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Pomegranate (*Punica Granatum L)* Peel Powder as a Partial Replacement for Wheat Flour in Chocolate Cookies: Effect of Particle Size on the Physical and Antioxidant Properties

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Abstract

This study aimed to evaluate the effect of different particle sizes of pomegranate peel powder (PPP) as a partial replacement for wheat flour on the physical and antioxidant properties of cookies. The PPPs were prepared in three different particle sizes (1.0 mm, 0.5 mm, and 0.25 mm) and incorporated into cookies at a 15% substitution level. The physical properties of the cookies were evaluated, and the antioxidant properties were assessed in both dough and cookies to determine the effect of particle size on antioxidant retention after exposure to heat during baking. The particle size of PPP significantly affected the overall appearance, hardness, spread ratio, and colour of cookies. Reducing particle size decreased the cookie diameter and spread ratio, resulting in increased hardness. The appearance of the cookies became more compact and smoother with smaller particle sizes (0.25 mm) and exhibited a higher L* value. Furthermore, dough and cookies containing the smallest PPP particles (0.25 mm) exhibited significantly higher total phenolic content (TPC) values, DPPH radical scavenging activity, and ferric-reducing antioxidant power compared (FRAP) compared to samples with the largest particle size. This was attributed to the higher release of bioactive compounds due to the larger surface area. Baking caused some thermal degradation of antioxidants, resulting in slightly lower TPC and DPPH values in cookies than in dough. These findings highlight PPP's potential as a functional ingredient, enhancing the nutritional quality of cookies while reducing pomegranate peel waste.

INTRODUCTION

Pomegranate (*Punica granatum L.*) is a seeded or granular apple, native to Afghanistan, Iran, China, and the Indian subcontinent [1]. Approximately 55% – 60% of the pomegranate fruit's weight is made up of its edible portion, or arils, which are composed of 80% juice and 20% seeds [2]. After extracting the juice, the peel and seeds are counted up to 54%, equal to 1.62 million tons per year [3]. The peel is a by-product of the juice processing industry and has been identified for its high antioxidant activity [4], attributed to phenolic compounds such as flavonoids and hydrolysable tannins [1, 3]. In a study comparing the antioxidant activity and total phenolic content (TPC) of orange and pomegranate peels, it was found that orange peel extracts had a substantially lower TPC of 35.73 mg/g GAE, while pomegranate peel extracts had a TPC of 139.40 mg/g GAE [5]. The pomegranate juice industry generates large quantities of pomegranate peel, which is disposed of as agricultural waste. Its high biological oxygen demand (BOD) contributes to greenhouse gas emissions, impacting the environment. In recent years, considerable attention has been given to the valorization of pomegranate peel in bakery products, leveraging its richness in dietary fiber and antioxidants. It has been successfully incorporated into biscuits [6], bread [7], and muffins [8], with studies reporting significant increases in fiber and polyphenol content at inclusion levels of 5-10%, with optimal acceptability limited to 5 – 7.5% only. To enhance the development of functional foods using agricultural by-products rich in bioactive compounds, it is desirable to increase their incorporation levels.

One critical factor influencing product acceptability is the particle size of the added material. Therefore, this study investigates the effect of pomegranate peel particle size (15%) on the physical characteristics and antioxidant properties of chocolate cookies. Powder particle size has a significant impact on the structural and antioxidant properties of food products. It is a fundamental factor influencing the functional and physicochemical properties of processed foods, which, in turn, affects the consumer acceptability of bakery products. Studies have shown that varying particle sizes (<0.15 mm to 0.18 mm) significantly impact the hardness and spread ratio of cookies [9]. Another study has demonstrated that smaller particle sizes (0.01–0.03 mm) in chocolate production result in a smoother texture, whereas larger particles (0.05–0.1 mm) create a slightly grainy or rough texture [10]. Additionally, cookies containing smaller particle sizes (0.104 mm) of grape seed flour have been reported to have higher polyphenol content but lower sensory acceptability [11].

Therefore, this study was conducted to evaluate the impact of varying particle sizes of pomegranate powder (1.0 mm, 0.5 mm, and 0.25 mm) on the physical and antioxidant properties of chocolate cookies. In the development of new products incorporating peel powder, particle sizes ranging from 0.25 to 1.0 mm are commonly used [12, 13]. The physical properties of the cookies were assessed, while the antioxidant properties were evaluated in both the dough and the cookies to determine the effect of particle size on preserving polyphenol content after exposure to heat during baking.

MATERIALS AND METHODS

Preparation of pomegranate peel powder

The pomegranates were washed under running tap water to remove any dirt and impurities. They were then chopped into smaller pieces before being steam-blanched for 3 min. The peels were subsequently dried in an oven at 60 °C for 24 h [14]. The dried pomegranate peels were ground into powder using a dry mill grinder (Panasonic, Malaysia), and the powder was sieved through 1.0 mm, 0.5 mm, and 0.25 mm mesh sizes. The pomegranate peel powder (PPP) was stored in sealed, airtight low-density polyethylene bags and kept in a freezer at -18°C for future analysis.

Preparation of Extract for Antioxidant Analysis

The extract was prepared using the method outlined by Mercado-Mercado et al. [15]. A 50 mL capped centrifuge tube was filled with 2 g of each sample and 20 mL of acidified methanol solution (50 0.8 M HCl: 50% methanol, v/v, 60 min). The tubes were then shaken continuously at room temperature using an orbital shaker at 200 rpm for 1 h. The samples were then centrifuged at 6243 *g* for 10 min at 4°C using a Hettich Zentrifugen D-78532 Tuttlingen (Germany) refrigerated centrifuge. After collecting the supernatants, the remaining residues were re-extracted with 20 mL of acetone-water (70:30, v/v), then shaken and centrifuged under the same conditions. For the determination of total phenolic content, DPPH, and FRAP of pomegranate peel powder for dough and cookies, the supernatants from the first and second extractions were combined (50:50, v/v) and kept in a freezer at -80 °C.

Preparation of cookies

Chocolate cookies were selected for this study because cocoa powder is an important ingredient that helps minimize bias during sensory evaluation and masks any potential unpleasant taste from PPP. Cookies were prepared by incorporating PPP at 15% of the wheat flour weight, following the method described by Abreu et al. [16] with slight modifications on the number of ingredients, as shown in **Table 1**. The 15% level was chosen to evaluate the feasibility of higher incorporation beyond the commonly studied range (5-10%), aiming to enhance the functional properties, particularly in antioxidant capacity. Cookies without PPP were used as the control sample. Margarine, castor sugar, and brown sugar were manually mixed for 1 minute or until homogeneous. Wheat flour, cocoa powder, and PPP were gradually added to the mixture and mixed evenly. Then, 5 g portions of dough were weighed and pressed to a thickness of 1 cm on a baking pan. The baking pans were placed in a deck oven and baked at 150 °C for 15 min.

Table 1. The formulations of chocolate cookies incorporated PPP with varying particle sizes.

Ingredient	Formulations (per 100 g)			
	Control	F1 (1.0 mm)	F2 (0.5mm)	F3 (0.25mm)
PPP	0	5.1	5.1	5.1
Wheat flour	34	28.9	28.9	28.9
Castor sugar	18	18	18	18
Brown sugar	10	10	10	10
Margarine	35	35	35	35
Cocoa powder	3	3	3	3

PPP = pomegranate peel powder

Antioxidant analysis

Antioxidant properties were assessed on both dough and cookies. This assessment was evaluated in terms of total phenolic content (TPC), Ferric Reducing Antioxidant Power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Determination of total phenolic content (TPC)

The TPC was determined using the Folin-Ciocalteu method of Tian et al. [17]. A test tube containing 0.5 mL of the antioxidant extract and 2.5 mL of 10% Folin-Ciocalteu reagent was left at room temperature in the dark for 2 min. Then, 2 mL of a 7.5% sodium carbonate solution was added and mixed thoroughly. The sodium carbonate solution was prepared by dissolving 7.5 g of sodium carbonate in 100 mL of distilled water in a volumetric flask. The mixture was allowed to stand at room temperature in the dark for 1 h. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Distilled water was used as a negative control. A gallic acid standard curve, prepared within a concentration range of 0 to 200 mg/L, was used to calculate the gallic acid equivalent (GAE). The TPC was expressed as milligrams of GAE per gram of the extract (mg GAE/g).

Determination of antioxidant activity by Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was performed following the method outlined by Mercado-Mercado et al. [15]. To prepare the FRAP working solution, sodium acetate buffer (0.3 M, pH 3.6), TPTZ-HCl (10 mM in 40 mM HCl), and ferric chloride hexahydrate (20 mM) were mixed in a ratio of 10:1:1 ratio. The resulting mixture was reheated in a water bath at 37°C for 30 min before being combined with the antioxidant extracts. The sodium acetate buffer was prepared by dissolving 0.31 g of sodium acetate trihydrate in 1.6 mL of glacial acetic acid, then adding distilled water to a final volume of 100 mL, adjusting the pH to 3.6. To prepare the TPTZ-HCl solution, 78 mg of TPTZ was dissolved in 25 mL of 40 mM hydrochloric acid (HCl), which was obtained by diluting 0.328 mL of 37% HCl in distilled water to a final volume of 100 mL.

Ferric chloride solution (20 mM) was prepared by dissolving 135 mg of ferric chloride hexahydrate in 25 mL of distilled water and stored at 4°C. For the assay, 240 µL of the antioxidant extract was mixed with 1800 µL of the FRAP working solution. The mixture was brought to room temperature and incubated in the dark for 30 min. Methanol was used in place of the extract as a negative control. Absorbance was measured at 595 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). A standard curve was prepared using ascorbic acid solutions ranging from 0 to 60 mg/L. Results were expressed as milligrams of ascorbic acid equivalents per gram of sample (mg AAE/g).

Determination of antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH)assay

The antioxidant extract was combined with 2 mL of a DPPH methanolic solution (100 µM) and allowed to stand for 30 min at room temperature in the dark. To prepare a 100 µM DPPH solution, 3.94 mg of DPPH was dissolved in 100 mL of 70% methanol, stirred for at least 1 h at room temperature in the dark until fully dissolved. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Methanol was used in place of the extract as a negative control. A standard curve was constructed using ascorbic acid solutions ranging from 0 to 30 mg/L. The DPPH scavenging activity % was calculated using the following formula:

$$\text{Percentage Inhibition of DPPH (\%)} = \frac{\text{Absorbance of Control-Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Texture analysis

The textural properties of cookies were measured using a TA-XT2 Texture Analyzer (Stable Micro Systems, UK) to determine hardness. The parameters used for the analysis included a trigger force of 5 g, a load cell of 30 kg, a pre-test speed of 2.0 mm/s, a test speed of 2.0 mm/s, a post-test speed of 2.0 mm/s, and a test distance of 15 mm. hardness was measured at the point when cookie broke into two large pieces and the highest peak force was recorded. The peak force (g) at the breaking point represented the breaking strength of the cookie [18]. The measurements were performed in triplicate, and the mean value was recorded as hardness (N).

Spread ratio

The spread ratio is an important parameter in evaluating cookie quality. A digital vernier caliper (Miltutoyo, Japan) was used to measure the diameter of the cookies by placing three cookies edge-to-edge. The total length of the three cookies was measured and divided by three to obtain the average diameter. After rotating each cookie by 90°, two additional diameter measurements were taken. To determine thickness, three cookies were stacked on top of each other and measured using the digital vernier caliper. The cookies were then restacked, and the thickness was measured twice more. The spread ratio was calculated using the formula: diameter/thickness.

Colour analysis

Chromameter CR-400 (Konica Minolta, Japan) was used to measure the light reflected from the cookie surface, providing precise colour information. Prior to analysis, the instrument was calibrated using a white tile (calibration plate). The Hunter colour values - L* (lightness), a* (redness), and b* (yellowness) were obtained from both the top and bottom surfaces of the cookies. Three readings were taken for each sample, and the average was calculated. The results were reported as mean ± standard deviation.

Statistical analysis

All data were expressed as mean ± standard deviation and analysed statistically using Minitab version 21. The data were recorded after triplicate analytical determinations for the sample. One-way ANOVA with Turkey's LSD test was used to analyse all of the data to find any significant differences.

RESULT AND DISCUSSION

The physical appearance of cookies incorporated with 15% pomegranate peel powder

Fig. 1 shows the physical appearance of chocolate cookies incorporating 15% PPP at three different particle sizes. The particle size of PPP significantly influenced the texture and structural appearance of the chocolate cookies, with the smallest particle size (F3) producing a smooth surface texture, while the largest particle size (F1) exhibited a coarse, gritty surface texture with visible PPP flecks throughout the cookie. Cookies prepared with a coarser PPP could have a rougher surface and a less consistent colour than those made with a finer powder, which tends to mix into the dough more easily, as mentioned by Srivastava et al. [6].

The physical properties of cookies incorporated with 15% pomegranate peel powder

The physical properties of chocolate cookies incorporated with 15% pomegranate peel powder (PPP), varying in particle size, were evaluated in terms of diameter, thickness, spread ratio, and hardness, as shown in **Table 2**. Initially, replacing wheat flour with PPP of 1.0 mm particle size (F1) resulted in larger chocolate cookies, probably due to the disruption of the gluten network by the coarse PPP particles, which facilitated greater cookie dough spread during baking. However, as the particle size of PPP was reduced to 0.5 mm and 0.25 mm, a decreasing trend in cookie diameter was observed from 124.21 mm to 86.84 mm, while cookie thickness showed a corresponding increase.

Fig. 1. Physical appearance of chocolate cookies incorporated with different particle sizes of 15% pomegranate peel powder.

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Since the spread ratio was calculated as the ratio of cookie diameter to thickness, the observed reductions in diameter and increases in thickness led to a decrease in the spread ratio as the PPP particle size decreased from 1.0 mm to 0.5 mm and 0.25 mm. A similar trend in the spread ratio was observed by Korese et al. [19], who reduced the particle size of orange-flesh sweet potato flour from 0.5 mm to 0.25 mm in cookies. Margarine, used as the sole fat source and continuous phase ingredient, plays a crucial role in facilitating dough spread. However, in formulation F3, which contained PPP with the smallest particle size and consequently the largest surface area, the amount of margarine was insufficient for effective particle dispersion. This likely contributed to the lowest spread ratio observed. Smaller particle sizes were reported to exhibit stronger intermolecular forces and a larger contact surface area [20], resulting in thicker, less spread cookies during baking [21].

Table 2. Physical parameters of cookies incorporated with different types of particles of pomegranate peel powder.

Formulations	Diameter (mm)	Thickness (mm)	Spread ratio (mm)	Hardness (g)
Control	111.13 ± 1.54 ^{c}	22.60 ± 0.54 ^{b}	4.92 ± 0.06 ^{c}	1663.0 ± 190.0 ^{b}
F1 (1.0 mm)	124.21 ± 2.84 ^{a}	18.32 ± 0.61 ^{c}	6.79 ± 0.36 ^{a}	1164.3 ± 38.7 ^{c}
F2 (0.5 mm)	117.98 ± 0.55 ^{b}	19.11 ± 0.49 ^{c}	6.17 ± 0.01 ^{b}	1358.9 ± 113.0 ^{c}
F3 (0.25 mm)	86.84 ± 0.23 ^{d}	33.29 ± 0.34 ^{a}	2.61 ± 0.03 ^{d}	4006.0 ± 191.0 ^{a}

Note: Values are mean ± SD (standard deviation) of three replicates. Values with different alphabet superscripts within columns are significantly different ($p \leq 0.05$)

The spread ratio of cookies significantly influences their textural properties, particularly hardness. Cookies with a higher spread ratio generally exhibited lower hardness values, likely due to their thinner structure as affected by the spreading effect. As shown in **Table 2**, F1 cookies, which exhibited the highest spread ratio, also showed the lowest hardness. In contrast, F3 cookies, characterized by the smallest spread ratio, demonstrated the highest hardness. This trend aligned with the changes in diameter, thickness, and spread ratio as discussed in the previous paragraph. A similar trend of increased hardness with smaller flour particle sizes was also reported by Troilo et al. [22] when grape pomace was incorporated into muffins.

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Colour properties of cookies incorporated with 15% pomegranate peel powder

The effect of incorporating PPP at different levels on the colour profile of chocolate cookies was shown in **Table 3**. The L* value indicated the lightness of a colour, ranging numerically from 0 to 100, where a higher number represents a lighter colour. The a* value represented the red-to-green spectrum, with positive values indicating red and negative values indicating green. The b* value represented the blue-to-yellow spectrum, with positive values indicating yellow and negative values indicating blue.

Table 3. Colour profile of chocolate cookies incorporated with different particle sizes of 15% pomegranate peel powder.

Treatments	L*	a*	b*
Control	44.19 ± 0.55 ^{a}	4.24 ± 0.20 ^{ab}	5.97 ± 0.31 ^{ab}
F1 (1.0 mm)	42.19 ± 1.33 ^{b}	3.87 ± 0.20 ^{b}	5.36 ± 0.42 ^{b}
F2 (0.5 mm)	42.89 ± 0.64 ^{ab}	4.29 ± 0.16 ^{ab}	5.50 ± 0.16 ^{b}
F3 (0.25 mm)	44.25 ± 0.33 ^{a}	4.75 ± 0.47 ^{a}	6.88 ± 0.86 ^{a}

Note: Values are mean ± SD (standard deviation) of three replicates. Values with different alphabet superscripts within columns are significantly different ($p \leq 0.05$)

The L* value decreased significantly from the control to F1 but showed no significant difference between F1 and F2 or F3, indicating that particle size affects the lightness of the cookies. A larger particle size (F1, 1.0 mm) had a lower total surface area, and its incorporation in place of wheat flour produced a coarse, gritty, uneven surface. In contrast, smaller particle sizes have a greater total surface area, resulting in a well-blended mixture with a smooth, even surface. Rough surfaces typically absorb more light than smooth surfaces, hence the reduction in lightness value. It has been reported that smooth, regular surfaces reflect more light than wrinkled, irregular ones, resulting in a higher lightness value [23].

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The a* and b* values were higher in the F3 sample, possibly due to better blending with other ingredients compared to other formulations.

Total phenolic content
The effect of particle size on the total phenolic content (TPC) of 15% PPP in dough samples is shown in **Fig. 2**. Incorporating 15% PPP into dough and cookies significantly enhanced their TPC, with the extent of this enhancement influenced by PPP particle size. The TPC for dough increased significantly ($p < 0.05$) from 17.08 mg GAE/g to 22.5 mg GAE/g as affected by the different particle sizes. The trend indicated a significant increase in the particle size of PPP as it decreased. This shows that the smaller particle size (F3, 0.25 mm) of PPP enhanced the release of phenolic compounds, due to the increased surface area available for extraction [24].

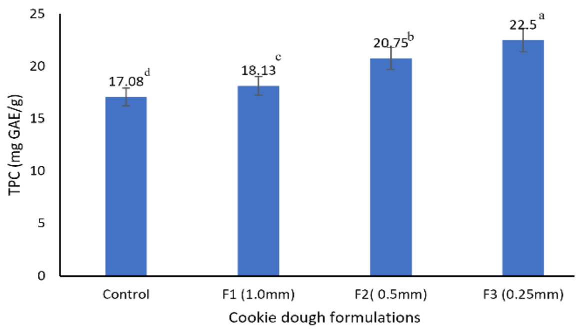

Fig. 2. The total phenolic content of chocolate cookie dough incorporated with different particle sizes of PPP.

Wang et al. [25] reported a similar result in PPP subjected to different levels of steam-explosion pressure, where the highest TPC was observed in samples with the smallest particle size. Specifically, they reported that PPP with a particle size of 0.149 mm exhibited the highest TPC (21.99 mg GAE/g), while PPP with a particle size of 0.841 mm yielded the lowest TPC (18.27 mg GAE/g). The increase in TPC with decreasing particle size is likely due to the enhanced release of phenolic compounds during mixing, as smaller particles offer a greater surface area for solvent interaction. This effect is particularly beneficial for enhancing the antioxidant capacity of the dough. However, it is important to note that the influence of particle size on antioxidant properties may vary across plant materials, as some studies have reported no significant effect [26].

Thermal processing, including roasting, pasteurization, and frying, can significantly impact the antioxidant properties of food. According to ElGamal et al. [27], the antioxidant capacity of most products is lower after drying processing compared to before. In this study, baking reduced the TPC due to thermal degradation of phenolics; hence, cookies exhibited a lower TPC than dough, as shown in **Fig. 2** and **Fig. 3**. The control sample had the lowest TPC (5.5 mg GAE/g). Incorporating PPP significantly increased the TPC of cookies and showed an increasing trend as the particle size of PPP decreased. The smallest particle size of PPP was more effective in providing the cookies with phenolic content and antioxidant properties. A study indicates that adding PPP to cookies improves their antioxidant qualities and increases TPC, while phenolics may be somewhat lost during baking at higher temperatures, there are still significant functional benefits [28].

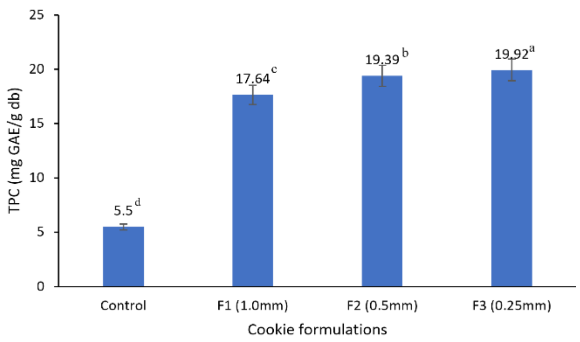

Fig. 3. The total phenolic content of chocolate cookies incorporated with different sizes of PPP.

DPPH (2,2-diphenyl-1-picrylhydrazyl)
The percentage of DPPH inhibition increased as the particle size of PPP decreased. **Fig. 4** and **Fig. 5** showed that the percentage of DPPH inhibition for dough and cookies increased significantly ($p < 0.05$) as the particle size decreased. A study reported by Wang et al. [25] found that decreasing particle size enhanced antioxidant activity, as measured by the DPPH radical scavenging assay, with the DPPH value of 0.149 mm for pomegranate peel powder being 1.19 times that of 0.841 mm. A similar finding was reported by Prasedya et al. [29], who found that smaller powder particles had a higher surface area, which, in turn, enhanced the transfer of bioactive compounds from the biological material solvent [30]. Some antioxidants, such as polyphenols in PPP degraded or lost activity due to high temperature during baking, potentially reducing the DPPH inhibition value of cookies compared to the dough. However, in some studies, the baking process can increase DPPH radical scavenging activity due to the formation of Maillard reaction products [31].

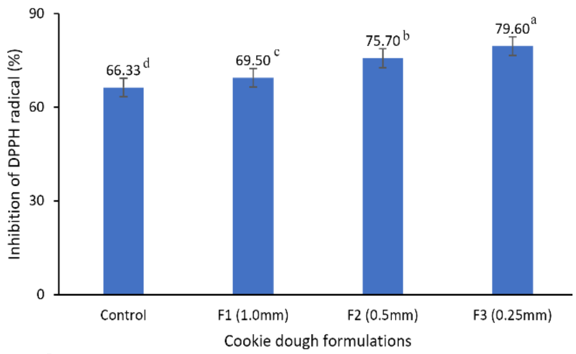

Fig. 4. DPPH radicals scavenging activity of chocolate cookie dough with different particle sizes of PPP.

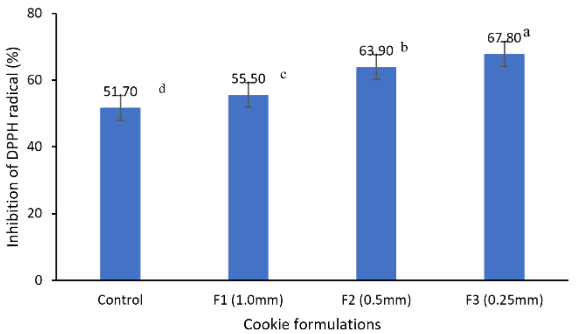

Fig. 5. DPPH radicals scavenging activity of chocolate cookie with different particle sizes of PPP.

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Ferric reducing antioxidant power (FRAP)

The FRAP assay provides a precise way to determine how much antioxidant activity a sample has by measuring the conversion of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). According to Payne et al. (2013), the capacity of electron-donating antioxidants to reduce ferric iron underlies the FRAP assay's unique quantification of their concentration. Similar to the trend of TPC and DPPH, FRAP also exhibited an increasing trend as the particle size of PPP increased for both dough and cookies, as shown in **Fig. 6** and **Fig. 7**. According to Rao et al. [32] pomegranate peel high antioxidant activity was linked to its phenolic compounds, which showed a strong correlation with increased reducing power and FRAP values. FRAP activity is likely to be higher in the dough than in cookies due to the heat degradation of antioxidants in cookies. High temperatures can degrade some heat-sensitive antioxidant compounds (e.g., polyphenols and flavonoids) in pomegranate peel powder. As a result, the FRAP activity in cookies is lower than in dough.

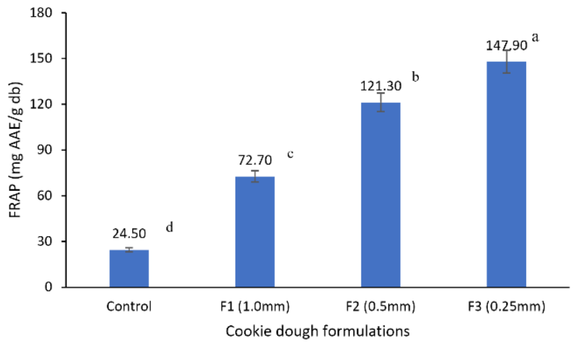

Fig. 6. FRAP activity of chocolate cookie dough containing PPP of different particle sizes.

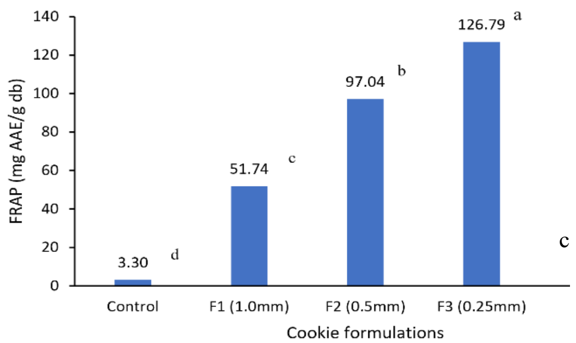

Fig. 7. FRAP activity of a chocolate cookie containing PPP of different particle sizes.

CONCLUSION

The particle size of pomegranate peel powder significantly affects the physical attributes of cookies, including hardness, spread ratio, colour, and appearance. The particle size of PPP also influenced the antioxidant properties of both dough and cookies. Larger particle sizes exhibit reduced antioxidant activity, whereas smaller particle sizes increase the release of bioactive chemicals and result in higher activity. The highest antioxidant properties were observed in cookies containing the smallest particle size (0.25 mm). This formulation exhibited the highest TPC, DPPH, and FRAP assays. However, this particle size also resulted in the smallest spread ratio, leading to the hardest texture. Therefore, future studies are suggested to vary particle size within each formulation by combining the smallest and largest particle sizes in different proportions. Additionally, it is recommended to compare cookies containing 0.25 mm particle size with those containing 0.5 mm PPP at varying levels of incorporation to identify the most acceptable formulation in terms of physicochemical and sensory properties.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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