental results demonstrated an inverse correlation between drying temperature and phenolic retention; while elevated temperatures (50 °C and 60 °C) accelerated moisture reduction, they detrimentally impacted the thermolabile bioactive profile. Statistical validation via ANOVA and DMRT confirmed that the 40 °C treatment maintained significantly higher phytochemical integrity compared to higher temperature treatments. Consequently, vacuum drying at 40 °C represents an effective, scalable strategy for preserving the functional and phytochemical properties of pomegranate peel, facilitating its application as a high-value, natural ingredient in the nutraceutical and food processing industries, consistent with industrial valorization trends.

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