

## Research Article

# Effect of Freeze-Drying on Apple Pomace and Pomegranate Peel Powders Used as a Source of Bioactive Ingredients for the Development of Functional Yogurt

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Agro-industrial by-products of fruits have turned into an essential source of bioactive products. This study examined the effect of freeze-drying on apple pomace powder (APP) and pomegranate peel powder (PPP) and their utilization in functional yogurt development at different concentrations. Freeze-dried powders in functional yogurt were investigated by chemical profile and bioactive characterization of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. The highest concentration of TPC (4.64) mg GAE/g, TFC (1.73 ± 0.00) CE mg/g, and antioxidant activity (83.87 ± 0.02) % was investigated in the yogurt sample  $T_6$ , having the maximum amount of PPP in it, which was significantly higher compared to the treatments having APP. Yogurt samples were analyzed for their sensory attributes, which showed a decline with the increase in both APP and PPP concentrations in contrast by introducing the optimum levels of APP and PPP (3% or 6%); hence, no significant loss in sensory profile was found as compared to the control samples. The results were found to be significant at the level ( $p < 0.05$ ). In terms of the freeze-dried APP and PPP results, the APP samples had the most complete chemical composition, with the exception of fiber and ash concentration. Treatments of functional yogurt were prepared for their physicochemical profile, which demonstrated a straight proportionate relationship between the proportions of both powders in the meantime. Protein and fat levels were likely to decrease as both dry powder levels increased. Hence, apple pomace and pomegranate peel can be used after freeze-drying as a rich source of bioactive compounds in functional yogurt in the food industry.

## 1. Introduction

The agro-industrial waste is rich in dietary fiber, phytochemicals, and other essential nutrients [1, 2]. A substantial amount of this waste is often burned or dumped; however, it holds many important and beneficial nutrients [3]. Apple pomace is considered one of the essential and major industrial wastes, which contain significantly higher amounts of dietary fiber, bioactive compounds, and other essential nutrients. With time, the term “food by-products” has been

progressively utilized. This term enlightens biological material as waste can be appropriately treated and altered into more valuable market-conscious derivatives [4]. The term “food waste” refers to “fractions of food and inedible parts of food” removed from the food supply chain [5]. Definitions become more complicated when it comes to the sector of fruits and vegetables. Fruit and vegetable waste can be defined as unpalatable parts of vegetables being discarded throughout the reception, handling, transportation, and different processing stages [6].

Due to the higher biodegradability of fruits and vegetable waste, this can cause environmental complications. Apart from biological and nutritional losses, economic loss is also present. That is why in the last few decades, great efforts were made for the development of advanced methods and policies for waste [7]. Fruits and vegetables are a significant source of nutrition, especially their residues or wastes left behind after processing in fruit juice processing industries [8]. Fruit wastes or residues can be converted into a consumable form or utilized as a processing aid in food products because they are a portion of the fruit left behind after juice extraction. Apple pomace and pomegranate peel are excellent sources of dietary fiber and higher bioactive potential; therefore, the food products developed by employing these waste plant residues will significantly affect the health of the textural properties of the prepared products. These fruits residues can play a vital role in regulating the body's proper working as they contain sufficient amounts of bioactive compounds, resulting in reducing oxidative stress [9]. All over the globe, apple pomace is being utilized to develop different functional dairy products such as bakery items (e.g., spinach-flavoured ice cream made up of vegetable fat and fiber-supplemented dairy yogurt) [10].

Human beings have been familiar with fermentation techniques since the Stone Age. It is one of the oldest preservation techniques that convert milk into more nutritional products and prolong its shelf life at a significant level. The accurate beginning of the fermentation technique is almost unknown, but it is considered that it could be existing from 15000 years ago [11]. Yogurt is one of the most consumed milk products around the globe [12]. The production of yogurt is mainly done by either fermentation of fresh milk or reconstituted milk with lactic acid bacteria. It is popular among customers due to its benefits on the intestinal environment and body immunity [13]. Yogurt has a unique flavour, texture and good sensory characteristics. Yogurt is also beneficial for our gut microflora. Yogurt can be obtained by treating the pasteurized milk with lactic acid fermentation via *Streptococcus thermophilus* and *Lactobacillus bulgaricus* or with other cultures of bacteria [14, 15].

Nevertheless, the abovementioned health-endorsing characteristics prompt food entrepreneurs to focus on pomegranate peel phytochemicals containing food preparations including food supplements, nutraceuticals, and phenolic enriched diets [16]. In addition to their nutraceutical significance, PoP and PoPx show essential practical purposes (antioxidant, antimicrobial, colorant, and flavouring). They might also act as excellent natural additives for food preservation and quality improvement. The medical industry observed a significant improvement due to peel-extracted derivatives' high nutritional and nutraceutical capabilities [17].

Freeze-dried products hold much of their original flavour, phytochemical properties and are found to be very light and crispy at the same time [18]. Still, there is no single method of moisture removal suitable for all commodities of food products since each organic material (food) has its exclusive characteristics and subsequently, the requirements are diverse. There is a dire need to stop burning and wasting

these nutritious leftovers that can be easily transferred into new products and recover the nutritional loss in existing products and enhancements in their shelf life. The purpose of this research is to confirm that apple pomace and pomegranate peel powder can be employed in the preparation of high-fiber fermented functional products, such as yogurt. For this, the freeze-dried apple pomace and pomegranate peel powder and their bioactive potential at various stages of the yogurt development process have been analyzed.

## 2. Materials and Methods

**2.1. Procurement of Raw Material.** Raw milk, apple, and pomegranate were purchased from dairy, fruits, and vegetable stores, respectively, situated in Lahore, India. Apples and pomegranates were transferred into plastic bags; however, milk was collected in a sterile glass bottle with a 500 mL capacity to avoid contamination. Furthermore, the raw materials were transferred to the fruits and vegetable laboratory at the Department of Food Science, Government College University Faisalabad, for further examination. The raw form of apple pomace and pomegranate peel is shown in Figure 1(b).

**2.2. Handling of Raw Material.** Apple, pomegranate, and milk were brought in to the laboratory for further processing. First, apple and pomegranates were thoroughly washed with distilled water and inner seeds in case of apple were removed using apple seed corer. However, milk container was placed in the refrigerator at 7°C until apple pomace and pomegranates peel were developed.

**2.3. Drying of Apple Pomace and Pomegranate Peel.** Apple pomace and pomegranate peel were dried using the freeze-drying technique as prescribed by [19] with some needed modifications. For this purpose, a laboratory freeze-dryer (ALPHA 1-2 LD Plus, Christ, USA) was employed. Different parameters were settled for wet apple pomace and pomegranate peel freeze-drying, such as freezing temperature,  $T_{sh}$  temperature,  $P_{kch}$  (mbr) vacuum pressure, and residual moisture content, as shown in Table 1. Furthermore, wet apple pomace and pomegranate peel were conveyed in a freeze-dryer for drying. Then, the dried powders were collected and stored in aluminum pouches at room temperature for further analysis. After drying, apple pomace and pomegranate peel were ground to make fine powder for further analysis and development of functional yogurt as shown in Figures 1(c) and 1(d).

**2.4. Product Development.** APP and PPP were weighed and directly added to the pasteurized milk before fermentation, as shown in Table 2. Furthermore, for fermentation, cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were introduced into milk and mixed at 46°C and allowed to ferment for 12–24 hrs. Further analysis was conducted after fermentation.

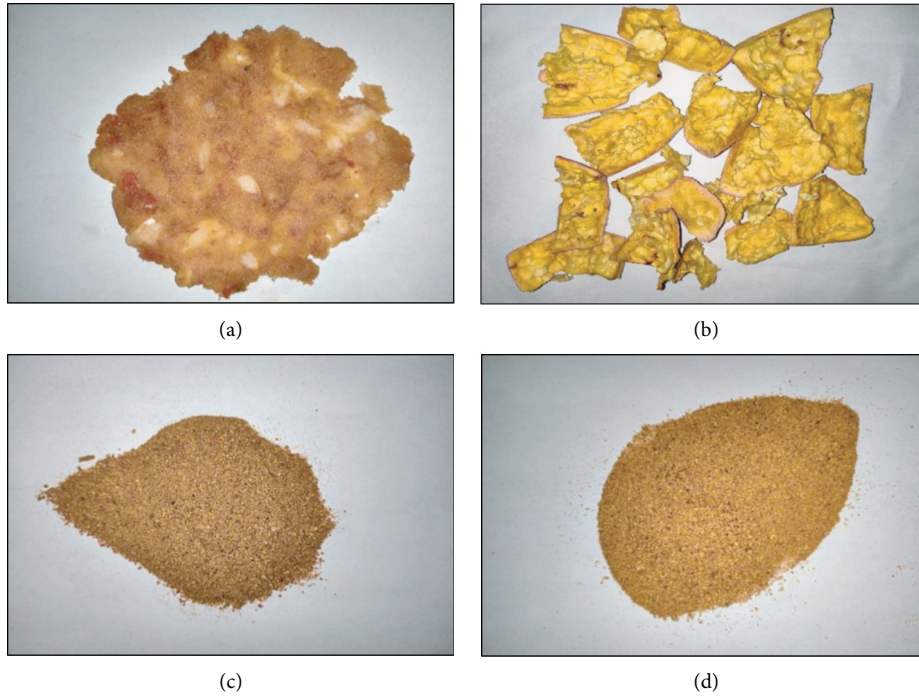


FIGURE 1: (a) Fresh apple pomace. (b) Fresh pomegranate peel. (c) Dried apple pomace. (d) Dried pomegranate peel.

TABLE 1: Different optimized conditions in freeze-dryer.

Product	Factors				
	Blanched at 100°C	Freezing temp. (°C)	$T_{sh}$ (°C)	$P_{kch}$ (mbr)	RM (%)
AP	+	-27	90	1.3	2.1
PP	+	-80	90	1.5	1.3

PP = pomegranate peel. AP = apple pomace.

TABLE 2: Treatment table.

Treatments	Levels (%)
$T_0$	Control
$T_1$	3%
$T_2$	6%
$T_3$	9%
$T_4$	3%
$T_5$	6%
$T_6$	9%
$T_7$	APP + PPP
	2.5 + 2.5%

PPP = pomegranate peel powder. APP = apple pomace powder.

**2.5. Chemical Composition.** The basic chemical composition such as moisture, fat, crude protein, ash, and carbohydrate

content of freeze-dried PPP and APP was examined according to the standard procedures prescribed by the Association of Official Analytical Chemists (AOAC, 2010). Moisture content was evaluated by taking exactly 2 g of dried powder sample and drying in an oven at about  $100 \pm 5^\circ\text{C}$  for approximately 3 hrs and then reweighed. Protein and fat content were further examined by employing the Kjeldahl and Soxhlet apparatus. In addition, the ash content was measured by subjecting 2 g sample under the controlled environment of the muffle furnace at  $400^\circ\text{C}$  for 3 hrs. The crude fiber was also examined in this particular effort. Meanwhile, carbohydrate- or nitrogen-free extracts were calculated by employing the following expression:

$$\% \text{Carbohydrates (NFE)} = 100 - (\text{Fat} + \text{Protein} + \text{Moisture} + \text{Ash} + \text{Crude Fiber}). \quad (1)$$

**2.6. Rheological Analysis of Yogurt.** The samples were measured right after making to reduce the effects of sample shear history. Tests were conducted using an Anton Paar Physica MCR 301 controlled stress rheometer (Anton Paar

Germany GmbH, Ostfildern, Germany) with a measuring cell (PPTD 120) equipped with a Peltier temperature control. A humidity chamber was used to prevent water loss during evaluation. A Julabo circulator (Julabo West, Inc., CA, USA)

was used as a temperature control system for the Peltier element. A parallel plate geometry (PP50) was used at 0.5 mm gap. The test was conducted at 4°C. Using a farinograph (Brabender, Duisburg, Germany) according to AAAC methods (2000), the influence of apple pomace and pomegranate peel powders on the mixing profile of the dough was investigated. Extensograph (Brabender, Duisburg, Germany) was used to investigate the dough's elastic characteristics according to AAAC methods (2000). The visco-amylograph (Brabender, Duisburg, Germany) was used to determine the blends' pasting properties according to AAAC methods (2000).

## 2.7. Bioactive Characterization of Yogurt

**2.7.1. Sample Extraction.** Freeze-dried pomegranate peels and apple pomace samples were ground into a fine powder using a KMF grinder at 9676.8 g. Prepared ground samples were kept in sterile bags to prevent contamination at -40°C until further extraction. Methyl alcohol, ethyl alcohol, and water were employed to prepare extracts. Accurately, 0.5 g of dried sample was added into a flask followed by exactly 100 mL ethyl acetate and stirred at 20°C for 3 hrs. The mixture was centrifuged (Harrier 18/80 refrigerated centrifuge) (SANYO, MSE, UK) at 9676.8 g for 30°C. Furthermore, the supernatant was filtered by Whatman filter paper (No. 1, Ø 155 mm). However, the prepared extracts were stored at 4°C for further analysis as followed by [20, 21].

**2.7.2. Antioxidant Activity (DPPH Assay).** Antioxidant activity was examined by the method (2,2-diphenyl-1-picryl hydroxyl) prescribed by [19] with slight modifications as mentioned above. For this purpose, accurately 15 µl extracts were added into a test tube followed by 735 µl methanol and 750 µl 0.1 mM DPPH solution and thoroughly mixed until the extract dissolved in methanol. Then, the mixture was incubated for exactly 30 min in the dark to avoid any exposure to light. A UV-visible spectrophotometer measured the absorbance was measured at 517 nm by employing a UV-visible spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA). A suitable calibration curve was prepared using ascorbic acid as the standard solution. The obtained results were expressed as mM ascorbic acid (AA) equivalent  $g^{-1}$  of extracts.

**2.7.3. Total Phenolic Content (TPC).** Prepared extracts were examined for their TPC by the Folin-Ciocalteu method as prescribed by [1]. For this purpose, 70 µL of the prepared extracts was accurately added in a test tube of 10 mL capacity, followed by 250 µL of Folin-Ciocalteu reagent and 750 µL of Na<sub>2</sub>CO<sub>3</sub> (1.9 M). However, a total volume of exactly 5 ml was made up by adding distilled water and then mixed by using a vortex mixer for about 1 minute before incubation for 2 hrs in the dark. Consequently, the absorbance was measured by utilizing a Spectrophotometer (Thermo-Spectronic, Surrey, England) at 765 nm wavelength. A calibration curve was prepared by employing controlled

solutions of gallic acid. Obtained results were expressed as gallic acid equivalents (GAE) in  $mg^{-8}$  dry solids.

**2.7.4. Total Flavonoid Content (TFC).** TFC of the freeze-dried apple pomace and pomegranate peel extract was determined by a method as prescribed by [1]. For this purpose, exactly 1 mL of the prepared extract was placed in to a test tube (10 mL) already containing 4 mL of distilled water. At an instant, 0.3 mL of 5% sodium nitrite was added into the test tube. However, after 5 min accurately, 0.3 mL of 10% aluminium chloride was placed in the same test tube. Then, after 6 min, exactly 2 mL of 1 M sodium hydroxide was added to the test tube and mixed. Instantly, the test tube was diluted with the addition of 2.4 mL of distilled water and thoroughly mixed.

At last, the absorbance of the pink coloured mixture was examined at 510 nm and water was used as a blank. Different amounts of catechin solutions were used to create an appropriate calibration curve. The results were expressed in mg catechin equivalent (CE) per g of dried solids.

**2.8. Syneresis Analysis.** Accurately 5 ml of sample was taken in falcon tubes and the tubes was centrifuged at 500 rpm for 15–20 minutes at 4–5 degrees centigrade. The whey was separated after 1–2 minutes. The whey amount was expressed as volume of whey separated per 100 ml of yogurt. The syneresis was checked and measured during 0, 7th, 14th, and 21st [13].

**2.9. Sensory Evaluation.** A 9-point hedonic scale was used to assess the sensory evaluation of yogurt samples. Sensory attributes were judged by a panel of different trained judges relevant to the field of study. The parameters on the scale were as follows: 1 = dislike, 2 = dislike slightly, 3 = neither like or dislike, 4 = like moderately, 5 = like very much, 6 = like extremely, 7 = good, 8 = very good, and 9 = excellent.

**2.10. Statistical Analysis.** The obtained data were analyzed by analysis of variance (ANOVA) through SPSS. Duncan's Multiple Range (LSD) test was utilized to determine the significance level between the mean values obtained.

## 3. Results

**3.1. Chemical Profile.** In Table 3, the average chemical compositions of PPP and APP were elaborated, which depicts that APP samples were examined to exhibit the highest protein (8.16) g/100 g and fat (1.10) g/100 g contents, whereas PPP samples were investigated to have much higher ash (3.53) g/100 g, fiber (35.19) g/100 g along with moisture (8.43) g/100 g, and carbohydrate (61.34) % levels in contrast to APP samples.

**3.2. Bioactive Potential of APP and PPP.** In Table 4, freeze-dried APP and PPP were studied for their bioactive potential, which clearly shows a significantly higher TPC (221.77 ± 1.79) mg GAE/g content and TFC (26.15 ± 1.00)

TABLE 3: Proximate chemical analysis of apple pomace powder and pomegranate peel powder.

Attributes	APP	PPP
Moisture (g/100 g)	7.88	8.43
Protein (g/100 g)	8.16	3.26
Fat (g/100 g)	1.10	0.55
Fiber (g/100 g)	12.70	35.19
Ash (g/100 g)	1.53	3.35
Carbohydrates (%)	53.12	61.34

APP: apple pomace powder. PPP: pomegranate peel powder.

CE mg/g levels in samples from PPP in contrast to the TPC ( $52.36 \pm 1.22$ ) mg GAE/g and TFC ( $8.40 \pm 0.13$ ) CE mg/g found in APP samples.

**3.3. Rheological Analysis of Yogurt.** The average yogurt contains ( $14.60 \pm 0.67$ ) g/100 g protein content, though, the maximum content was observed in  $T_0$  ( $15.27$ ) g/100 g sample having 0% dried APP and PPP; on the other hand, least protein content ( $13.98$ ) g/100 g was determined in  $T_6$  sample with 9% freeze-dried PPP. The fat analysis depicts that on average ( $3.89 \pm 0.38$ ) g/100 g yogurt contains fat content, though, the highest content was found in  $T_0$  ( $4.11$ ) g/100 g sample having 0% freeze-dried APP and PPP, whereas the minimum fat concentration was determined in  $T_6$  ( $3.69$ ) g/100 g sample having 9% PPP. Brix determination clearly shows that on average ( $13.61 \pm 0.35$ ) w/w % yogurt contains total soluble solids content; however, the maximum concentration was detected in  $T_6$  ( $14.26$ ) w/w % sample having 9% freeze-dried PPP; on the other hand, minimum TSS content was examined in  $T_0$  (control) ( $13.07$ ) w/w % sample with 0% freeze-dried powder. Solid not fat analysis depicts that on average ( $10.24 \pm 0.60$ ) w/w % yogurt contains solid, not fat content though, the highest amount was examined in  $T_6$  ( $11.21$ ) w/w % sample having 0% dried APP and PPP, whereas least SNF content was examined in  $T_0$  (Control) ( $9.17 \pm 0.01$ ) w/w % sample with 0% freeze-dried APP and PPP. pH determination can be used as an accurate acidity indicator of milk and provide H<sup>+</sup> value or absorption in milk. The association between acidity and pH value is only a loose-end estimation. pH determination clearly shows that on average ( $4.49 \pm 0.04$ ) yogurt contains pH content; however, the highest value was detected in  $T_3$  ( $4.56$ ) sample having 9% freeze-dried APP; in contrast, the least pH value was examined in  $T_6$  ( $4.43 \pm 0.01$ ) sample with 9% PPP.

After a sample of furnace oil has been entirely burnt, the ash content indicates the incombustible component that remains. The ash content of the developed functional yogurt was determined. This certain type of analysis was conducted to observe the concentration and nature of minerals (inorganic mass) in food. Ash analysis depicts that, on average, the ash content is ( $2.08 \pm 0.16$ ) g/100 g yogurt while the maximum content was found in  $T_6$  ( $3.37 \pm 0.01$ ) g/100 g sample having 9% freeze-dried PPP; on the other hand, least ash content was detected in  $T_0$  (control) ( $1.85 \pm 0.01$ ) g/100 g sample with 0% dried powder. The rheological profile of yogurt is exhibited in Table 5.

TABLE 4: Bioactive profile of apple pomace powder and pomegranate peel powder.

Attributes	APP	PPP
TPC (GAE mg <sup>-g</sup> )	$52.36 \pm 1.22^b$	$221.77 \pm 1.79^a$
TFC (CE mg <sup>-g</sup> )	$8.40 \pm 0.13^b$	$26.15 \pm 1.00^a$

Means that do not share a letter in a column respective to their factor are significantly different at level ( $p < 0.05$ ). APP: apple pomace powder. PPP: pomegranate peel powder. TPC: total phenolic content. TFC: total flavonoid content. Values in mean column are given in (mean  $\pm$  SD).

The Brix, ash, SNF, and pH values except for fat and protein levels of different treatments were tending to increase with the increase in APP or PPP concentrations as shown in Figure 2. Moreover, adding freeze-dried PPP contents in the developed functional yogurt increased significantly higher than APP incorporated treatments at level ( $p < 0.05$ ).

**3.4. Bioactive Profile of Functional Yogurt.** In Table 6, the descriptive analysis for total phenolic contents analysis depicts that on average ( $4.07 \pm 0.37$ ) mg GAE/g concentrations were found. However, the maximum content was detected in  $T_6$  ( $4.64$ ) mg GAE/g sample having 9% freeze-dried PPP; on the other hand, least TPC was inspected in  $T_0$  (control) ( $3.39$ ) mg GAE/g sample with 0% freeze-dried powder. TFC analysis elaborates that on mean ( $1.44 \pm 0.17$ ) CE mg/g concentrations were found. However, the highest level was investigated in  $T_6$  ( $1.73$ ) CE mg/g sample having 9% freeze-dried PPP, whereas the lowest TFC was inspected in  $T_0$  (control) ( $1.21$ ) CE mg/g sample with 0% freeze-dried powder. Antioxidant activity examination explains that on average ( $70.58 \pm 9.43$ ) % was calculated. Meanwhile, the extreme activity was diagnosed in  $T_6$  ( $83.87$ ) % sample having 9% freeze-dried PPP; besides, the least antioxidant activity or DPPH inhibition was measured in  $T_0$  (control) ( $59.51$ ) % sample with 0% freeze-dried powder as shown in Figure 3.

**3.5. Syneresis in Functional Yogurt.** Prepared functional yogurt samples comprising of  $T_0, T_1, T_2, T_3, T_4, T_5, T_6,$  and  $T_7$  were analyzed for their syneresis at different intervals of time (day 0, day 7, day 14, and day 21) by using a method as described in Materials and Methods. Descriptive analysis was conducted; however, maximum concentration was observed and detected in  $T_0$  ( $1.82 \pm 0.01$ ) g/100 g at 21st day of storage, whereas minimum syneresis concentration was observed and detected in  $T_6$  ( $1.32 \pm 0.01$ ) g/100 g at day 0 of storage. Three times replications were made for each treatment as shown in Table 7.

**3.6. Sensory Evaluation.** In Table 8, the developed functional yogurt with the addition of APP and PPP at different levels was conducted. As far as the descriptive analysis is concerned, the maximum scores for appearance ( $8.50$ ), texture ( $7.70$ ), flavour ( $7.40$ ), taste ( $8.50$ ), and consistency ( $7.70$ ) were observed and detected in  $T_0$  (control) sample having absolutely no \*APP and \*PPP concentrations; on the other

TABLE 5: Effect of adding apple pomace powder and pomegranate peel powder on the rheological profile of yogurt. Values in mean column are given in (mean  $\pm$  SD).

Treatments	Parameters					
	TSS %	SNF %	pH	Ash %	Fat %	Protein %
$T_0$	13.07 $\pm$ 0.02 <sup>f</sup>	9.17 $\pm$ 0.01 <sup>g</sup>	4.48 $\pm$ 0.05 <sup>cd</sup>	1.85 $\pm$ 0.03 <sup>g</sup>	4.11 $\pm$ 0.02 <sup>a</sup>	15.27 $\pm$ 0.02 <sup>a</sup>
$T_1$	13.35 $\pm$ 0.01 <sup>e</sup>	9.77 $\pm$ 0.02 <sup>f</sup>	4.51 $\pm$ 0.03 <sup>c</sup>	1.96 $\pm$ 0.01 <sup>f</sup>	4.03 $\pm$ 0.02 <sup>b</sup>	15.09 $\pm$ 0.02 <sup>b</sup>
$T_2$	13.61 $\pm$ 0.04 <sup>c</sup>	10.13 $\pm$ 0.02 <sup>e</sup>	4.54 $\pm$ 0.04 <sup>b</sup>	2.08 $\pm$ 0.03 <sup>d</sup>	3.91 $\pm$ 0.01 <sup>c</sup>	14.57 $\pm$ 0.06 <sup>e</sup>
$T_3$	13.83 $\pm$ 0.01 <sup>b</sup>	10.34 $\pm$ 0.01 <sup>c</sup>	4.56 $\pm$ 0.01 <sup>a</sup>	2.21 $\pm$ 0.02 <sup>b</sup>	3.84 $\pm$ 0.04 <sup>d</sup>	14.17 $\pm$ 0.08 <sup>g</sup>
$T_4$	13.53 $\pm$ 0.01 <sup>cd</sup>	10.20 $\pm$ 0.01 <sup>d</sup>	4.46 $\pm$ 0.03 <sup>e</sup>	2.02 $\pm$ 0.01 <sup>e</sup>	3.85 $\pm$ 0.06 <sup>d</sup>	14.75 $\pm$ 0.02 <sup>c</sup>
$T_5$	13.87 $\pm$ 0.17 <sup>b</sup>	10.90 $\pm$ 0.03 <sup>b</sup>	4.45 $\pm$ 0.04 <sup>e</sup>	2.16 $\pm$ 0.04 <sup>c</sup>	3.77 $\pm$ 0.01 <sup>e</sup>	14.29 $\pm$ 0.03 <sup>f</sup>
$T_6$	14.26 $\pm$ 0.01 <sup>a</sup>	11.21 $\pm$ 0.02 <sup>a</sup>	4.43 $\pm$ 0.06 <sup>f</sup>	2.37 $\pm$ 0.03 <sup>a</sup>	3.71 $\pm$ 0.02 <sup>f</sup>	13.98 $\pm$ 0.04 <sup>h</sup>
$T_7$	13.39 $\pm$ 0.03 <sup>e</sup>	10.16 $\pm$ 0.01 <sup>de</sup>	4.46 $\pm$ 0.04 <sup>e</sup>	1.98 $\pm$ 0.00 <sup>ef</sup>	3.89 $\pm$ 0.04 <sup>c</sup>	14.68 $\pm$ 0.06 <sup>d</sup>
Average	13.61 $\pm$ 0.35	10.24 $\pm$ 0.60	4.49 $\pm$ 0.34	2.08 $\pm$ 0.16	3.89 $\pm$ 0.38	14.60 $\pm$ 0.67

Means that do not share a letter in a column are significantly different at level ( $p < 0.05$ ).  $T_0$  = 0% control.  $T_1$  = 3% apple pomace powder.  $T_2$  = 6% apple pomace powder.  $T_3$  = 9% apple pomace powder.  $T_4$  = 3% pomegranate peel powder.  $T_5$  = 6% pomegranate peel powder.  $T_6$  = 9% pomegranate peel powder.  $T_7$  = 2.5 + 2.5% apple pomace powder + pomegranate peel powder.

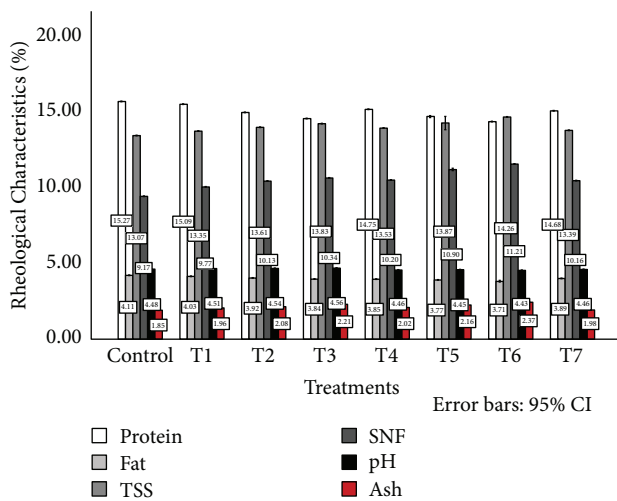


FIGURE 2: Effect of adding APP and PPP on the rheological properties of yogurt.

TABLE 6: Effect of adding apple pomace powder and pomegranate peel powder on the bioactive profile of yogurt. Values in mean column are given in (mean  $\pm$  SD).

Treatments	Parameters		
	Antioxidant activity (%)	TPC (mg GAE/g)	TFC (CE mg/g)
$T_0$	59.51 $\pm$ 0.04 <sup>h</sup>	3.39 $\pm$ 0.01 <sup>h</sup>	1.21 $\pm$ 0.01 <sup>g</sup>
$T_1$	61.80 $\pm$ 0.02 <sup>g</sup>	3.67 $\pm$ 0.04 <sup>g</sup>	1.24 $\pm$ 0.03 <sup>f</sup>
$T_2$	63.85 $\pm$ 0.01 <sup>f</sup>	3.99 $\pm$ 0.03 <sup>e</sup>	1.35 $\pm$ 0.02 <sup>e</sup>
$T_3$	65.13 $\pm$ 0.02 <sup>e</sup>	4.12 $\pm$ 0.04 <sup>c</sup>	1.46 $\pm$ 0.04 <sup>d</sup>
$T_4$	79.46 $\pm$ 0.01 <sup>c</sup>	4.08 $\pm$ 0.02 <sup>d</sup>	1.53 $\pm$ 0.00 <sup>c</sup>
$T_5$	82.67 $\pm$ 0.02 <sup>b</sup>	4.28 $\pm$ 0.03 <sup>b</sup>	1.61 $\pm$ 0.03 <sup>b</sup>
$T_6$	83.87 $\pm$ 0.02 <sup>a</sup>	4.64 $\pm$ 0.05 <sup>a</sup>	1.73 $\pm$ 0.05 <sup>a</sup>
$T_7$	68.33 $\pm$ 0.03 <sup>d</sup>	3.79 $\pm$ 0.02 <sup>f</sup>	1.34 $\pm$ 0.04 <sup>e</sup>
Average	70.58 $\pm$ 9.43	4.00 $\pm$ 0.37	1.44 $\pm$ 0.17

Means that do not share a letter in a column are significantly different at level ( $p < 0.05$ ). TFC: total flavonoid content. TPC: total phenolic content.  $T_0$  = 0% control.  $T_1$  = 3% apple pomace powder.  $T_2$  = 6% apple pomace powder.  $T_3$  = 9% apple pomace powder.  $T_4$  = 3% pomegranate peel powder.  $T_5$  = 6% pomegranate peel powder.  $T_6$  = 9% pomegranate peel powder.  $T_7$  = 2.5 + 2.5% apple pomace powder + pomegranate peel powder.

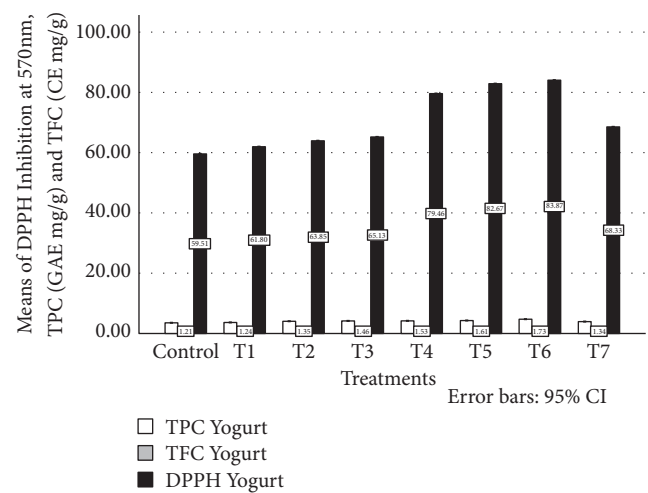


FIGURE 3: Effect of adding APP and PPP on the biological properties of yogurt.

hand, least scores for appearance (4.80), texture (4.70), flavour (4.20), and taste (3.30) were investigated in  $T_6$  sample with 9% PPP. Overall acceptability scores were found to be significantly higher than other analyzed treatments. In this study, sensory attributes tended to decrease with the increase in freeze powder concentrations; however, by adding APP in yogurt, sensorial scores did not decrease significantly compared to the treatments having PPP in them. Estimated marginal means for sensory evaluation of samples were conducted and textured to ease in comparison as shown in Figure 4.

## 4. Discussion

Fruit juice processing enterprises generate a large amount of industrial waste, such as apple pomace and pomegranate peel. The moisture is removed from pomegranate peel and apple pomace using a freeze-drying method. The moisture content of PPP was determined to be in the range of 8.3–7.9 g/100 g using the freeze-drying procedure [22].

TABLE 7: Descriptive analysis for syneresis in different treatments. Values are given in (mean  $\pm$  SD).

Attribute	Treatments	Time intervals				Means
		Day 0	Day 7	Day 14	Day 21	
Syneresis (g/100 g)	Control	1.65 $\pm$ 0.00	1.73 $\pm$ 0.00	1.78 $\pm$ 0.01	1.82 $\pm$ 0.00	1.75 $\pm$ 0.07 <sup>A</sup>
	T <sub>1</sub>	1.61 $\pm$ 0.00	1.64 $\pm$ 0.00	1.67 $\pm$ 0.01	1.71 $\pm$ 0.00	1.66 $\pm$ 0.04 <sup>B</sup>
	T <sub>2</sub>	1.58 $\pm$ 0.00	1.61 $\pm$ 0.00	1.63 $\pm$ 0.00	1.66 $\pm$ 0.00	1.62 $\pm$ 0.03 <sup>CD</sup>
	T <sub>3</sub>	1.37 $\pm$ 0.00	1.39 $\pm$ 0.00	1.44 $\pm$ 0.00	1.48 $\pm$ 0.00	1.42 $\pm$ 0.05 <sup>E</sup>
	T <sub>4</sub>	1.60 $\pm$ 0.00	1.62 $\pm$ 0.00	1.65 $\pm$ 0.00	1.68 $\pm$ 0.00	1.64 $\pm$ 0.03 <sup>C</sup>
	T <sub>5</sub>	1.55 $\pm$ 0.00	1.59 $\pm$ 0.00	1.61 $\pm$ 0.00	1.64 $\pm$ 0.00	1.60 $\pm$ 0.03 <sup>D</sup>
	T <sub>6</sub>	1.32 $\pm$ 0.00	1.35 $\pm$ 0.00	1.39 $\pm$ 0.00	1.42 $\pm$ 0.00	1.37 $\pm$ 0.04 <sup>F</sup>
	T <sub>7</sub>	1.59 $\pm$ 0.00	1.61 $\pm$ 0.00	1.66 $\pm$ 0.00	1.69 $\pm$ 0.00	1.64 $\pm$ 0.04 <sup>BC</sup>
Means	1.54 $\pm$ 0.12 <sup>D</sup>	1.57 $\pm$ 0.13 <sup>C</sup>	1.61 $\pm$ 0.12 <sup>B</sup>	1.64 $\pm$ 0.12 <sup>A</sup>		

Means that do not share a letter are significantly different at level ( $p < 0.05$ ).  $T_0 = 0\%$ .  $T_1 = 3\%$  apple pomace powder.  $T_2 = 6\%$  apple pomace powder.  $T_3 = 9\%$  apple pomace powder.  $T_4 = 3\%$  pomegranate peel powder.  $T_5 = 6\%$  pomegranate peel powder.  $T_6 = 9\%$  pomegranate peel powder.  $T_7 = 2.5 + 2.5\%$  apple pomace powder + pomegranate peel powder.

TABLE 8: Effect of adding apple pomace powder and pomegranate peel powder on the sensory profile of yogurt. Values are given in (mean  $\pm$  SD).

Sensory attributes	Treatments							
	T <sub>0</sub> (Control)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>
Appearance	8.50 <sup>a</sup>	7.40 <sup>b</sup>	6.30 <sup>e</sup>	5.40 <sup>g</sup>	7.30 <sup>c</sup>	6.10 <sup>f</sup>	4.80 <sup>h</sup>	6.30 <sup>d</sup>
Texture	7.70 <sup>a</sup>	7.30 <sup>b</sup>	5.80 <sup>d</sup>	5.20 <sup>g</sup>	7.10 <sup>c</sup>	5.60 <sup>e</sup>	4.70 <sup>h</sup>	5.30 <sup>f</sup>
Flavour	7.40 <sup>a</sup>	7.00 <sup>b</sup>	6.10 <sup>d</sup>	5.40 <sup>g</sup>	6.90 <sup>c</sup>	5.90 <sup>e</sup>	4.20 <sup>h</sup>	5.80 <sup>f</sup>
Taste	8.50 <sup>a</sup>	7.20 <sup>b</sup>	6.50 <sup>d</sup>	5.70 <sup>f</sup>	6.80 <sup>c</sup>	5.40 <sup>g</sup>	3.30 <sup>h</sup>	5.60 <sup>e</sup>
Consistency	7.70 <sup>a</sup>	7.50 <sup>c</sup>	7.20 <sup>e</sup>	6.80 <sup>h</sup>	7.60 <sup>b</sup>	7.30 <sup>d</sup>	6.90 <sup>g</sup>	7.10 <sup>f</sup>
Overall acceptability	7.80 <sup>a</sup>	7.30 <sup>b</sup>	6.40 <sup>d</sup>	5.70 <sup>g</sup>	7.15 <sup>c</sup>	6.10 <sup>f</sup>	4.80 <sup>h</sup>	6.00 <sup>e</sup>

Means that do not share a letter in a row are significantly different at level ( $p < 0.05$ ).  $T_0 = 0\%$  control.  $T_1 = 3\%$  apple pomace powder.  $T_2 = 6\%$  apple pomace powder.  $T_3 = 9\%$  apple pomace powder.  $T_4 = 3\%$  pomegranate peel powder.  $T_5 = 6\%$  pomegranate peel powder.  $T_6 = 9\%$  pomegranate peel powder.  $T_7 = 2.5 + 2.5\%$  apple pomace powder + pomegranate peel powder.

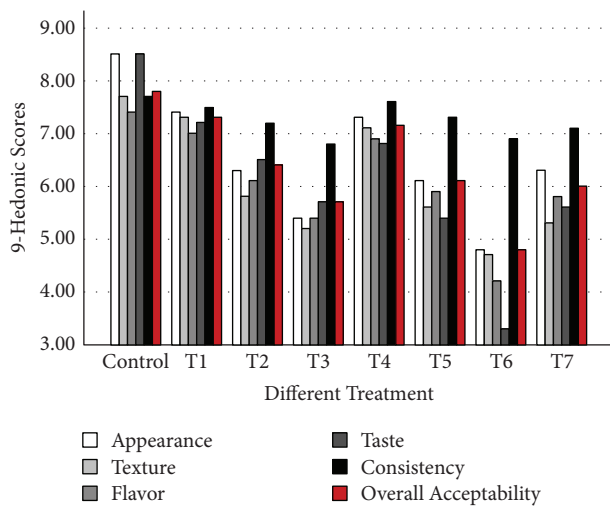


FIGURE 4: Estimated marginal means for sensory evaluation in functional yogurt samples were conducted and textured to ease in comparison.

Another study [1] undertaken during the production of fiber-enriched cookies found that by incorporating freeze-drying, moisture content of 8.90–9.15 g/100 g could be attained, which was observed in a similar range of 7.88 g/100 g as reported in the current effort. In contrast to APP, PPP samples had the greatest fiber 35.19 g/100 g and ash 3.53 g/100 g; these results were observed in form as resulted

in the range of 34.05–39.13 g/100 g and 3.30–3.41 g/100 g for protein and ash, respectively, in PPP samples [23].

The TPC of APP samples ranging from 28.91 to 30.17 mg GAE/g as studied by [24] contradicts with the results of current report and this contradiction may be due to the difference in extraction solution aqueous, methanolic or ethanolic. The highest TPC (221.71) mg GAE/g and TFC (26.15) mg GAE/g was discovered in freeze-dried PPP samples; similar outcomes were reported by [21] in methanolic extracts. Thermal treatments have negative impact on polyphenols in the food system as predicted by [19].

Another study was reported by [25], stating that during the air-drying of red pepper as temperature rises polyphenolic concentrations tend to decrease significantly. According to [26], this might be because the majority of phenolic compounds are attached to cellular structures, and subsequent dehydration procedures tend to liberate the bound biologically active compounds from the food system, making them more bio-accessible during the extraction process. A study also stated that freeze-drying is generally considered as one of the best and most effective dehydration approaches coupled with enhanced shelf span [19], while [27] states that freeze-drying is the technique that allows the best preservation of phytochemicals and their bioactivity in fruit and vegetable powders. With reference to the present study, freeze-drying can be adopted for the maximum recovery of bioactive compounds (i.e., TPC and TFC from apple pomace and pomegranate peel).

pH level in response to the addition of APP has been observed to be increased, whereas after the incorporation of PPP the pH levels were examined to decrease significantly at higher concentrations. Similar phenomenon was observed during the development of peanut milk fermented curd as reported by [28]. Thus, in the case of apple pomace, pH tends to increase which shows a decline in the acidity of yogurt, which may be due to the dilution factor. After introducing APP and PPP in inclining order, total soluble solid along with solid not fat and ash levels was found to be increased significantly. Some similar increases reported in TSS and SNF levels when incorporated in the development of fiber-enriched yogurt [14, 29]. Fat and protein levels were found to be remarkably affected by the introduction of increasing APP and PPP concentration order; in validation, the Food Drug Administration (FDA) standards for drinkable yogurt postulate >8.25% milk (SNF), fat levels to satisfy nonfat yogurt (<0.5%), low-fat yogurt (2%), and yogurt (>3.25%) before the addition of other ingredients. Thus, fiber-enriched yogurt with APP and PPP can be termed as low-fat yogurt.

Syneresis was also examined where maximum concentration was observed and detected in  $T_0$  ( $1.82 \pm 0.01$ ) g/100 g at 21st day of storage, whereas minimum syneresis concentration was observed and detected in  $T_6$  ( $1.32 \pm 0.01$ ) g/100 g at day 0 of storage. Syneresis concentrations tended to decrease by the addition of APP and PPP. Meanwhile, treatments having pomegranate peel powder ( $1.42 \pm 0.01$ ) g/100 g as compared to apple pomace powder ( $1.71 \pm 0.01$ ) g/100 g were observed to show significantly less syneresis concentrations at the 21st day of storage.

Highest TPC (4.69) GAE/g and TFC (1.78) CE/g level was observed in  $T_6$  having 9% PPP in it. In the current study, TPC as well as TFC was found to be increased significantly as APP and PPP concentration tend to incline in yogurt. Meanwhile, by introducing PPP and APP, remarkably higher increment was found in bioactive components in oppose to the treatments having APP in it. A similar increase has been stated in TPC and TFC compounds during the development of probiotic yogurt fortified with apple pomace flour [30]. Highest TPC and TFC were found in a study during the characterization of 12 widely used spices [31]. It has been reported that by fortifying pomegranate peel extracts during the development of stirred yogurt, TPC and TFC were found to be significantly increased and found in the ranges of 3.39–5.97 mg GAE/g and 1.11–2.18 mg CE/g, respectively [29]. TPC and TFC concentrations of different developed treatments were found directly proportional to APP and PPP concentrations; moreover, by incorporating freeze dried PPP, TPC concentrations in developed functional yogurt was observed increased significantly higher as compared to freeze-dried APP. These results were found in engagement with the studies led by [13, 23].

Antioxidant activity scavenges free radicals and it is directly proportional to the phenolic and flavonoid compounds in a particular food systems. As the previous trend extends, a significant increment in the antioxidant activity was examined as APP and PPP concentrations, which tend to increase gradually. The antioxidant activity of different developed treatments were observed to be directly

proportional to APP and PPP concentrations; moreover, by incorporating freeze dried PPP, antioxidant activity in developed functional yogurt increased significantly higher as compared to freeze-dried APP [13, 23]. Previously, studies reported by [23, 28, 30] emphasize that by the introduction of APP as well as PPP, the antioxidant activity of yogurt increases.

## 5. Conclusion

In this study, we prepared functional yogurt by the addition of APP and PPP that was examined for its bioactive profile such as TPC, TFC, and DPPH inhibition, also known as antioxidant activity. First, apple pomace and pomegranate peel were freeze-dried and further introduced into yogurt. Chemical composition and the bioactive investigation clearly showed that PPP was far more superior to APP except for protein and fat content, which was found to be significantly higher in APP samples. Moreover, adding APP or PPP rheological attributes of functional yogurt was found to be increased marginally in comparison to the control sample. In addition, bioactive characterization of functional yogurt was also done, which exhibits significantly higher outcomes in treatments having PPP. Developed functional yogurt samples were also investigated for their sensory parameters, which describes that by introducing higher levels of either APP or PPP in both cases, the sensory profile tends to decrease; this decline was found to be more prominent in PPP ( $T_4$ ,  $T_5$ , and  $T_6$ ) added treatments in contrast to APP ( $T_1$ ,  $T_2$ , and  $T_3$ ) introduced ones. The APP-enriched and PPP-enriched yogurt were found to be the most suitable in terms of appearance, body/consistency, flavour, and overall acceptance in this study, indicating that apple pomace and pomegranate peel can be utilized as a source of bioactive compounds in yogurt after freeze-drying in the food industry.

## Data Availability

The data set supporting the conclusions of this article is included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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