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Functional properties, phenolic constituents and antioxidant potential of industrial apple pomace for utilization as active food ingredient

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Abstract

Apple pomace is a waste biomass generated after apple fruit processing. In present investigation, efforts were made to comprehend influence of differently dried pomace on cell wall properties and phenolic profile. Different drying techniques were employed to remove moisture content from fresh apple pomace. Total dietary fiber yield (74%) and array of functional properties such as density, water and oil holding capacity, swelling capacity and glucose dialysis retardation index (36.91%) was found better in freeze dried fraction. The higher total phenolics (5.78 ± 0.08 mg GAE/g dry weight) content was also recorded in freeze dried fraction followed by oven and sun drying. The 50% aqueous acetone was found as more efficient solvent for extraction of phenolic constituents. RP-HPLC analysis has revealed presence of quercetin, phloridzin and phloretin as major phenolics. Thus, it is evident from the results that pomace generated at industrial scale can be utilized as a source of dietary food ingredient.
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Keywords: Dietary fiber; Drying; Apple pomace; Polyphenols; Antioxidant

1. Introduction

Dietary fibers demonstrated to have imperative role in improvement and management of human health, especially gastrointestinal system. However, type and source of dietary fiber greatly influence their functional properties. At present, the primary sources employed in food industries are of cereal origin with minimal contribution from fruits and vegetables. However, later has additional benefits owing to the presence of array of bioactive components particularly antioxidant molecules [1]. 'Apple pomace' is one of such source advocated to have enormous potential as dietary food component [2]. It is the waste residue left after extraction of juice from apple (*Malus domestica*) fruits. The major part (approx. 95%) of the generated biomass is skin/pulp tissues, which consists of cell wall polysaccharides (e.g. pectin, cellulose, hemicellulose, lignin and gums) and skin bound phenolic compounds i.e. dihydrochalcones, flavonols, flavanols and phenolic acids [3]. Apple pomace possess strong antioxidant properties due to the presence of phenolics like epicatechin, its dimer, quercetin glycosides, chlorogenic acid, phloridzin and 3-hydroxy-phloridzin [4]. The phenolics rich extract of pomace were found to exhibit anticarcinogenic activity by preventing colon cancer [5]. The non-starch polysaccharides are known as dietary fiber [2] and diet generally enriched with fiber is associated with good digestive health, with reduction in gastrointestinal problems, helps in weight management, lower risk of coronary heart disease, better glycemic control and lower possibility of certain type of cancer [6]. However, these physiological effects are exerted by specific dietary fiber, which may vary depending upon the fiber source and processing method [7]. Fruit fibers reported to have an edge over cereal in terms of better soluble: insoluble ratio, lower phytic acid content, and presence of associated bioactive molecules such as antioxidant [8]. In processing of high moisture biomass like apple pomace, method of moisture removal could

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be an important factor affecting actual properties of dietary fiber and release of skin-bound phenolic. Hence, the present study pursued to assess the functional propeties, phenolic content and antioxidant potential of differently dried fiber fractions.

2. Materials and methods

2.1. Industrial apple pomace collection

Pomace was collected from fruit processing unit of Himachal Pradesh Horticultural Produce Marketing and Processing Corporation Ltd (HPMC), Parwanoo, Himachal Pradesh, during September 2011. Potassium meta-bisulfite (at 600 ppm) was added as preservative to avoid spoilage of the pomace during transportation. Preserved pomace brought to CSIR-IHBT and stored at 10 ± 2 °C until drying was done.

2.2. Chemicals and reagents

2,2′-Diphenyl-1-picrylhydrazyl, Folin-Ciocalteu's phenol reagent, gallic acid, trolox, phloridzin, phloretin, quercetin was purchased from Sigma–Aldrich (Germany). All other solvents and chemicals were of analytical grade and obtained from Merck (Mumbai, India). The dialysis membranes procured from Thermo Scientific.

2.3. Extraction and drying of dietary fiber fraction

Industrial apple pomace was put in 10 L plastic can and mixed with tap water for separation of seed/twigs. After mixing, extraneous material separated from the slurry. Seedless pomace was washed thrice to reduce concentration of soluble solids (5.2–0.2 °Bx). The seedless apple pomace then recovered from slurry by filtration using muslin cloth, as shown in flow diagram (Fig. 1). Hot-air oven, sun drying and freeze or low temperature drying method was used to remove the moisture of extracted fiber fraction from pomace. In case of hot-air oven method, fibrous material was spread over aluminum trays in thin layer (0.5–0.75 cm) for removal of moisture at 60 ± 2 °C in industrial hot-air tray drying oven (MSW-215), until no further decrease in weight was observed. The fraction was converted into fine powder using cutting mill (Retsch) (1 mm) and packed in polybags (HiDispo™ Bag-12, HIMEDIA). This dried fiber fraction was considered as 'FI' and stored at room temperature. In case of sun drying, seedless pomace was spread thinly (0.5–0.75 cm) in aluminum trays and kept under sun in an open area and jumble every 1 h to ensure uniform drying. The average humidity and temperature was 48%–72% and 11.0–26 °C, respectively during the drying period. After drying, pomace fraction (FII) was processed similar to F1. The third fraction (FIII) was obtained by low-temperature drying (−55 °C) using freeze drier (CHRIST Alpha 1–2 L Dplus, Germany). Similar to FI, the dried fraction was powdered, packed and stored till further analysis.

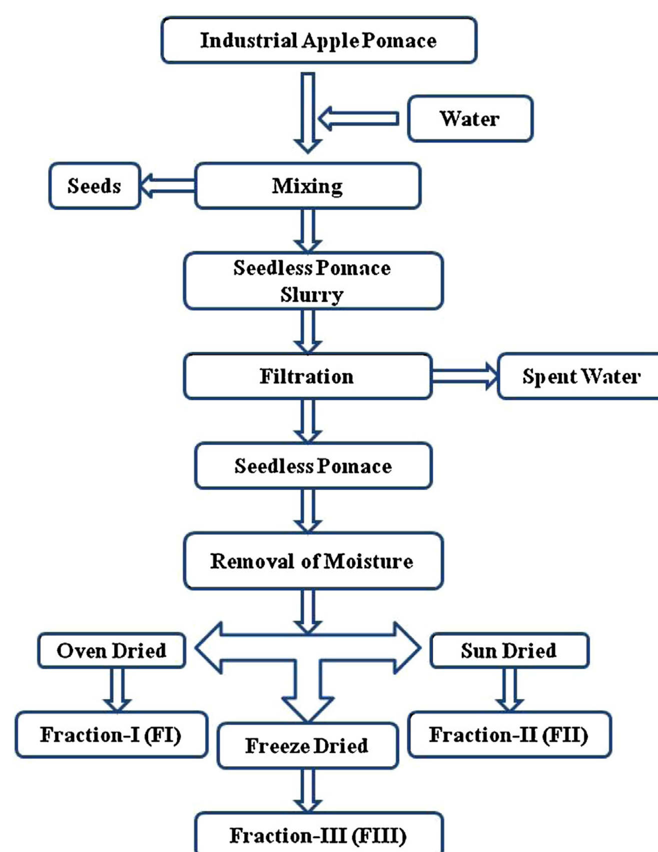

Fig. 1. Flow diagram for preparation of dietary fiber fractions from apple pomace.

2.4. Dietary fiber content

Total dietary fiber (TDF), doluble dietary fiber (SDF) and insoluble dietary fiber (IDF) contents were determined according to phosphate buffer based enzymatic-gravimetric method AOAC (labeled 991.42, 993.19 and 985.29) with slight modifications [9]. In brief, fiber fractions were gelatinized with heat stable α-amylase, digested with protease and amyloglucosidase to make samples protein and starch free. Subsequently, IDF were filtered and washed with warm distilled water, which was pooled and combined with 4 times volume of 95% ethanol, before heating at 60 °C to precipitate SDF. The precipitates were weighed after drying at 105 °C in hot-air oven until reaching constant weight.

2.5. Measurement of functional properties

2.5.1. Density

Bulk density was measured using the method of Elkhalifa et al. [10]. A 50 mL graduated cylinder was filled with 10 g fiber fraction followed by gentle tapping of the cylinder. The volume of fiber powder was read directly and results were expressed as g/mL. In packed density, a calibrated 10 mL graduated syringe filled with fiber fraction and pressure was applied manually until additional pressure would not further reduce the volume.

The packed density was calculated as: Packed density = Sample weight (g)/Sample volume (mL).

In hydrated density, a calibrated 10 mL graduated cylinder was filled with distilled water and fiber fractions were added carefully to avoid adhesion of particles to cylinder walls and let them stand for 15 min. Difference between volume of water before and after adding the samples were recorded as mL of water displaced and expressed as g/mL of water displaced:

$$\text{Hydrated Density} = \frac{\text{Weight of sample (g)}}{\text{Water displaced (mL)}}$$

2.5.2. Water holding capacity (WHC)

Fiber fractions (250 mg) were added to 50 mL centrifuge tubes and 25 mL of distilled water was added. The suspension was mixed and allowed to stand at room temperature for 1 h. Thereafter samples were centrifuged (15 min, 3,000 × *g*) and supernatant discarded. The weight of the pellet was recorded and expressed as gram of water retained per gram of fiber sample.

2.5.3. Oil holding capacity (OHC)

Fiber fractions (2 g each) added to pre-weighed centrifuge tube and 20 mL soybean oil was added. Samples mixed with vortex shaker and kept for 30 min at 25 ± 2 °C without disturbance. Thereafter tubes were centrifuged for 25 min at 4,000 r per minute and supernatant oil decanted. OHC was calculated as gram oil retained per gram sample [10].

2.5.4. Swelling capacity (SWC)

Samples (1 g) were kept in graduated centrifuged tube and 30 mL of water was poured. Samples were hydrated for 18 h [11]. The final volume attained by fiber fraction was measured as:

$$\text{Swelling Capacity (mL/g)} = \frac{\text{Volume occupied by sample(mL)}}{\text{Original sample weight(g)}}$$

2.6. Glucose dialysis retardation index (GDRI)

Glucose diffusion was measured by Ou et al. [12] reported method with slight modifications. Samples (0.5 g) were mixed with 25 mL of glucose solution (50 mmol/L) and mixed. The solution was dialysed against 80 mL of deionized water (pH 7.0) at 37 °C using a dialysis membrane having 12,000 Da cut-off Molecular Weight. After 20, 30, 60, 90, and 120 min, the glucose content in the dialysate was measured by Biochemical Analyzer (YSI 7100) using enzymatic method (glucose oxidase). The glucose dialysis retardation index (GDRI) was calculated as:

$$\text{GDRI} = 100 - \left(\frac{\text{Glucose diffused from fibre sample}}{\text{Glucose diffused from control sample}}\right) \times 100$$

2.7. Extraction of phenolics

Different samples (5 g) were extracted with 100 mL of respective solvents 50% methanol (APM1), 50% acetone (APA1) and 50% ethanol (APE1) for 30 min at 60 °C. The extracts were filtered with Whatman No. 1 filter paper and concentrated under vacuum (at 40–45 °C temperature) using rotary evaporator (Buchi 210), and lyophilized until a constant weight was reached. Lyophilized extract was then re-dissolved in methanol to obtain final concentration of 1 mg/mL. All the extracts were filtered through 0.45 μm filter (Millipore) before HPLC analysis.

2.8. Determination of total polyphenol content

Polyphenol content was determined using Folin-Ciocalteu's method [13]. TPC of samples were calculated based on prepared calibration curve (20, 40, 60, 80, 100 μL aliquots of 0.1% aqueous gallic acid) and expressed as mg gallic acid equivalent (GAE)/g dry apple pomace.

2.9. Determination of total flavonoid content

Total flavonoid content (TFC) was measured by colorimetric method [14]. The calibration curve of 0.01% quercetin was prepared by using different concentration (20, 40, 60, 80 and 100 μL). Absorbance was measured after 30 min at 415 nm. TFC was expressed as quercetin equivalent in mg/g dry weight sample powder.

2.10. HPLC quantification of phenolics

Reversed-phase high performance liquid chromatography (RP-HPLC) analysis of phenolics were performed using Waters HPLC system equipped with autosampler-2707 and photodiode array (PDA) 2998 detector and multisolvent pump (600 Controller). In-house developed RP-HPLC-DAD method was used for polyphenolic assessment [15]. Synergi MAX RP80, C_{12} column (4.6 mm × 250 mm length, 4 μm particle size) was used for the separation of compounds. Stock solutions of all the standards were prepared at 1.0 μg/μL concentration using HPLC grade methanol. Spectral data were scanned between 200 and 700 nm, and final data reading was taken at 280 nm.

2.11. Antioxidant activity

2.11.1. Ferric-reducing antioxidant power assay (FRAP)

Assay was carried out according to Benzie and Strain method [16]. FRAP reagent was prepared adding 0.3 mol/L acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. Reagent was warmed at 37 °C prior to use. 50 μL of different extracts were added to 1.5 mL of the FRAP reagent. Absorbance of the reaction mixture was recorded at 593 nm using UV–vis spectrometer after 4 min against blank. A standard calibration curve was prepared at 415 nm using various concentrations (10, 20, 30, 40 and 50 μg) of 0.01% Trolox.

Antioxidant activity in the extracts was calculated from the calibration curve and expressed as mg TEAC/g dry weight sample powder.

2.11.2. Radical scavenging activity

Free radical scavenging activity of different samples extracts against stable 2,2′-diphenyl-1-picrylhydrazyl (DPPH) was determined spectrophotometrically method with slight modification [17]. 100 μL extract from various samples were added to 2.9 mL of the 100 μmol/L DPPH solution. The mixture was vortexed and allowed to stand in dark for 30 min at room temperature. Absorbance was measured at 517 nm against a blank, consisted of 100 μL of ethanol and 2.9 mL of DPPH solution. Decrease in absorbance was measured at 517 nm after incubation for 30 min at room temperature. The radical scavenging activity was calculated as % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_A is the absorption of tested extract solution and A_B is the absorption of blank sample. Results were expressed as mg TEAC/g dry weight sample powder.

2.12. Statistical analysis

All measurements were done in triplicate samples, if otherwise not mentioned. Experimental values are given as means ± standard deviation. Significance was determined by GLM analysis (ANOVA) using Statistica 7.0. Differences at $P < 0.05$ considered significant using Box and Whisker Plot analysis.

3. Results and discussion

3.1. Effect of drying process on dietary fiber yield

The preserved apple pomace was mixed with tap water in 10 L plastic vessel for preparation of slurry. Seeds/twigs separated manually from pomace slurry. The seedless pomace converted into different fiber fractions (Fig. 1) and analyzed for their functional and antioxidant properties.

The results showed higher moisture content in freeze dried fiber fraction (FIII, 7.09%) as compared to oven (FI, 5.90%) and sun dried (FII, 5.60%). The dietary fiber having low moisture content (<10%) is preferred, as it improves shelf-life and reduces bulk, which eases the packaging, handling and transportation of the material.

The total dietary fiber (TDF) contents were 74, 69 and 66.56% in FIII, FI and FII fiber fraction, respectively (Fig. 2a). Significantly, higher TDF and insoluble dietary fiber (IDF) were recorded in FIII fraction. Borchani and his coworkers [18] also observed higher content of TDF and IDF in freeze dried date fiber concentrate extracted from different varieties in comparison to sun and oven drying method. In contrast, Wang and Thomas recorded lower TDF (35.29%) contents in freeze dried apple pomace [19]. In another study, Sato et al. [20] have found 33.4%–51.85% TDF content containing both soluble and insoluble fiber in oven dried apple pomace of eleven cultivars. Whereas, Figuerola et al. [7] have reported 60.7%–89.8% TDF in oven dried apple fiber concentrate, as compared to 69.13% TDF in our study. In case of soluble dietary fiber (SDF), a non-significant difference was observed with respect to drying process. SDF/IDF ratio in FI, FII and FIII fiber fractions was 1:3.37, 1:4.55 and 1:4.07, respectively. Nevertheless, this can further be improved by modification in processing condition. It was evident from the results that samples exposed to varied times and temperatures for moisture removal, exhibits temperature-related modifications to cell wall matrix and its constituents.

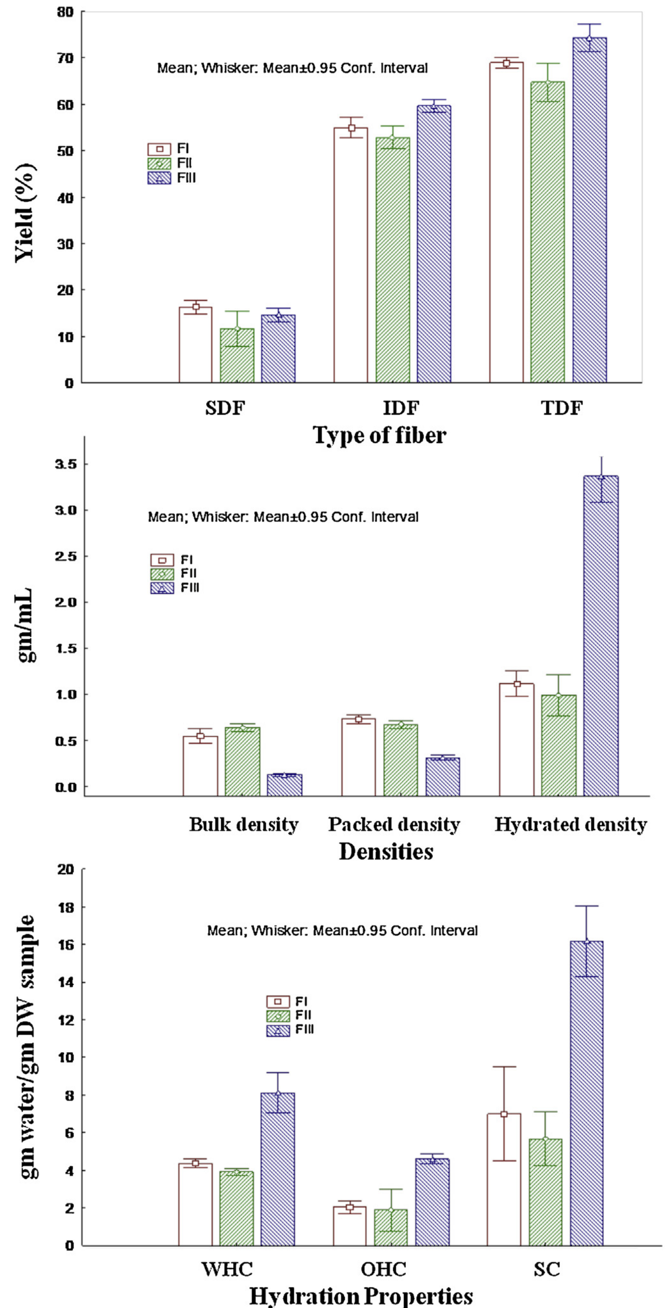

Fig. 2. (a) Soluble, insoluble and total dietary fiber content, (b) bulk, packed and hydrated density and (c) WHC, OHC and SWC capacity of dietary fiber fractions.

Table 1
Effect of drying on glucose dialysis retardation index of pomace fractions.

Fiber fractions	Glucose in dialysate g/L (GDRI % ± SD) Time					
	10 min	20 min	30 min	60 min	90 min	120 min
Control	0.595 ± 0.005 (0.00)	0.843 ± 0.008 (0.00)	1.03 ± 0.023 (0.00)	1.46 ± 0.040 (0.00)	1.71 ± 0.020 (0.00)	1.97 ± 0.021 (0.00)
FI	0.4 ± 0.001 (32.77 ± 0.17)	0.477 ± 0.008 (24.87 ± 0.88	0.835 ± 0.016 (19.05 ± 1.70)	1.29 ± 0.020 (11.18 ± 1.43)	1.54 ± 0.015 (9.52 ± 0.92)	1.81 ± 0.026 (8.62 ± 1.01)
FII	0.467 ± 0.005 (21.56 ± 0.83)	0.705 ± 0.001 (16.36 ± 0.11)	0.918 ± 0.019 (10.87 ± 1.81)	1.39 ± 0.006 (4.56 ± 0.39)	1.63 ± 0.006 (4.47 ± 0.33)	1.86 ± 0.006 (5.24 ± 0.29)
FIII	0.375 ± 0.004 (36.91 ± 0.58)	0.450 ± 0.004 (29.01 ± 0.48)	0.788 ± 0.001 (23.49 ± 0.10)	1.25 ± 0.006 (13.92 ± 0.39)	1.52 ± 0.006 (10.71 ± 0.34)	1.75 ± 0.000 (11.16 ± 0.00)

3.2. Functional properties

The functional properties of apple pomace fiber fractions were also investigated and results are shown in Fig. 2b. The bulk density (g/mL) was recorded to be higher in FII (0.64 g/mL) and FI (0.55 g/mL) fiber fractions as compared to FIII (0.13 g/mL). The higher bulk density of oven and sun dried fiber fraction can be associated with high shrinkage of cell wall material at elevated temperature than freeze drying process. Earlier Sudha et al. [21] reported 0.52 g/mL bulk density in oven dried apple pomace, alike our observation. Bakre and Jaiyeoba [22] found relatively higher bulk density in oven dried (0.61 g/mL) okra powder than sun dried (0.47 g/mL) powder. Higher packed density was recorded in FI (0.74 g/mL) and FII (0.68 g/mL) as compared to FIII (0.32 g/mL) fiber fractions. Comparable results were reported for oven dried (0.71 g/mL) apple fiber fractions by Sudha et al. [21] whereas Chen et al. [23] recorded 0.66 g/mL packed density in spray dried apple fiber similar to FII fraction. The maximum hydrated density was recorded in FIII (3.37 g/mL) fiber fraction as compared to FI (1.12 g/mL) and FII (0.99 g/mL). High hydrated and lower bulk and packed density of FIII could be attributed to removal of moisture at low temperature without causing shrinkage of cell wall material [24].

3.3. Hydration properties

Hydration properties referred to the ability of cell wall material to retain water in its matrix. The results showed much higher water holding capacity (WHC) of FIII (8.12 g/g dry weight) dietary fiber fraction than FI (4.3 g/g dry weight) and FII (3.92 g/g dry weight) (Fig. 2c). WHC obtained in present work is higher as compared to values given by Figuerola et al. [7] (1.62–1.87 g water/g dry matter) for oven dried fiber concentrates of different apple cultivars, and lower than the values reported by Sudha et al. [21] (8.39 g water/g solid water) for oven dried apple pomace. In case of freeze drying process, exclusion of ice crystals shield the matrix (honeycomb type of structure), which tends to rehydrate rapidly and more completely and preserving cell wall matrix, whereas moisture removal at higher temperature may cause breakdown of the cell wall polysaccharides network [25,26].

The oil absorption capacity (OHC) was found to be significantly ($p < 0.05$) higher in FIII (4.59 g/g dry weight) fiber fraction as compared to FI (2.04 g/g dry weight) and FII (1.88 g/g dry weight) fractions (Fig. 2c). It has been reported that the presence of lignin (insoluble dietary fiber) might play some role in the oil absorption [27]. The swelling capacity (SC) of FIII (16.16 g/g of sample) was significantly higher as compared to FI (7.0 g water/g of sample) and FII (5.6 g water/g of sample) apple pomace fiber fraction (Fig. 2c). Higher SC was also reported in freeze dried date fiber concentrate (DFC) as compared to oven dried fraction [18]. Porous structures developed within the cell wall matrix during the process of water removal at low temperature enables easy and complete rehydration [24], thus showing higher SC in FIII fiber fractions. In addition, functional properties such as swelling, water retention and fat adsorption capacity were found to decrease significantly with increase in drying temperature. This decrease was observed as a result of the structural modifications of cell wall polysaccharides of parenchyma tissues [28]. The results revealed better hydration properties of freeze dried fraction (FIII) and thus it has potential as a low caloric bulk ingredient in dietary fiber enrichment of variety of food products.

3.4. Glucose diffusion and glucose dialysis retardation index (GDRI)

The difference of glucose in the dialysate between control and treatment can be regarded as the amount of glucose adsorbed [12]. On the basis of delay in glucose diffusion with respect to control, GDRI for different fiber fraction could be obtained. During diffusion process, glucose content in the dialysate was elevated from ((0.375 ± 0.004)–(0.467 ± 0.005) g/L) (at 10 min) to ((1.75 ± 0.000)–(1.86 ± 0.006) g/L) (at 120 min) in all the fiber fractions (Table 1). Higher GDRI percentage was recorded in FIII fiber fraction as compared to FI and FII throughout the diffusion period. Maximum GDRI value was recorded at 10 min in FI ((32.77 ± 0.17)%), FII ((21.56 ± 0.83)%) and FIII ((36.91 ± 0.58)%) fiber fractions, respectively. With increment in diffusion time from 20 to 120 min, FIII ((10.71 ± 0.34)%–(29.01 ± 0.48)%) had greater GDRI values than FI ((8.62 ± 1.01)%–(24.87 ± 0.88)%) and FII ((4.47 ± 0.33)%–(16.36 ± 0.11)%) fiber fractions, respectively. FIII fraction was found to have better absorption capacity than

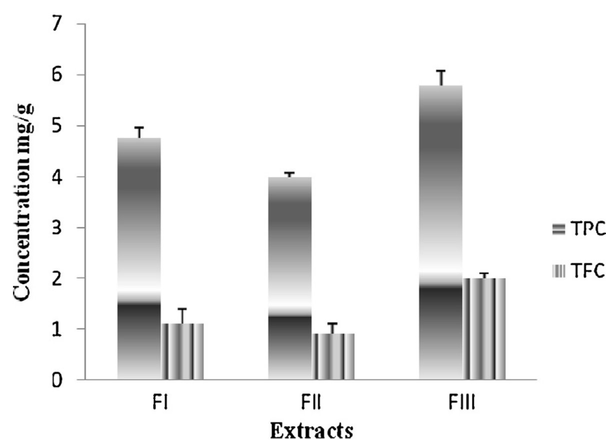

Fig. 3. Total phenolic content and total flavonoid content of dietary fiber fractions.

Table 2
Total phenolics, flavonoids, antioxidant activity and major phenolics in different solvent extracts.

Parameters	Extracts		
	APE1	APM1	APA1
TPC (mg GAE/g dry weight sample powder)	2.17 ± 0.20	1.00 ± 0.10	3.31 ± 0.31
TFC (mg QE/g dry weight sample powder)	0.45 ± 0.02	0.15 ± 0.01	0.99 ± 0.02
DPPH (mg TEAC/g dry weight sample powder)	2.68 ± 0.28	2.09 ± 0.51	3.74 ± 0.34
FRAP (mg TEAC/g dry weight sample powder)	0.79 ± 0.02	0.77 ± 0.09	0.91 ± 0.19
Phloridzin (μg/mg extract)	1.03 ± 0.00	0.82 ± 0.31	1.23 ± 0.08
Quercetin (μg/mg extract)	5.11 ± 0.02	3.72 ± 0.03	5.72 ± 0.08
Phloretin (μg/mg extract)	3.10 ± 0.03	1.10 ± 0.07	2.01 ± 0.01

Note: The values represent mean values ± SD per sample.

FI and FII, as it absorbed maximum glucose in less time. GDRI is well aligned with the physical and functional properties of respective fiber. The retention of physical structure (porosity and density of cell wall material) during freeze-drying promotes absorption of glucose by FIII fiber fractions. In addition, insoluble fiber was also reported to have physical barriers against glucose molecules that entrap them in cell wall matrix [29]. From the results, it can be deduced that FIII fiber fraction is more efficient in delaying glucose absorption through delaying the glucose diffusion in gastrointestinal tract.

3.5. Total polyphenol content (TPC) and (TFC)

Phenolic compounds are plants' secondary metabolites that posses wide-range of biological activities. These are found to be associated with antioxidant activity by playing a significant role in stabilizing lipid peroxidation [30]. In this study, the effect of drying on the yield of total polyphenol and flavonoid contents were evaluated. TPC in different extracts were determined from regression equation of calibration curve ($y = 0.0024x + 0.0285$, $R^2 = 0.9983$). It was ranged between 3.98 ± 0.09 and 5.78 ± 0.08 mg gallic acid equivalent (GAE)/g of dry pomace (Fig. 3). The highest amount of phenolics was found in the FIII followed by FI and FII extracted with 70% ethanol for 30 min at 60 °C. Correspondingly, the highest content of flavonoids was also found in FIII fraction (Fig. 3). Total flavonoids were determined from regression equation of calibration curve ($y = 0.0050x - 0.0069$, $R^2 = 0.9990$) and ranged between 0.91 ± 0.01 and 2.00 ± 0.01 mg quercetin equivalent (QE)/g of dry pomace. Corroborating to our results, Lavelli and Corti [31] reported higher phenolic contents (5,626 mg/kg dry weight) in freeze dried apple pomace as compared to air (5,521 mg/kg dry weight) and vacuum (5,176 mg/kg dry weight) dried. It is evident that drying of apple pomace does not significantly affect the phenolic contents in industrial apple pomace. Oven drying is economical reasonable and therefore it is the most acceptable method of choice. Hence, efforts were made to assess the efficiency of different extraction solvents (acetone, ethanol, and methanol) on yield of phenolic content and antioxidant activity in oven dried industrial apple pomace was studied in present investigation. Results revealed that 50% aqueous acetone was effective in extraction of phenolics as compared to other solvent systems. The highest TPC (3.31 ± 0.31 mg GAE/g dry weight sample powder) was recorded in APA1 extract, ranged between 1.00 ± 0.10 and 3.31 ± 0.31 mg GAE/g dry weight sample powder. Similarly, the highest TFC (0.99 ± 0.02 mg QE/g dry weight sample powder) was also observed in APA1 extract, which ranged between 0.15 ± 0.01 and 0.99 ± 0.02 mg QE/g dry weight sample powder. Present results have illustrated that the solvents with disparate polarities had considerable effect on total polyphenols (Table 2).

3.6. Identification and quantification of major phenolics by RP-HPLC

Earlier developed RP-HPLC-DAD method was used for quantification of individual phenolics, which contribute largely toward antioxidant properties [15]. It is evident from the results (Table 2) that the apple pomace extract APA1 possesses higher content of major phenolics, which further corroborates the trends observed in total phenolic content. The major phenolics reported in APA1 extract include quercetin (5.72 ± 0.08 μg/mg), phloretin (2.01 ± 0.01 μg/mg) and phloridzin (1.23 ± 0.08 μg/mg). The lowest content of phenolics was reported in APM1 (50% aqueous methanol) extract. The HPLC chromatogram of APA1 extract and individual phenolic standards i.e. phloridzin, phloretin and quercetin is shown in Fig. 4. Earlier, Lavelli and corti reported that 70% acetone is an efficient solvent for extraction of phenolics from apple pomace [31]. The presence of phloridzin and phloretin like molecules in industrial pomace will be of great importance to ever-growing demand for such molecules in nutraceutical as well as in pharmaceutical sectors. Dihyrochalcones phloridzin and phloretin were found to posses numerous biological properties. Phloridzin helps in the reduction of blood glucose level whereas phloretin was found to exhibit strong antitumor activity [32,33]. Najafian studied the effect of phloridzin on streptozotocin-induced rat model of diabetes and found that it significantly reduces blood glucose

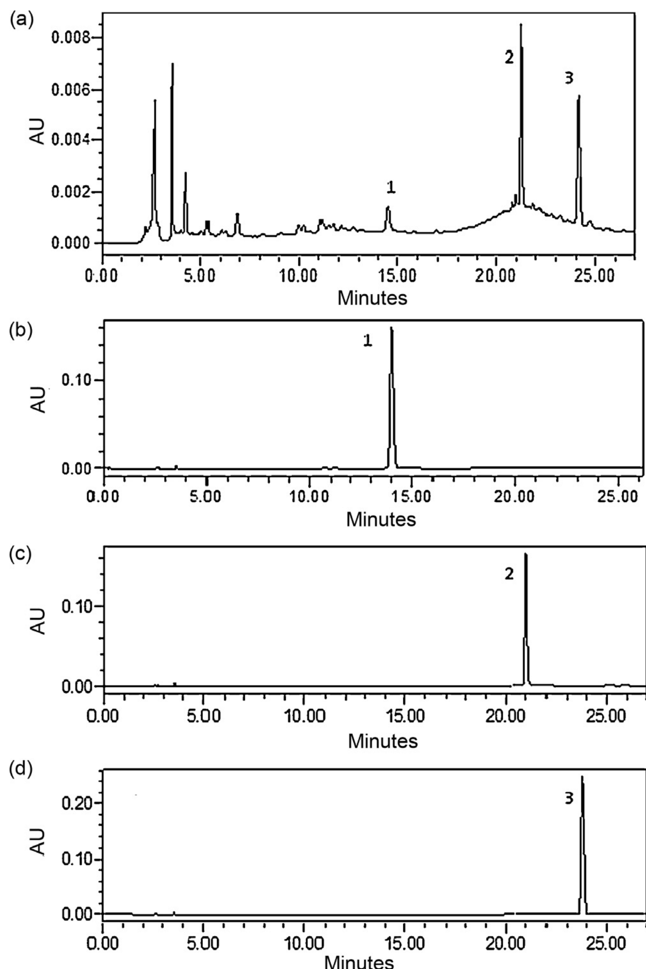

Fig. 4. (a) HPLC chromatogram of APA1 extract showing major phenolics, (b) phloridzin, (c) quercetin and (d) phloretin.

level, and recuperate dyslipidemia in diabetic rats. Researchers demonstrated that flavanol quercetin act as a strong antioxidant and anti-inflammatory agent [34]. In silico studies performed to identify key targets of type 2 diabetes mellitus (T2DM) by our group also showed that phloridzin potentially interacts with proteins, especially with MAPK1, central to T2DM mechanisms [35].

3.7. Antioxidant activity

Antioxidant capacity of apple pomace is accredited to the quantity of total polyphenols present in the biomass. The effect of various extraction solvents on the antioxidant activity of phenolics was also examined employing DPPH and FRAP assay. The results showed that APA1 extract posseses stronger antioxidant activity as compared to other solvent extracts irrespective of assays (DPPH and FRAP). TEAC values for the samples ranged between 0.77 ± 0.09 and 0.91 ± 0.19 mg Trolox/g by FRAP assay, while 2.09 ± 0.51–3.74 ± 0.34 mg Trolox/g by DPPH assay. A linear relationship was also established between total polyphenol and flavonoids contents with TEAC values and antioxidant activity was observed to increase proportionally to the polyphenol and flavonoid content (Fig. 5). In another

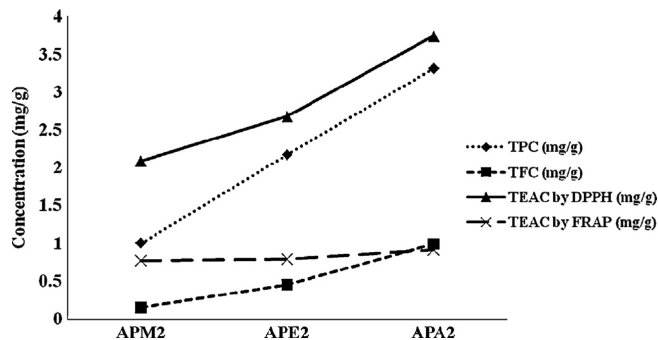

Fig. 5. Total polyphenol, flavonoid and antioxidant activity of apple pomace.

study, Wijngaard and Brunton [36] reported that apple pomace extracted with 60% ethanol at 102 °C showed 1091 ± 82 mg TE/100 g antioxidant activity by DPPH radical scavenging assay. Thus, it can be deduced from the results that these fractions have potential in food application as dietary fiber with antioxidant properties.

4. Conclusions

Beneficiation of high moisture residues such as apple pomace without affecting their nutritional profile and nature of cell wall polysaccharide is a major bottleneck in their economical utilization. Results showed that freeze dried fiber fraction is able to retain the functional properties desired from any dietary fiber supplement. This was further supported by higher glucose absorption capacity of freeze dried fraction as compared to oven and sun. However, oven dried fraction recorded higher SDF/IDF ratio, than rest of the fiber fractions. The presence of phenolic compounds such as phloridzin, quercetin and phloretin provide additional advantage to extracted dietary fiber fraction i.e. antioxidant rich dietary fiber. In conclusion, freeze dried and oven dried dietary fiber fraction exhibited good potential to be considered as a functional ingredient for dietary fiber enriched food product development. However, the final selection will depend upon the type of the food product being enriched and overall economic feasibility of the process.

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