Fidriyanto *et al.*
Food Production, Processing and Nutrition (2023) 5:6
https://doi.org/10.1186/s43014-022-00119-8

Food Production, Processing and Nutrition

RESEARCH

Open Access

Multivariate analysis of structural and functional properties of fibres from apple pomace using different extraction methods

Rusli Fidriyanto[1,2], Brij Pal Singh[1], K. M. Manju[1], Yantyati Widyastuti[2] and Gunjan Goel[1*]

Abstract

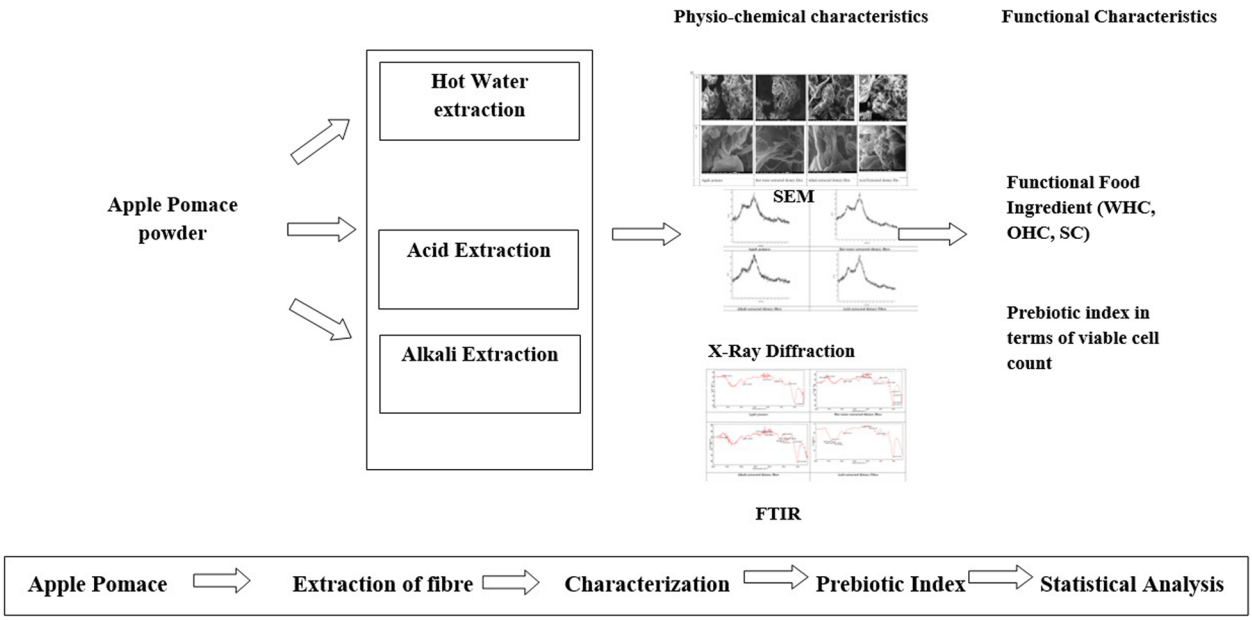
In recent years, diets rich in fibres have become more popular due to their well-documented beneficial health effects. This has driven exploration of novel dietary fibres from various bioresources. Apple pomace, an industrial waste rich in fibres was used in this study to extract the insoluble dietary fibres. The effect of various extraction methods (hot water, acid, and alkali) on the physico-chemical, structural and functional properties, and prebiotic activity of dietary fibres was evaluated. Hot water extraction resulted in highest yield of dietary fibres in comparison to other methods ($p < 0.05$). All the fractions resulted in different organization of fibrous components as depicted by scanning electron micrographs, Fourier Transform Infrared spectroscopy (FTIR), X-Ray Diffraction (XRD) pattern and Thermo Gravimetric Analysis (TGA). The acid extracted fibre fraction was observed to be amorphous with loose and porous structure whereas the alkali extracted fraction was more thermal stable based on TGA profile. Among the functional properties, acid extracted dietary fibres fraction possessed highest water and oil holding capacity ($p < 0.05$). The hot water extracted dietary fraction resulted in maximum increase in viable cell count of standard probiotic strains *Lactobacillus sporogenes* and *Streptococcus faecalis*. The Principal Component Analysis revealed that acid extracted fraction possessed better functional activity which also correlates with the structural properties whereas for prebiotic activities, the fibre obtained from hot water extraction method served the best method. These results indicate that dietary fibres extracted through hot water can be employed as a potential prebiotic substrate for the probiotic cultures and could be further explored in foods to improve textural, functional, and bioactive properties of foods.

Keywords: Dietary Fibre, Apple Pomace, Prebiotic, Probiotic, Functions

*Correspondence: gunjanmicro@gmail.com

[1] Department of Microbiology, School of Interdisciplinary and Applied Sciences, Central University of Haryana, 123031 Mahendergarh, India
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Apple pomace is the solid byproduct of apple processing units consisting mainly the apple peel, core, seed, calyx, stem, and soft tissue. It is primarily comprised of insoluble polysaccharides including cellulose, hemicellulose, and lignin and has high water content. The pomace has lower contents of minerals, proteins, vitamins and simple carbohydrates such as glucose, fructose, and sucrose (Vendruscolo et al. 2008; Waldbauer et al. 2017). Millions of tonnes of this waste are produced annually throughout the world which has high biochemical oxygen demand (BOD), thereby posing a threat to the environment if it is dumped in landfills. Therefore, valorisation of apple pomace biomass is necessary for its effective utilization in food industry. Despite being a poor animal feed additive due to its relatively low protein content and high amount of sugar, apple pomace is nonetheless largely used as animal feed (Vendruscolo et al. 2008; Kammerer et al. 2014). Alternatively, apple pomace is also utilized for the production of enzymes, organic acids, phenolic compounds, ethanol, aroma compounds, and natural antioxidants. As the pomace biomass is a rich source of fibres, the most practical methods for using it would be to extract fibres and to evaluate their potential as functional food ingredients (Vendruscolo et al. 2008; Bhushan et al. 2008; Issar et al. 2017; Lyu et al. 2020).

In general, dietary fibres are divided into two categories based on their solubility in water: insoluble dietary fibre includes cellulose, hemicelluloses, and lignin, and soluble dietary fibre, includes pentosans, pectin, gums, and mucilage (Li et al. 2014). The extraction of soluble dietary fibres from apple pomace has been reported using water, acid-alkali digestion methods (Sharma et al. 2017; Li et al. 2014;

Dietary fibres are well established to be beneficial to human health. The prevention, mitigation, and treatment of a number of ailments, such as coronary heart disease, colon cancer, obesity, and diabetes, have been linked to fiber-rich diets (Vendruscolo et al. 2008). These prebiotics are the key substrates for probiotics resulting in beneficial effects on the health of the host, due to the modulation of intestinal microbiota. These substrates help in maintaining the gut homeostasis through proliferative activity of beneficial gut microbiota. Edible plant fibre (polysaccharides, oligosaccharides, etc.) serves as the best sources of prebiotics (Cunningham et al. 2021; Gibson et al. 2017), however, efforts have been made to extract newer prebiotics as these preparations are in high demand and indications for their use are expanding. In this context, apple pomaces serve as a ready to use underutilized substrate due to its low cost and easy availability for extraction of prebiotics. Moreover, the fibre component in pomace is reported to be of higher quality than the fibres from popular cereals (Kruczek et al. 2017). In addition, incorporation of apple pomace fibres improves the structure of the finished food product since they have better water-holding capacity, solubility, and swelling properties (Wang et al. 2019).

Although the characteristics of apple pomace as a source of nutraceuticals have been reported so far, however the effect of different extraction methods on structure, physico-chemical properties and prebiotic activities have not been investigated. Taking into consideration the importance of dietary fibres and overall trend and demand for natural healthy compounds, this study was conducted to extract the dietary fibres from apple pomace using different extraction methods. The present study targeted mainly the insoluble fibre fraction whereby each extracted fibre fraction was characterized using Fourier Transform Infrared (FT-IR) spectrum, Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Thermo Gravimetric Analysis (TGA). The activity of the extracted fibre fraction as potential prebiotic substrate was determined using its effect on viable cell counts of *Lactobacillus sporogenes* and *Streptococcus faecalis*.

Materials and methods

Apple pomace preparation

The apples were procured from the local market and juice was extracted using a home juicer. After juice extraction, the residual material was collected, given a single rinse with tap water, and then filtered through two layers of muslin cloth. The rinsed apple pomace was placed in a glass tray and spread out thinly before being dried at 60 °C with airflow unitl it is dried. The weight of the dried pomace was determined; afterwards the pomace was grounded in laboratory grinder and passed through 50 mm mesh. The finished apple pomace powder was kept in sealed containers in a cold, dry place.

Extraction of dietary fibre

Three different methods viz., hot water, alkali, and acid extraction, were used to extract the insoluble dietary fibres from apple pomace. For hot water extraction, a 20 g of apple pomace was mixed with 400 mL of distilled water and stirred at 90 °C for 1 h in a hot water bath. For alkali extraction, 20 g of apple pomace was mixed with 400 mL of 50 mM NaOH, and the pH of the resulting slurry was adjusted to 7.0 using 50 mM HCl. The mixture was then stirred for 4 h at 45 °C. For acid extraction, 20 g of apple pomace were mixed with 400 mL of distilled water, the pH of the slurry was adjusted to 2.0 using sulphuric acid, and the mixture was agitated at 80 °C for 4 h. After incubation, the supernatants of all the treatments were removed by centrifugation (3000 g for 15 min) and the pellets obtained were washed with distilled water twice and dried at room temperature to get the fibre fraction. All the dried fibre fractions were lyophilized to complete drying. The yield was calculated as a percentage of the obtained dietary fibre to apple pomace powder initially used.

Physico-chemical characteristics

Scanning electron microscope (SEM)

For assessing the impacts of different extraction methods on surface morphology of extracted fibre scanning electron microscopy (ZEISS 7610 F Plus/JEOL) was used. The micrographs were obtained after fixing each individual fraction onto the specimen holder, covered using the gold powders, and monitored at 100x and 1000x magnification (Gan et al. 2020).

X-ray diffraction (XRD)

XRD patterns of extracted dietary fibre preparations were analysed in a poly-crystal X-ray diffractometer PANalytical X'Pert Pro, using CuKα radiation (λ = 1.5406 Å, 45 kV, 40 mA).

Fourier transform infrared spectroscopy (FT-IR)

The Fourier transform infrared spectroscopy (FT-IR) of the different apple pomace dietary fibre preparation was determined using Agilent Technologies Cary 630 FT-IR instrument. The spectra were recorded in transmission mode from 4000 to 650 cm^{-1} at a resolution of 16 cm^{-1}. The samples were grounded using the KBr powders, followed by pressing to pellets.

Thermogravimetric analysis (TGA)

Thermal degradation of dietary fibre samples was studied by thermogravimetric (TG) analysis. The TGA measurements were performed on TA Instruments Thermal Analysis Workstation (Shimadzu Corporations, Japan) with the aid of TA-60WS software. An average of 5 mg of sample was weighed in an alumina pan with an empty pan as reference. The conditions of the TG analysis were heating from 30 to 700 °C, by 5 °C/ min and Argon flow of 100 mL/min (Zlatanović et al. 2019).

Functional characteristics

Water holding capacity (WHC), oil holding capacity (OHC) and swelling capacity (SC)

The hydration properties of DFs from the apple pomace were determined according to the method described by Robertson et al. (2000) with some modifications. The water holding capacity (WHC) and oil holding capacity (OHC) were determined by mixing the dry sample with distilled water (1:20, w/v) for 24 h and with olive oil (1:10, w/v) for 1 h at 4 °C, respectively, and then centrifuged at 7000 *g* for 15 min. The swelling capacity (SC) was determined by adding 0.2 g of sample into 15 mL distilled water and incubated for 18 h at 4 °C. The WHC/OHC and SC were calculated as per the Eqs. 1 and 2, respectively.

Table 1 Total yield and functional characteristics of apple pomace and extracted dietary fiber fractions

Treatment	Yield (%)	Water Holding Capacity	Oil Holding Capacity	Swelling Capacity
Apple Pomace	-	1.76 ± 0.04^{a}	1.17 ± 0.02^{a}	1.98 ± 0.04^{a}
Hot Water Extraction	63.28 ± 1.58^{c}	1.97 ± 0.04^{b}	1.64 ± 0.04^{b}	6.07 ± 0.15^{b}
Alkali Extraction	59.29 ± 1.48^{b}	2.23 ± 0.05^{c}	2.35 ± 0.05^{c}	8.95 ± 0.22^{c}
Acid Extraction	49.32 ± 1.23^{a}	4.23 ± 0.10^{d}	3.62 ± 0.09^{d}	8.95 ± 0.21^{c}

a-d Means with different superscripts within columns significantly differed ($P<0.05$)

$$\mathrm{WHC/OHC(g/g)} = \frac{(Hydrated\ weight\ of\ DF - Dried\ weight\ of\ DF)}{Dried\ weight\ of\ DF} \quad (1)$$

$$\mathrm{SC} = \frac{volumeoccupiedbysample}{originalsampledryweight} \quad (2)$$

Assessment of prebiotic activity

Probiotic cultures

The probiotic cultures used in the present study were two standard probiotic strains of *Lactobacillus sporogenes* and *Streptococcus faecalis* (Jamwal et al. 2019).

Fermentation experiment

The prebiotic activity of dietary fibre fractions was determined as described by Tadayoni et al. (2015). Sugar free MRS medium (dipotassium hydrogen phosphate 2.0 g/L; Tween 80 1.0 g/L; di ammonium hydrogen citrate 2.0 g/L; sodium acetate 5.0 g/L; magnesium sulfate 0.2 g/L; manganese sulfate 0.04 g/L, meat extract 8.0 g/L, peptone 10.0 g/L, yeast extract-4.0 g/L) enriched with 2% extracted dietary fibre preparation. Inulin was taken as a standard prebiotic substrate for comparison. The MRS base medium was used as negative control. The overnight activated probiotic cultures (1.0×10^{7} CFU/ml) were inoculated to the control and dietary fibre supplemented MRS media. The tubes were incubated for 24 h at 37 °C. The proliferative effect of different substrate was determined based on viable cell count after 24 h of incubation. Prebiotic Index (PI) was calculated according to Huebner et al. (2007) taking *E.coli* strain as enteric control Huebner et al. (2007). It is the ratio of growth of probiotic in the experimental prebiotic substrate to the growth of probiotic in a control carbohydrate. The value of prebiotic index higher than 1 indicates that the substrate has a positive effect on the growth of probiotic culture whereas a value near to 1, indicates a low effectiveness of the evaluated substrate.

Statistical analysis

The experiments were performed in completely randomized design with four different treatments (apple pomace, hot water extraction, alkali extraction, and acid extraction methods) in three replications. To determine the significant differences, a one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test was employed at $p<0.05$. Statistical analysis was performed using SPSS, Version 23.0 (SPSS Inc., Chicago, IL, 215 USA). Using principal component analysis (PCA), we determined the effect of different extraction techniques towards the different physiochemical and prebiotic properties of apple pomace. For this purpose, PCA was conducted on different extraction techniques (adopted as columns) and different physiochemical and prebiotic properties (as rows). The numbers of dimensions were determined based on Eigen values of higher than 1.0 and also by the total percentage of variance as explained by the number of components selected. As our data set includes different extraction techniques, the PCA might reveal the overall responses towards functional attributes that might have been hidden among the other features. In the present study, PCA was carried out on mean-normalized data of the physiochemical properties and viable cell count of probiotic cultures under different dietary fibre extracted through different techniques.

Results and discussion

Yield

The effect of different extraction methods on yield of dietary fibre is mentioned in the Table 1. The total percentage yield of dietary fibre from apple pomace in hot-water, alkali and acid extracts was 63.28, 59.29 and 49.32, respectively. The hot-water extract found with higher percent yield, which was higher significantly ($p<0.05$) from alkali and acid extract. Acid and alkali methods are used to extract dietary fibre therefore from the results hot water extraction method seems to have better recovery and serve as an economical method. The higher recovery of insoluble fraction could be attributed to the damage of insoluble fraction by acid or alkali which might have been converted to soluble fraction as observed for other fruit wastes such as kiwi fruit, soybean residue (Sun et al. 2018) and date palm flowers (Karra et al. 2020). Moreover, the use of alkali and acid mediated extraction can damage the molecular structure of dietary fibre as observed in the present study.

Physico-chemical characteristics

SEM analysis

Scanning electron microscopy images of hot water, alkali and acid extracted fibre are shown in Fig. 1. The SEM images were taken to observe surface morphology and relative geometry of the particles present in extracted fibres. The morphology of untreated apple pomace was found smooth and compact. The acid extracted fibres were aggregated with irregular smooth layer and cavities on outer surfaces as compared to water or alkali extracted fibres. Conversely, the water extracted fibres had a loose and compact fragmented structure with less pores. The porous structure found in acid extracted samples may be attributed to the damage to the cellulosic components in the fractions. Furthermore, the tightly packed fibrous structures of the untreated apple pomace can be attributed to the strong internal bonds in the fibres (Gouw et al. 2017). The cellulose fibres such as pectin, hemicellulose, and lignin make the irregular surface of treated apple pomace (Melikoğlu et al. 2019). Similar to our study Liang et al. (2018) also found crumbled and loose structure with holes on the surface of apple pomace after steam explosion.

FT-IR analysis

The FTIR spectra of all the fibre extracts are shown in Fig. 2. All samples except acid extracted pomace showed broad peak region from around 3600 cm^{-1} to 3000 cm^{-1}, which relates to the O-H and N-H bonds stretching, present in lignocellulose components of pomace materials (Zarrinbakhsh et al. 2016). The peak at 2921 cm^{-1} signifies the asymmetric –CH2–, symmetric –CH3 and –CH2– stretching vibrations attributed specifically to the C-H in the lignin components (Xu et al. 2013). The peaks in the region 1,800–650 cm^{-1} represented the characteristic bands for all the plant cell wall polysaccharides which could be overlapped. More specifically, the bands in the region of 1620 cm^{-1} were observed in acid and alkali extracted fibre and 1740 cm^{-1} in untreated and hot water extracted pomace which corresponds to strong C=O stretching vibration of hemicelluloses and lignin (Lin et al. 2019). A peak at 1022 cm^{-1} was observed in all the pomace fractions. The peak in this wave number indicates the vibrations of C-6 cellulosic component. The peak in this area is reported as typical for glucose residue of disaccharides (Pastorova et al. 1994; Zarrinbakhsh et al. 2013).

XRD analysis

The dietary fibre usually contains cellulose as the major non-digestible polysaccharides representing 70% ordered crystalline region and the remaining as disordered non-crystalline region. The 2θ for cellulose and hemicellulose fraction is dominated by the diffraction peaks in the range of 15°–25° (Zhang et al. 2017). The XRD pattern of all the fractions appeared similar in the shape between the range of 15–25°, with the main diffraction peak at 15.5°, 21.5° indicating that all the fibre fractions contained cellulose type I (Dong et al. 2019; Wan et al. 2010). The prominent peak detected at 2θ (22°) and minor peaks

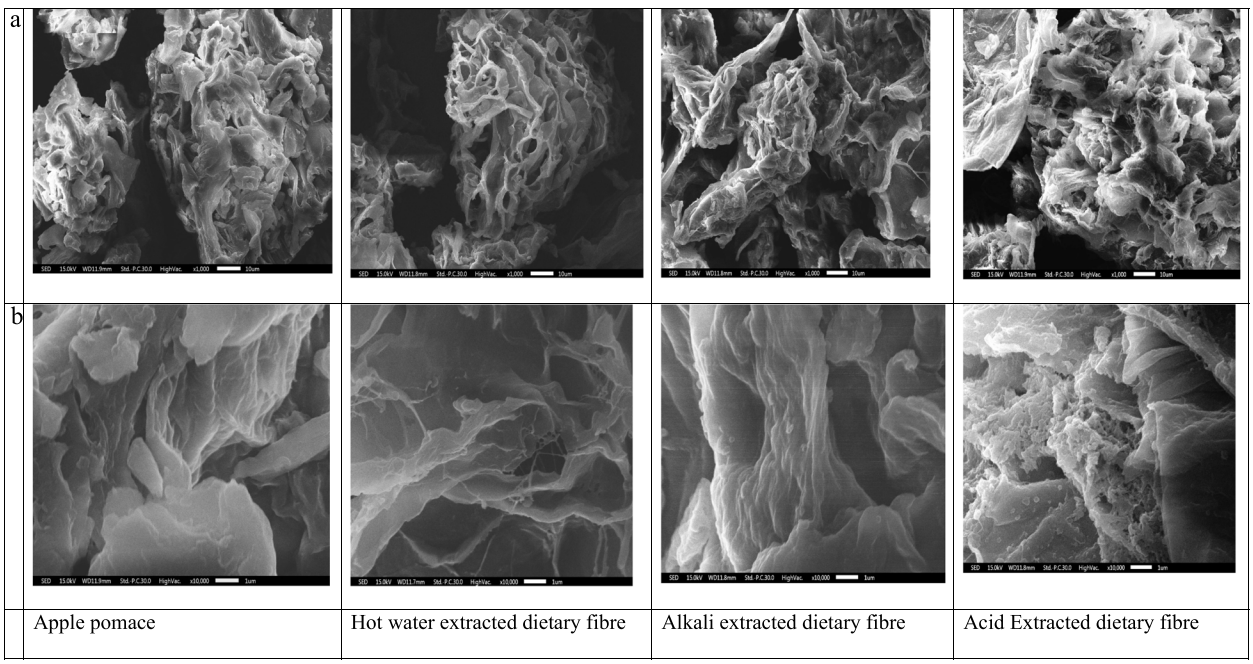


Fig. 1 Micrographs of apple pomace extracted through different methods as determined by SEM at different magnifications

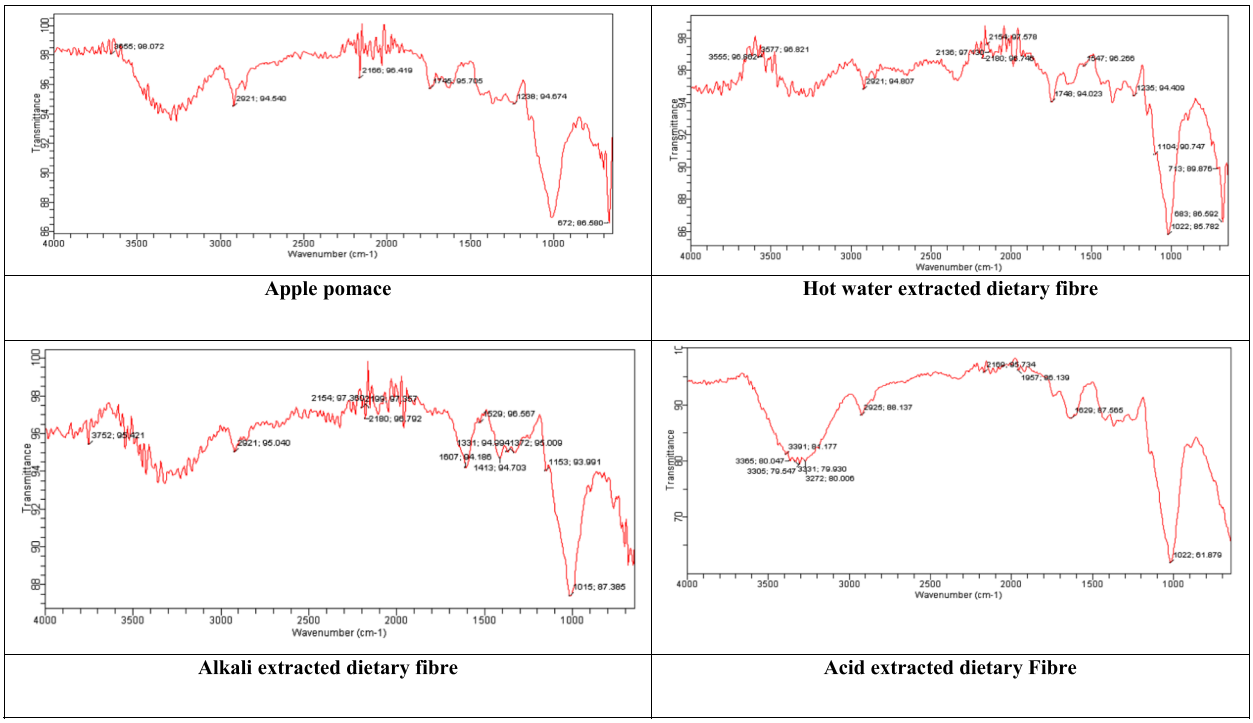


Fig. 2 FT-IR transmittance value for IR wavenumber from 650 to 4000 cm-1 for apple pomace and extracted dietary fiber fraction

at 2θ (15° and 33°), confirmed the presence of crystalline region. Similarly, Melikoğlu et al. (2019) also reported crystalline peaks at 2θ = 16.1° of apple pomace.

Thermogravimetric analysis (TGA)

TGA was used to determine the thermal degradation behaviour of fibre extracted through three different methods (Fig. 3). According to the TGA thermograms, apple pomace shows three weight loss stages between 30 and 700 °C. An initial weight loss (1 to 4%) between 70 and 120 °C was attributed to moisture loss and some volatile organic components. At initial stage of decomposition, the loss at a temperature around 120 °C has been reported due to evaporation of absorbed water (Ma & Mu 2016). The mass loss rate of acid extracted dietary fiber from apple pomace was significantly higher than those of the other insoluble dietary fibers, which reflected that more free water and bound water. The second stage (193–400 °C) of decomposition had a significant weight loss (upto 65%), due to degradation of polysaccharides and lipids and strong reactions like dehydroxylation, deoxygenation or decarboxylation (Forero et al. 2016). The third stage (400–700 °C) resulted in complete weight loss attributed to the degradation of cellulose or degradation of substances formed by polymerization of the degradation products generated in the earlier steps. The similar results were accorded by Zlatanović et al. 2019 with apple pomace flours. Degradation of lignin has been reported over a wide temperature range (250–700 °C), due to presence of different oxygen functional groups (e.g., carbonyl, phenolic hydroxyl, benzylic hydroxyl functional groups) which may have different thermal stability and char thermal decomposition (Hua et al. 2019). The second and third degradation stage of alkali extracted dietary fiber indicated less percentage of weight loss by high temperature of depolymerisation and degradation of cellulosic components (Pelissari et al. 2014).

Functional characteristics

Water (WHC) and oil holding capacity (OHC)

The water/oil holding capacity and swelling capacity of the dietary fibre indicates the hydration properties of the fibre component. These properties play a significant role in providing suitable texture to the final product. The water holding capacity of different dietary fibre extracts is shown in Table 1. The WHC of acid extracted dietary fibre was significantly higher ($p < 0.05$) as compared to alkali, hot water, and control pomace which might be due to reorganization of fibre structure to be

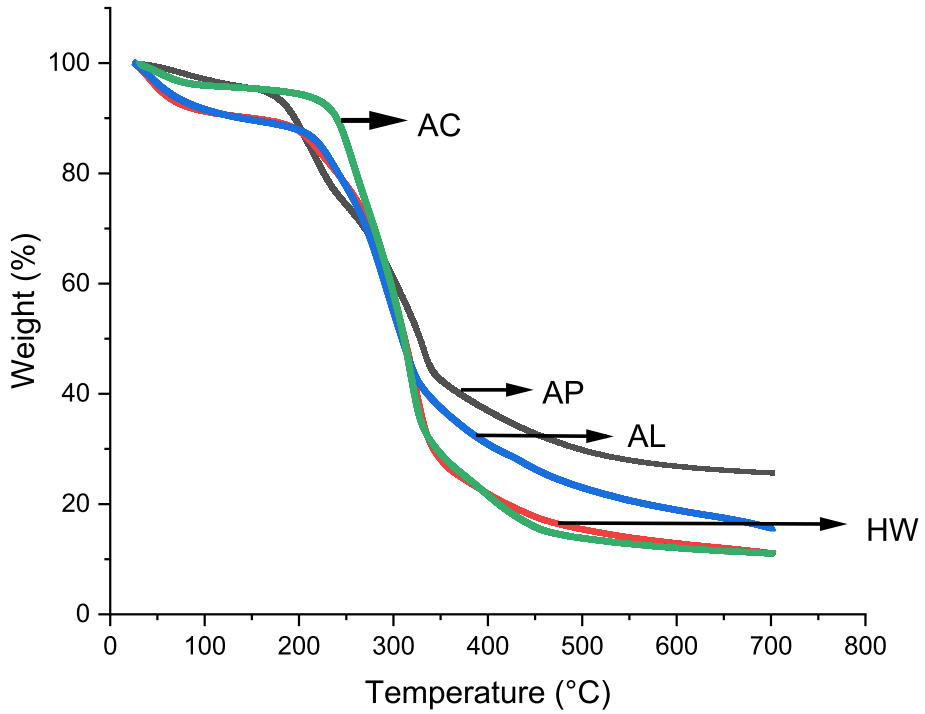


Fig. 3 Thermo gravimeteric curves of apple pomace and extracted dietary fibre fraction

more porous and exposure of more hydrophillic groups by the acid treatment (Yaich et al. 2015). Moreover, the extracted fractions were freeze dried which may have an effect of WHC of the extracted fraction compared to pomace powder as such the freeze drying may induce wider cavities in the structure that can retain more water as in the case of acid extracted fibres (Heras et al. 2017). On the other hand, XRD pattern of acid extracted fiber have relatively low crystallinity because of presence of non-crystalline cellulose that absorb more water as indicated by SEM images (Wang et al. 2022). Although, there is only slightly difference between untreated and hot water extract fibre, but all the samples differ significant from each other. In line with our study, Li et al. (2014) reported WHC (3.0 g/g) of acid extracted apple pomace, which was lower as compared to our acid extract (4.23 g/H2O.g^{-1}). On the contrary, Gouw et al. (2017) reported higher WHC (8.70 g water/g DW) of dried apple pomace as compared to our results. Similarly, Wang, Kristo, and LaPointe (2019) also reported higher WHC (16.3 g/g). Liang et al. (2018) also reported higher WHC (11.51 g/g) of steam explosion treated apple pomace. Similar pattern was also observed with OHC, acid extracted dietary fibre showed higher OHC as compared to other tested samples (Table 1). The OHC of dietary fibre is related to its surface properties such as charge density and hydrophobicity. In line with our study, Wang et al. (2019) also reported fat adsorption capacity of 4.1 g oil/g DM of AP powder. The higher OHC of acid extract in our study may be due to the presence of components other than fibre in the sample that contribute to hydrophobicity. This might be ascribed to the increase in the porosity, exposed surface area of the fibre and consequent enhancement of the physical entrapment of oil by capillary attraction. Similarly, Gouw et al. (2017), also reported oil absorption capacity of dried apple pomace of 1.48 g oil/g DW, which was within our average range (1.17 to 3.62 g/oil. g–1) in different apple pomace samples. Similarly, Li et al. (2014) reported OHC (1.3 g/g) in acid extracted apple pomace. However, Liang et al. (2018) reported higher OHC (4.25 g/g) in steam explosion treated apple pomace as compared to our hot water extract (1.64 g/oil. g–1) apple pomace. As per the SEM analysis, fibres formed bundles of the matrix due to close compactness of fibres through hydrogen bonds and van der Waals forces, which exposed the hydrophobic surface of pomace to adsorb more oil.

Swelling capacity (SC)

For SC, large difference was observed between untreated sample and their different extracts. Acid and alkali extracts showed similar SC, but significant difference was between untreated and hot water extract (Table 1). Similar patten (6.51 ml/g DW) was reported by Gouw et al. (2017) with dried apple pomace, but higher SC was

reported (20 ml/g) by Wang et al. (2019). The SC of steam explosion treated apple pomace (5.66 ml/g) reported by Liang et al. (2018) was slightly lower as compared to hot water extract apple pomace (6.07 ml/g) found in our study. Moreover, the SC of acid extracted apple pomace reported by Li et al. (2014) was lower (2.5 ml/g) as compared to our result (8.95 ml/g).

Prebiotic activity

The prebiotic activity of hot water, alkali and acid extracted dietary fibre was evaluated in sugar free MRS base medium supplemented with 2% fibre extract separately. *L. sporogenes* and *Streptococcus faecalis* were used as standard probiotic strain and inulin as a standard prebiotic (Table 2). The results indicated that both the probiotic strains showed significant difference in viable log cell count in MRS supplement with different dietary fibre extract as compared to MRS supplement with inulin. Both the strain showed significantly higher ($p < 0.05$) log count in hot water extracted dietary fibre as compared to acid or alkali extracts. The apple pomace powder showed higher log count than extracted dietary fibre preparations in tested probiotic strains. Although, the log count of both the strains in MRS supplement with different dietary fibre extract was slightly decline as compared to glucose or inulin added MRS, but count was still within the recommended range for probiotic (> log 7 CFU/g). The prebiotic index indicated that both the probiotics possessed different ability to use all the fractions. In comparison to inulin, the apple pomace and fraction extracted using hot water had prebiotic index of more than 1 indicating its potential as a prebiotic substrate. The acid and alkali extracted fraction had a prebiotic index lower than 1 indicating that these fractions are not suitable a prebiotic substrate. The difference among these values are dependent on presence of specific metabolic enzymes and transport systems within the bacterial cultures. Moreover, the acid and alkali treatment might have induced degradation of potential other prebiotic components during processing of the fibre fraction. In line with our study, Jovanovic´ et al. (2020) studied the growth pattern of probiotic strain of *L. acidophilus*, *S. thermophilus* and *B. bifidum* during yoghurt preparation with apple pomace flour (APF) fortification. They found insignificant changes in viable counts of *L. acidophilus*, and *S. thermophilus*, but slightly decline in *B. bifidum* log count. Similarly, Fernandes et al. (2019) also reported decline count of *S. thermophilus* in yogurt supplemented with 3.3% of apple pomace hot water extract as compared to plain yogurt in the end of fermentation. The higher prebiotic activity of untreated apple pomace could be attributed to availability of simpler sugars and higher water solubility of pomace as the water-soluble carbohydrates could more easily, rapidly and completely utilized by probiotics (Montagne et al. 2003). The other factors which determine the prebiotic efficacy of any substrate include degree of polymerisation, monosaccharide composition branching pattern and molecular weight which are not studied in the present investigation (Hughes et al. 2007).

The lowering of pH by probiotics determines the acidifying properties of probiotics as well as utilization of carbohydrates. A slight difference in pH of the spent media was found in apple pomace and inulin, and apple pomace extracts showed higher pH than other samples. In contrary of our result, Jovanovic´ et al. (2020) reported significant decrease in the initial pH of yogurt made from cow milk fortified with different concentration of apple pomace flour (APF), which may be largely attributed to the presence of natural acids in APF. Similar results were also obtained by Fernandes et al. (2019) with hot water extract of apple pomace. While working with *B. bifidus* Mateos-Aparicio et al. (2020) reported slight decline in pH in a medium containing apple pomace. The decrease in pH of the spent media also indicated the utilization of fibre fraction by tested cultures to produce organic acids.

Principal component analysis (PCA)

To rank the effect of different extraction methods on physicochemical and functional activities, PCA was used. PCA

Table 2 Functional properties of dietary fibre fractions on probiotic cultures

Treatment	*Lactobacillus sporogenes*			*Streptococcus faecalis*		
	Log CFU/ml	pH	Prebiotic Index	Log CFU/ml	pH	Prebiotic index
Basal media + 2% Glucose	10.11 ± 0.25^{f}	4.46 ± 0.11^{a}		10.07 ± 0.25^{f}	4.46 ± 0.11^{a}	
Basal media + 2% Inulin	9.59 ± 0.23^{e}	5.65 ± 0.14^{c}	1.77 ± 0.04^{e}	9.56 ± 0.23^{e}	5.70 ± 0.14^{c}	1.77 ± 0.04^{e}
Basal media + 2% Apple Pomace	9.35 ± 0.23^{d}	5.31 ± 0.13^{b}	1.01 ± 0.02^{c}	9.02 ± 0.22^{d}	5.24 ± 0.13^{b}	1.06 ± 0.02^{c}
Basal media + 2% Hot water extraction	8.79 ± 0.21^{c}	6.32 ± 0.15^{e}	1.19 ± 0.02^{d}	8.85 ± 0.21^{c}	6.28 ± 0.15^{e}	1.25 ± 0.03^{d}
Basal media + 2% Alkali Extraction	8.66 ± 0.21^{b}	6.65 ± 0.16^{f}	0.87 ± 0.01^{b}	8.61 ± 0.22^{b}	6.65 ± 0.16^{f}	0.85 ± 0.02^{b}
Basal media + 2% Acid extraction	8.44 ± 0.23^{a}	6.09 ± 0.15^{d}	0.74 ± 0.02^{a}	8.06 ± 0.20^{a}	6.14 ± 0.15^{d}	0.55 ± 0.01^{a}

a-f Means with different superscripts within columns significantly differed ($P<0.05$)

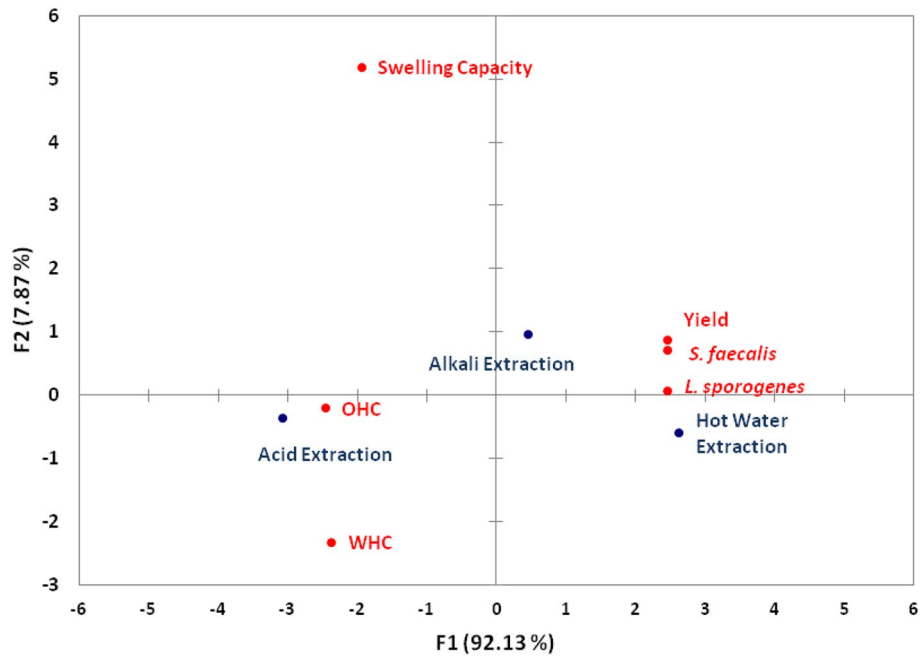


Fig. 4 PCA biplot of effect of different extraction techniques towards the different physiochemical and prebiotic properties of apple pomace

results in increased interpretability covering most of the explained variance within the variables (extraction methods in present case). It reduces the dimensionality of the data set and convert it into form of principal components. For out dataset, the PCA revealed the overall effect of extraction methods on different characteristics. PCA was conducted on the results obtained for each activity (as rows) and different extraction methods (as columns). The number of dimensions were determined based on eigen value (>1.0). The analysis of mean-normalised data of physicochemical and prebiotic activities represented the PCA with two components (F1 and F2) indicating a total variance of 100%. Among these two components, a variability of 92.13% and 7.87% was observed for the first principal component (F1) and second principal component (F2), respectively (Fig. 4). The positioning of hot water extraction method to the right of the PCA scatter-plot was due to highest factor score (Table S1) which resulted in enhanced growth of probiotic cultures whereas higher values for physiochemical properties were obtained in case of apple pomace obtained through acid extraction. The individual correlation of different physiochemical and prebiotic culture in terms of PCA is described in Table S2.

A Scree Plot on different principal components is a simple line segment plot indicating the fraction of total variance in the data as determined by individual components. The scree plot helps in identifying only one factors (F1) with eigen values > 1, demonstrating that the studied variables can plausibly be grouped into only one factor with 85.12% of the entire variance (Fig S1). Therefore, there was no need to report the rest of the factors which account for a very little of the variability.

Conclusion

In conclusion, the study investigated the effect of different extraction methods on the physico-chemical and functional characteristics of dietary fibres from apple pomace. Different methods affect the quantity and quality of the fibres. The highest yield of fibre was obtained using hot water extraction method. Among the functional attributes, the acid extracted fraction exhibited higher functional activities if used as food ingredients whereas the highest efficiency to be a prebiotic substrate was obtained for hot water extracted fraction. The extracted crude fibres have potential in promoting the growth of probiotic cultures. The hot water extraction method could be used to obtain fibre fraction from apple pomace in terms of its advantage being a green process. Overall, the results indicate that the dietary fibre from apple pomace could be used as a functional food ingredient; however, the functions of extracted fraction in different food matrices should also be assessed before their use.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-022-00119-8.

Additional file 1: Table S1. Factor score of principal components for effect of different extraction techniques towards the different physiochemical and prebiotic properties of apple pomace. **Table S2.** Correlation matrix (Pearson's correlation coefficients) among different extraction methods and functional activities of dietary fibre. **Fig. S1.** A Scree plot of relative eigenvalues for principal components.

Acknowledgements
Mr. Rusli Fidriyanto acknowledges the ASEAN-India Research Training Fellowship (AI-RTF) for carrying out this work at Central University of Haryana.

Authors' contributions
GG designed the study, GG and RF performed the experiment, GG, RF, BPS, KMM and YW analysed and interpreted the data, GG, BPS and KMM write and edited the manuscript. All the authors read and approved the final manuscript.

Funding
Mr Rusli Fidriyanto acknowledges the ASEAN-India Research Training Fellowship (AI-RTF) for carrying out this work at Central University of Haryana.

Availability of data and materials
The data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declares that there is no competing interest on this research work.

Author details
[1]Department of Microbiology, School of Interdisciplinary and Applied Sciences, Central University of Haryana, 123031 Mahendergarh, India. [2]Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Cibinong, Indonesia.

Received: 22 July 2022 Accepted: 4 December 2022
Published online: 03 February 2023

References

Bhushan, S., Kalia, K., Sharma, M., Singh, B., & Ahuja, P. S. (2008). Processing of apple pomace for bioactive molecules. *Critical Reviews in Biotechnology*, *28*(4), 285–296.

Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., Holscher, H. D., Hunter, K., Manurung, S., Obis, D., & Petrova, M. I. (2021). Shaping the future of probiotics and prebiotics. *Trends in Microbiology*, *29*(8), 667–685.

Dong, J. L., Wang, L., Lü, J., Zhu, Y. Y., & Shen, R. L. (2019). Structural, antioxidant and adsorption properties of dietary fiber from foxtail millet (Setaria italica) bran. *Journal of the Science of Food and Agriculture*, *99*(8), 3886–3894.

Fernandes, P. A., Ferreira, S. S., Bastos, R., Ferreira, I., Cruz, M. T., Pinto, A., Coelho, E., Passos, C. P., Coimbra, M. A., Cardoso, S. M., & Wessel, D. F. (2019). Apple pomace extract as a sustainable food ingredient. *Antioxidants*, *8*(6), 189.

Forero, D. P., Carriazo, J. G., & Osorio, C. (2016). Effect of different drying methods on morphological, thermal, and biofunctional properties of lulo (Solanum quitoense Lam.) Fruit powders. *Drying Technology*, *34*(9), 1085–1094.

Gan, J., Huang, Z., Yu, Q., Peng, G., Chen, Y., Xie, J., Nie, S., & Xie, M. (2020). Microwave assisted extraction with three modifications on structural and functional properties of soluble dietary fibers from grapefruit peel. *Food Hydrocolloids*, *101*, 105549.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., & Verbeke, K. (2017). Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*(8), 491–502.

Gouw, V. P., Jung, J., & Zhao, Y. (2017). Functional properties, bioactive compounds, and in vitro gastrointestinal digestion study of dried fruit pomace powders as functional food ingredients. *LWT*, *80*, 136–144.

Heras, R. M., Landines, E. F., Heredia, A., Castello, M. L., & Andres, A. (2017). Influence of drying process and particle size of persimmon fibre on its physicochemical, antioxidant, hydration and emulsifying properties. *Journal of food Science and Technology*, *54*(9), 2902–2912.

Hua, M., Lu, J., Qu, D., Liu, C., Zhang, L., Li, S., & Sun, Y. (2019). Structure, physicochemical properties and adsorption function of insoluble dietary fiber from ginseng residue: a potential functional ingredient. *Food Chemistry*, *286*, 522–529.

Huebner, J., Wehling, R. L., & Hutkins, R. W. (2007). Functional activity of commercial prebiotics. *International Dairy Journal*, *17*(7), 770–775.

Hughes, S. A., Shewry, P. R., Li, L., Gibson, G. R., Sanz, M. L., & Rastall, R. A. (2007). In vitro fermentation by human fecal microflora of wheat arabinoxylans. *Journal of agricultural and food chemistry*, *55*(11), 4589–4595.

Issar, K., Sharma, P. C., & Gupta, A. (2017). Utilization of apple pomace in the preparation of fiber-enriched acidophilus yoghurt. *Journal of Food Processing and Preservation, 41*(4), e13098.

Jamwal, A., Sharma, K., Chauhan, R., Bansal, S., & Goel, G. (2019). Evaluation of commercial probiotic lactic cultures against biofilm formation by Cronobacter sakazakii. *Intestinal Research*, *17*(2), 192.

Jovanović, M., Petrović, M., Miočinović, J., Zlatanović, S., Laličić Petronijević, J., Mitić-Ćulafić, D., & Gorjanović, S. (2020). Bioactivity and sensory properties of probiotic yogurt fortified with apple pomace flour. *Foods*, *9*(6), 763.

Kammerer, D. R., Kammerer, J., Valet, R., & Carle, R. (2014). Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients. *Food Research International*, *65*, 2–12.

Karra, S., Sebii, H., Yaich, H., Bouaziz, M. A., Blecker, C., Danthine, S., Attia, H., & Besbes, S. (2020). Effect of extraction methods on the physicochemical, structural, functional, and antioxidant properties of the dietary fiber concentrates from male date palm flowers. *Journal of Food Biochemistry*, *44*(6), e13202.

Kruczek, M., Gumul, D., Kačániová, M., Ivanišhová, E., Mareček, J., & Gambuś, H. (2017). Industrial apple pomace by-products as a potential source of prohealth compounds in functional food. *Journal of Microbiology, Biotechnology and Food Sciences, 7*(1), 22–26.

Li, X., He, X., Lv, Y., & He, Q. (2014). Extraction and functional properties of water-soluble dietary fiber from apple pomace. *Journal of Food Process Engineering*, *37*(3), 293–298.

Liang, X., Ran, J., Sun, J., Wang, T., Jiao, Z., He, H., & Zhu, M. (2018). Steam-explosion-modified optimization of soluble dietary fiber extraction from apple pomace using response surface methodology. *CyTA-Journal of Food*, *16*(1), 20–26.

Lin, Y., Chen, K., Tu, D., Yu, X., Dai, Z., & Shen, Q. (2019). Characterization of dietary fiber from wheat bran (*Triticum aestivum* L.) and its effect on the digestion of surimi protein. *LWT Food Science and Technology*, *102*, 106–112.

Lyu, F., Luiz, S. F., Azeredo, D. R. P., Cruz, A. G., Ajlouni, S., & Ranadheera, C. S. (2020). Apple pomace as a functional and healthy ingredient in food products: a review. *Processes*, *8*(3), 319.

Ma, M. M., & Mu, T. H. (2016). Effects of extraction methods and particle size distribution on the structural, physicochemical, and functional properties of dietary fiber from deoiled cumin. *Food chemistry*, *194*, 237–246.

Mateos-Aparicio, I., Peña Armada, R., Pérez-Cózar, M. .L. ., Rupérez, P., Redondo-Cuenca, A., & Villanueva-Suárez, M. J. . (2020). Apple by-product dietary fibre exhibits potential prebiotic and hypolipidemic effectsin high-fat fed Wistar rats. *Bioactive Carbohydrates and Dietary Fibre, 23*, 100219.

Melikoğlu, A. Y., Bilek, S. E., & Cesur, S. (2019). Optimum alkaline treatment parameters for the extraction of cellulose and production of cellulose nanocrystals from apple pomace. *Carbohydrate Polymers*, *215*, 330–337.

Montagne, L., Pluske, J. R., & Hampson, D. J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology*, *108*(1–4), 95–117.

Pastorova, I., Botto, R. E., Arisz, P. W., & Boon, J. J. (1994). Cellulose char structure: a combined analytical Py-GC-MS, FTIR, and NMR study. *Carbohydrate Research*, *262*(1), 27–47.
Pelissari, F. M., Sobral, P. J. D. A., & Menegalli, F. C. (2014). Isolation and characterization of cellulose nanofibers from banana peels. *Cellulose*, *21*(1), 417–432.
Robertson, J. A., de Monredon, F. D., Dysseler, P., Guillon, F., Amado, R., & Thibault, J. F. (2000). Hydration properties of dietary fibre and resistant starch: a european collaborative study. *LWT-Food Science and Technology*, *33*(2), 72–79.
Sharma, P. C., Gupta, A., & Issar, K. (2017). Effect of packaging and storage on dried apple pomace and fiber extracted from pomace. *Journal of food processing and preservation*, *41*(3), e12913.
Sun, J., Zhang, Z., Xiao, F., Wei, Q., & Jing, Z. (2018). Ultrasound-assisted alkali extraction of insoluble dietary fiber from soybean residues. *IOP Conference Series: Materials Science and Engineering, 392*(5), 052005.
Tadayoni, M., Sheikh-Zeinoddin, M., & Soleimanian-Zad, S. (2015). Isolation of bioactive polysaccharide from acorn and evaluation of its functional properties. *International Journal of Biological Macromolecules*, *72*, 179–184.
Vendruscolo, F., Albuquerque, P. M., Streit, F., Esposito, E., & Ninow, J. L. (2008). Apple pomace: a versatile substrate for biotechnological applications. *Critical Rreviews in Biotechnology*, *28*(1), 1–12.
Waldbauer, K., McKinnon, R., & Kopp, B. (2017). Apple pomace as potential source of natural active compounds. *Planta Medica*, *83*(12/13), 994–1010.
Wan, J., Wang, Y., & Xiao, Q. (2010). Effects of hemicellulose removal on cellulose fiber structure and recycling characteristics of eucalyptus pulp. *Bioresource Technology*, *101*(12), 4577–4583.
Wang, S., Fang, Y., Xu, Y., Zhu, B., Piao, J., Zhu, L., & Wu, J. (2022). The effects of different extraction methods on physicochemical, functional and physiological properties of soluble and insoluble dietary fiber from Rubus chingii Hu. Fruits. *Journal of Functional Foods*, *93*, 105081.
Wang, X., Kristo, E., & LaPointe, G. (2019). The effect of apple pomace on the texture, rheology and microstructure of set type yogurt. *Food Hydrocolloids*, *91*, 83–91.
Xu, F., Yu, J., Tesso, T., Dowell, F., & Wang, D. (2013). Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. *Applied Energy*, *104*, 801–809.
Yaich, H., Garna, H., Bchir, B., Besbes, S., Paquot, M., Richel, A., & Attia, H. (2015). Chemical composition and functional properties of dietary fibre extracted by Englyst and Prosky methods from the alga Ulva lactuca collected in Tunisia. *Algal Research*, *9*, 65–73.
Zarrinbakhsh, N., Mohanty, A. K., & Misra, M. (2013). Fundamental studies on water-washing of the corn ethanol coproduct (DDGS) and its characterization for biocomposite applications. *Biomass and Bioenergy*, *55*, 251–259.
Zarrinbakhsh, N., Wang, T., Rodriguez-Uribe, A., Misra, M., & Mohanty, A. K. (2016). Characterization of wastes and coproducts from the coffee industry for composite material production. *Bioresources*, *11*(3), 7637–7653.
Zhang, W., Zeng, G., Pan, Y., Chen, W., Huang, W., Chen, H., & Li, Y. (2017). Properties of soluble dietary fiber-polysaccharide from papaya peel obtained through alkaline or ultrasound-assisted alkaline extraction. *Carbohydrate Polymers*, *172*, 102–112.
Zlatanović, S., Ostojić, S., Micić, D., Rankov, S., Dodevska, M., Vukosavljević, P., & Gorjanović, S. (2019). Thermal behaviour and degradation kinetics of apple pomace flours. *Thermochimica Acta*, *673*, 17–25.

Publisher's Note