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Insights into Smoothies with High Levels of Fibre and Polyphenols: Factors Influencing Chemical, Rheological and Sensory Properties

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***Abstract*—**Attempts to add fibre and polyphenols (PPs) into popular beverages present challenges related to the properties of finished products such as smoothies. Consumer acceptability, viscosity and phenolic composition of smoothies containing high levels of fruit fibre (2.5-7.5 g per 300 mL serve) and PPs (250-750 mg per 300 mL serve) were examined. The changes in total extractable PP, vitamin C content, and colour of selected smoothies over a storage stability trial (4°C, 14 days) were compared. A set of acidic aqueous model beverages were prepared to further examine the effect of two different heat treatments on the stability and extractability of PPs. Results show that overall consumer acceptability of high fibre and PP smoothies was low, with average hedonic scores ranging from 3.9 to 6.4 (on a 1-9 scale). Flavour, texture and overall acceptability decreased as fibre and polyphenol contents increased, with fibre content exerting a stronger effect. Higher fibre content resulted in greater viscosity, with an elevated PP content increasing viscosity only slightly. The presence of fibre also aided the stability and extractability of PPs after heating. A reduction of extractable PPs, vitamin C content and colour intensity of smoothies was observed after a 14-day storage period at 4°C. Two heat treatments (75°C for 45 min or 85°C for 1 min) that are normally used for beverage production, did not cause significant reduction of total extracted PPs. It is clear that high levels of added fibre and PPs greatly influence the consumer appeal of smoothies, suggesting the need to develop novel formulation and processing methods if a satisfactory functional beverage is to be developed incorporating these ingredients.

***Keywords*—**Apple fibre, apple and blackcurrant polyphenols, consumer acceptability, functional foods, stability.

I. Introduction

THE positive roles of dietary fibres (DFs) and polyphenols (PPs) in health and prevention of disease provides the justification for increasing DF and PP contents in the daily diet [1]–[5]. Plant PPs possess diverse health-promoting properties including antioxidant and anti-inflammatory activities, and protection against diseases such as cardiovascular disease and some forms of cancer [4], [5]. A high intake of DF has been associated with improved regulation of energy intake, satiety, digestive health, as well as reduction of cancer, heart, obesity and diabetes problems [2], [3], [6]–[8]. The potential health benefits of PPs and DF put pressure on the food industry to develop more palatable means of delivery. Potable formulations are an increasingly popular delivery mechanism and hence were selected for use in this study.

DF covers a group of substances with distinct chemical structures and physical properties, influencing the functionality and sensory properties of finished foods [9]–[11]. Typically, fibres, especially insoluble fibres, pose a number of processing and sensory problems in beverage applications, including separation and precipitation issues during food processing, and detrimental effects on textural and visual properties [12]–[16]. This makes it challenging to develop products with a fibre level that meets the requirements of The *Code of Federal Regulations* (Title 21, Part 101.54), which allows "good source of fibre" and "excellent source of fibre" claims to be made for a product if it is low in fat and provides at least 10% (or 2.5 g), or at least 20% (or 5 g) of the daily value for fibre, respectively.

While consumers demand proven health benefits, flavour and taste remain the most critical requirements for any food product [17]–[19]. A functional food enriched with PPs and DF must not only retain these ingredients, but also ameliorate any undesired sensory attributes of the finished foods after food processing and storage [20]. This requires knowledge about the effects of the incorporated bioactives on the properties of the finished foods and how these effects may be counteracted. Bitterness and astringency have been identified as key sensory attributes that need to be overcome in products containing high PP content [21]–[23]. Stability of PPs and DF is a vital requirement for foods carrying bioactive compounds, and this may be influenced by food format, formulation, processing and storage conditions [1], [24]–[28].

This study aimed to provide insights into the effects of high levels of PPs and DFs on the chemical, physical and sensory properties of beverages, and the impact of heat treatment on the stability and extractability of PP in the absence or presence

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of fibre, in an effort to guide new product development. Smoothies were identified as a convenient carrier format to deliver high levels of PPs and DF. Both apple DF and PPs were selected because of their abundant health-promoting activities and desirable processing properties [29]–[33]. A blackcurrant PP extract was also included as it contained vitamin C and additional phenolic compounds such as anthocyanins, hydroxybenzoic acids, hydroxycinnamic acid compounds [34], [35], with their associated health benefits [36], [37]. The fibre and PP levels in the beverage formulations were in a range that would enable label content and/or health claims to be made. This paper reports the results of a consumer evaluation, and the viscosity and phenolic composition of smoothies containing 2.5-7.5 g added fibre, and 250-750 mg PPs; then compares the changes in colour, extractable PPs and vitamin C contents of selected smoothies during storage; and finally quantifies the effects of heating (75°C x 45 min, or 85°C x 1 min) on phenolic compounds in a simplified acidic aqueous model system with/without apple fibre (7.5 g per 300 mL serve).

II. Materials and Methods

A. Materials and Ingredients

Apple PP extract (APE, 78.5% phenolic) was purchased from Penglai Marine Biochemicals Ltd (Shandong, China). Blackcurrant PP extract (BPE, Anthomix 30, 29.04% phenolics) was sourced from Just the Berries Ltd (Palmerston North, New Zealand). High methoxy citrus pectin (CM203) and apple fibre (Herbacel Classic AF01, total fibre content 67.4%, of which 16% is soluble) were purchased from Herbstreith & Fox KG (Neüenburg, Switzerland). Apple puree (9 °Brix) and cloudy apple juice concentrate (CAJC, 55 °Brix) were purchased from ENZAFOODS New Zealand Ltd (Hastings, New Zealand). Citric acid was obtained from Davis Trading (Petone, New Zealand).

Folin–Ciocalteu phenol reagent, catechin, epicatechin, phloridzin, phloretin, quercetin, rutin, and *p*-coumaric, chlorogenic and caffeic acids were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cyanidin 3-*O*-*β*-glucopyranoside chloride was sourced from Polyphenols Laboratories AS (Hanaven, Sandnes, Norway). Methanol, acetone, *n*-hexane and formic acid were sourced from Ajax Finechem (Auckland, New Zealand). Milli-Q Plus water was used for all reagent preparation.

B. Preparation of Smoothies

Seven smoothies were formulated, with fibre contents of 2.5, 4.5 and 7.5 g and PP contents of 250, 500 and 750 mg per 300 mL serve. Smoothie production was undertaken at the pilot plant of Massey University, Palmerston North: the process included pasteurisation at 85ºC for ~15 s. The phenolic and fibre contents were adjusted using BPE and APE (BPE and APE at a fixed ratio of 20:80), and apple fibre, respectively. Apple juice concentrate, apple puree, citrus pectin and citric acid were included to improve the flavour, texture and acidity of smoothies. The resulting smoothies had varied textures, slightly different flavours and were intensely coloured (ranging from a dull to a bright maroon). The smoothies were assessed as microbiologically safe for consumption by AsureQuality (Auckland, New Zealand), prior to being stored at 2ºC for sensory evaluation and rheological analysis. A subsample was taken and stored at -80ºC for chemical analyses.

C. Effects of Fibre and Polyphenol Levels on the Acceptability of Smoothies

Smoothie acceptability was examined by evaluating the seven smoothies using a panel of 73 consumers, both male and female, without the disclosure of potential health benefits of these smoothies. Consumer panellists were recruited from members of the public via advertisements in two local community newspapers, and their travel expenses were reimbursed. To be eligible for participation, consumers had to have consumed fruit smoothie-type beverages, either commercial or homemade, in the last year and aged between 35 and 65 years old.

Smoothies were transferred from cool storage to a water bath set at 10ºC about half of an hour before evaluation to reach a serving temperature of 10ºC. Smoothies were mixed thoroughly before serving to ensure good distribution of fibre. Serving size was 35 mL. Smoothie presentation order was determined based on a Williams Latin square design [38] to minimise presentation order effects associated with fatigue and carry-over effects due to astringency from the preceding sample. Red lighting was used to mask colour differences between samples to ensure that differences in acceptability ratings were based on flavour and texture attributes without the interference of visual cues. The seven smoothies were rated for flavour, texture and overall acceptability using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely). Consumer panellists were invited to make comments to support their rating scores. Individual assessment booths were used to ensure independent ratings and avoid talking. Booth temperature was maintained at 20ºC and positive air pressure was used to minimise the risk of external odours influencing flavour evaluations.

D. Viscosity Analysis

The viscosity of the seven smoothies was examined at 10°C using a stress-controlled rheometer (Anton Parr Physica MCR301, Anton Parr GmbH, Graz, Austria) equipped with a Peltier temperature control device and cone plate geometry (angle between the cone and the plate was 1°). A humidity chamber was used to prevent water loss during measurement. A 0.49-mm measurement gap was set. The smoothie, which was stored at 2°C, was transferred into the assessment cup (set at 10°C) and left to stand for 5 min to ensure a consistent temperature within the testing sample. Viscosity of one or more duplicates of each sample was measured (30 measurements, in oscillation mode) over the shear rate range of 0.1 to 100 s^{-1}. Data were acquired and elaborated with

Rheoplus V2.66 (Anton Paar GMBH, Graz, Austria), and the values with variations < 5% were recorded. If the variation was > 5%, more duplicates were measured.

E. Storage Trials

A 14-day storage trial was conducted, and the smoothies were kept in glass screw-cap bottles (capped and wrapped in foil) at 4°C. Colour values (see G below), total phenolic (see H and I below) and vitamin C (see J below) contents were measured at Days 0, 8 and 14.

F. Total Soluble Solids Content and pH Values

The total soluble solids of each smoothie was measured in triplicate using a handheld refractometer (Pocket Pal-1, Atago, Tokyo, Japan) at 20°C and expressed as °Brix. The pH of each smoothie was determined in triplicate using a pH meter (CG837, Schott Instruments, Germany) equipped with a glass electrode (850, Schott Instruments, Mainz, Germany).

G. Colour Measurement

Sample colour was measured in triplicate using a Minolta CR-300 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) and expressed as Hunter *L*a*b** values. The *L**, *a** and *b** values define the lightness, red-greenness and blue-yellowness, respectively.

H. Preparation of Polyphenol Extracts from Smoothie by Accelerated Solvent Extraction

An aliquot of smoothie (5 g) was mixed with Celite™ (diatomaceous earth) at a ratio of 1:1 w/w and then transferred into Dionex 33 mL stainless steel extraction cells. A cellulose filter paper (30 mm, Whatman, Maidstone, UK) was placed at the end of the thimble. Extraction was carried out under nitrogen gas in a pressurised multiple-sample Accelerated Solvent Extractor (ASE 300, Dionex, Sunnyvale, CA) (operating conditions: 40ºC and 1500 psi, with 5 min heating and 10 min static time). Three extraction cycles were performed using 95% methanol. The PP extracts obtained were concentrated (to remove methanol) using the Ultra-Low Cold Trap Centrivap (Model 78100–01, Labconco Corp., Kansas City, MO) followed by freeze-drying. The extracts were kept at -80ºC, and reconstituted with water at a concentration of 10 mg/mL for Folin–Ciocalteu assay and 100 mg/mL for HPLC analysis.

I. Total Phenolic Content Determination

Solid phase extraction was carried out to prevent the interference of ascorbic acid with the total phenolic results. All the experimental steps were carried out rapidly and in reduced lighting. A Strata C18-E cartridge (2 g/12 mL Giga tubes, 55 µm, 70A; Strata, Phenomenex, Auckland, New Zealand) was preconditioned with 5 mL absolute methanol followed by 5 mL Milli-Q water. This step was repeated twice. An aliquot (5 mL) of the aqueous extract from Method *H* was settled onto the C-18 cartridge, allowed to drip slowly out and collected for the determination of *L*-ascorbic acid (Method *J*). Absolute methanol (3 x 5 mL) was then used to elute the phenolics trapped in the C-18 cartridge, and the eluate from each 5 mL flush was collected and kept at -20°C for analysis. The total phenolic content was measured using the Folin-Ciocalteu assay [39], [40] and expressed as catechin equivalents. A microplate reader (SpectraMax Plus 384; Molecular Devices, Sunnyvale, California, USA) was used to record the absorbance at 760 nm.

J. Determination of L-ascorbic Acid

The collected fraction from (Method *I*) was immediately used for the determination of L-ascorbic acid (in triplicate), following the method of the Association of Official Analytical Chemists [41].

K. High-Performance Liquid Chromatography Analysis

Individual phenolics in each aqueous extract of smoothie (from Method *H*) were analysed [42] using a Shimadzu analytical HPLC with a column oven (C40-10ASVP), auto-sampler (SIL-10AF), vacuum solvent degas module and diode-array detector (SPD-M10AVP), fitted with a Synergi® Polar-RP ether-linked column (250 x 4.6 mm, 4 µm particle size, 80 Å ether-linked column; Phenomenex, Auckland, New Zealand). The identification of PPs was carried out through using external standards (including catechin, chlorogenic acid, epicatechin, caffeic acid, phloridzin, *p*-coumaric acid, phloretin, quercetin and rutin), and with the aid of the Liquid chromatography-Mass Spectroscopy database of The New Zealand Institute for Plant & Food Research Limited. The mobile phases (A) acetonitrile + 0.1% formic acid and (B) acetonitrile:water:formic acid (5:92:3) were pumped at 1.5 mL/min at 45ºC. Each smoothie extract (1 mL) was centrifuged (Eppendorf Centrifuge 5702, Hamburg, Germany) at 3000 rpm for 15 min. Injection volume was 40 µL.

L. Effect of Heat Treatments on PP Stability and Extractability in Absence or Presence of Apple Fibre

A set of simplified acidic aqueous model formulations (pH 3.5) was established, in the absence or presence of apple fibre (7.5 g per 300 mL serve). The same ingredients as those in the smoothie formulations were used: apple pectin, citric acid, and APE, BPE or phenolic chemicals including chlorogenic acid, phloridzin, *p*-coumaric acid (1.67 mg phenolics per mL serve). These model formulations were subjected to two different heat treatments (75°C for 45 min or 85°C for 1 min) that are normally used for beverage production. Analysis of total phenolic content and phenolic profiling (by HPLC) were conducted on the samples before and after heat treatment.

M. Statistical Analysis

For the sensory evaluation, a parametric analysis of the data was performed using a REML mixed model analysis (Genstat Release 10 [(PC/Windows XP) Copyright 2006, Lawes Agricultural Trust (Rothamsted Experimental Station)]) with fixed effects for presentation order, preceding sample (included because of the large differences between some of the samples), fibre level (adjusted for PP level), PP level (adjusted for fibre level) and fibre x PP interaction, as well as

random effects for tasting session, subject and sample bottle to examine flavour, texture and overall acceptability scores [43]. The results obtained from chemical tests were statistically analysed using two-way analysis of variance.

III. Results and Discussion

A. Effects of Fibre Level and Polyphenol Concentration on the Acceptability of Smoothies

In general, the seven smoothies prepared for consumer acceptability testing had a good consistency with no phase separation. The total soluble solid content ranged from 11.0 to 12.5 °Brix, and the pH ranged from 3.4 to 3.5 (data not shown). These seven smoothies were evaluated by 73 consumers. Thirtysix percent (n=26) of consumers were male and 64% (n=47) were female with 58% (n=42) being in the 35–50 age group and 42% (n=31) in the 51–65 year age group. Most participants identified themselves as European (n=55) or Kiwi/New Zealander (n=9). In general, acceptability of the prototype smoothies was low and decreased with elevated fibre (P<0.001) and PP (P<0.001) contents (Table 1). Fibre content had a stronger effect than PP content on consumer acceptance.

As shown in Table 1, the 2.5 g fibre x 250 mg PP smoothie was given the highest overall ratings for flavour, texture and overall acceptability, with average hedonic ratings of 6.7, 6.4 and 6.7 (like slightly through to like moderately) respectively. The least acceptable smoothie was the 7.5 g fibre x 750 mg PP formulation with average flavour, texture and overall acceptability ratings of 4.4, 3.8 and 3.9 respectively, equating to “dislike slightly”. There was a significant effect of the fibre x PP interaction for flavour (P = 0.029) and overall acceptability (P = 0.025), but the effects were less strong than for the individual main effects, possibly due to the lack of effect of PP content on the two 4.5 g fibre formulations. A similar trend was detected for texture ratings, but the fibre x PP interaction was not significant (P = 0.292). Acceptability was lower for the smoothies with 7.5 g fibre than those with 2.5 g fibre at PP levels of 250 and 500 mg. There were no significant attribute differences between the 4.5 g smoothies at the two PP concentrations, whereas differences were seen for those from the 2.5 and 7.5 g fibre levels.

Bitterness from the PPs is likely to be one reason for decreasing acceptability with increasing PP [21], [22]. Undesirable flavours described by consumers as "slightly muddy”, “earthy” and “dried plant" were associated with the apple fibre occurring in the formulations; this may be responsible for the decreased flavour acceptability of elevated fibre content smoothies. PPs might bind to fibre polysaccharides [26], [44]; however, the potential binding effect is not sufficient to mask the unpleasant flavours in this study, like bitterness and astringency. The information obtained from this sensory study will help to determine the maximum and/or optimum fibre and PP concentrations for a sensorially acceptable smoothie-type beverage.

B. Viscosity of Smoothies

The viscosity of the seven smoothies (as described in Table 1) was analysed as a function of shear rate. A shear rate range from 0.1 to 100 s^{-1} was used for initial examination. It was found that in general, all the smoothies appeared to be non-Newtonian fluids showing pseudoplastic flow behaviour: all had similar viscosity values when the shear rate was less than 9.2 s^{-1} (data not shown). The flow curve pattern of all the smoothies, however, dramatically changed at a shear rate greater than 10 s^{-1}, and this change occurred at a slightly lower shear rate for the smoothies that contained the lower level of fibre, i.e. 2.5 g fibre (data not shown). Fig. 1 shows just the viscosity values at shear rates in the range of 30-60 s^{-1}, a range close to the effective shear rate range (40-50 s^{-1}) in the mouth [45]. The viscosity of heated suspensions of starches or hydrocolloids solutions depends markedly on the measuring conditions. The viscosity within the selected shear rate range (Fig. 1) would have implied actual sensory consistency [45].

TABLE I
Consumer Acceptability Ratings of Smoothies

Attribute	Fibre (g)	PP (mg)			Treatment effects	F test probability
		250	500	750		
Flavour[a]	2.5	6.43	5.48		Fibre (adj. for PP)	< 0.001
	4.5	5.71	5.74		PP (adj. for fibre)	< 0.001
	7.5	5.28	4.83	4.37	Fibre, PP	0.029
Texture[b]	2.5	6.69	6.09		Fibre (adj. for PP)	< 0.001
	4.5	5.87	5.73		PP (adj. for fibre)	< 0.001
	7.5	4.61	4.08	3.79	Fibre. PP	0.292
Overall[c]	2.5	6.44	5.46		Fibre (adj. for PP)	< 0.001
	4.5	5.59	5.51		PP (adj. for fibre)	< 0.001
	7.5	4.91	4.27	3.91	Fibre, PP	0.025

Note: *a*, Approx. LSD (5% level) 0.505; *b*, Approx. LSD (5% level) 0.451; *c*, Approx. LSD (5% level) 0.456. Acceptability 9-point hedonic scale: 9 = like extremely, 1 = dislike extremely. PP = polyphenols.

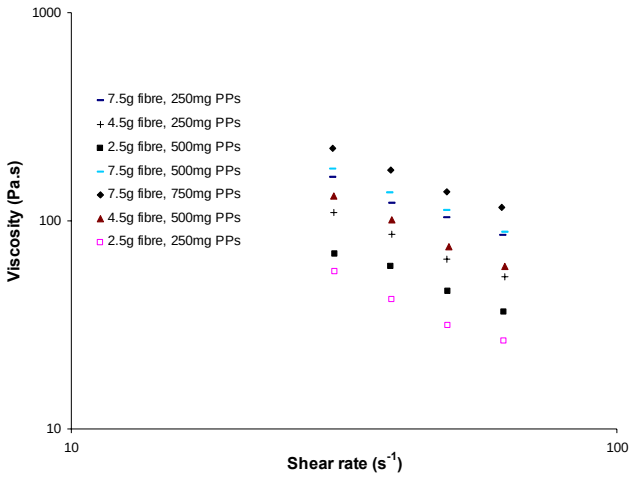

Fig. 1 Viscosity of smoothies containing 250, 500 or 750 mg polyphenols (PPs) and 2.5, 4.5 or 7.5 g fibre tested after production, measured as a function of shear rate

Fig. 1 shows that the viscosity decreased when the shear rate increased. At the same shear rate (within 30 to 60 s^{-1}), the

viscosity increased in the order of the smoothie containing 2.5 g fibre & 250 mg PPs < 2.5 g fibre & 500 mg PPs < 4.5 g fibre & 250 mg PPs and 4.5 g fibre & 500 mg PPs < 7.5 g fibre & 250 mg PPs and 7.5 g fibre & 500 mg PPs < 7.5 g fibre & 750 mg PPs. The change in viscosity as a function of fibre level did not follow a linear trend. The viscosity of the smoothie with 4.5 g fibre changed from the values closer to those with 7.5 g fibre, to the median value of the smoothies containing 2.5 and 7.5 g fibre, when the shear rate was increased from 30 to 60s^{-1}. Given that there were identical concentrations of ingredients other than fibre and PP added to each smoothie (i.e. only apple fibre, APE and BPE contents varied), it can be concluded that apple fibre content has played the dominant role in smoothie viscosity, with the PP level only slightly influencing the viscosity. The higher the fibre content, the greater the viscosity. Previous studies have also reported that added fibre affects the rheological properties of foods because of their water binding and swelling properties [12]–[14].

C. HPLC Phenolic Profiling

Fig. 2 shows the typical HPLC phenolic profiles at 280 and 530 nm of the smoothies investigated in this study. The same type of PPs were found in all smoothies, with the relative proportion of individual compounds varying with the amounts of added APE, BPE and apple fibre. These HPLC profiles were characteristic of the PP profiles for both apple and blackcurrant [24], [25], [34]. HPLC results show that major PPs (including delphinidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, catechin and its derivative, chlorogenic acid, phloridzin and quercetin) were retained in the smoothies after processing. The apple fibre, which contains chlorogenic acid, quercetin derivative and phloridzin, might also introduce additional amounts of these PPs [32].

D. Changes in Total Phenolic Content, Vitamin C Content and Colour During Storage at 4°C

Addition of high levels of DF and PPs, and the influence of such added fibre or PPs on the total phenolic content, vitamin C content and colour attributes of smoothies were the objectives of this study. The impact of added fibre was of particular interest, because most of the previous studies focussed on the beverages with high level of PPs only [21], [25]. To provide this insight, the obtained results were analysed where one parameter (PP or fibre) had been fixed at the same level.

Total Phenolic Content

The effect of fibre level on the total PP content was examined over the 14-day storage at 4°C. The fibre level of a smoothie appeared to affect the extractable PPs over time. At the same added PP level, the reduction in total extractable PP content after 14 days was the greatest for the smoothie with 2.5 g fibre, followed by that with 4.5 g fibre and then with 7.5 g fibre (Fig. 3). This was not readily apparent after the first eight days of storage (maximum 13% reduction in the PP content), but became obvious after 14 days of storage (up to 54% reduction in the PP content).

While only 250 mg PPs were added in the form of APE and

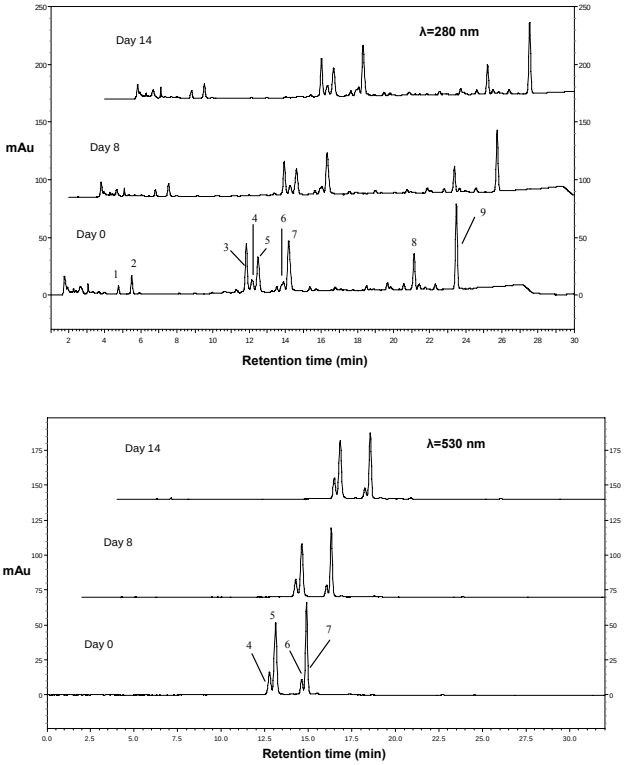

Fig. 2 HPLC chromatograms at 280 and 530 nm for a smoothie containing 750 mg polyphenols and 7.5 g fibre during storage at 4°C for 14 days. Peak 1: Unknown; Peak 2: Catechin derivative; Peak 3: Chlorogenic acid; Peak 4: Delphinidin-3-*O*-rutinoside; Peak 5: Delphinidin-3-*O*-glucoside; Peak 6: Cyanidin-3-*O*-rutinoside; Peak 7: Cyanidin-3-*O*-glucoside; Peak 8: Phloridzin; Peak 9: Quercetin

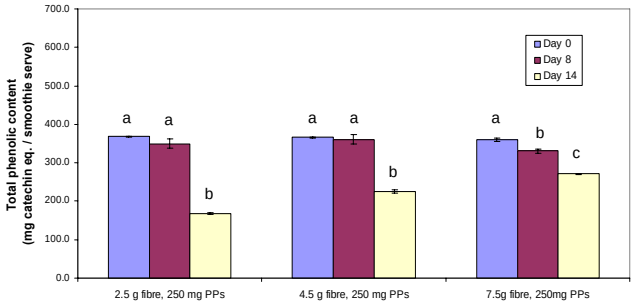

Fig. 3 Total phenolic content of smoothies (250 mg polyphenols, and 2.5, 4.5 or 7.5 g fibre) during storage at 4°C for 14 days. For each smoothie, columns with different letters (a, b, c) are significantly different (α = 0.05). Error bars are the standard deviation of the mean

BPE ingredients, the actual total phenolics detected in the smoothies at the start of the storage period (Day 0) using the same Folin-Ciocalteu assay were 360~368 mg. This suggests

that "free" PPs might be released from phenolic-containing complexes during processing, including those originally bound to the apple fibre and/or in a complex form with other food components [32], [46]–[49]. Perhaps this is not surprising as phenolic compounds of low molecular weight are reported to be easy to release from complexes during heat treatment [46], [50] and there was a pasteurisation step in the smoothie production process. Previous studies have also found an effect of the food matrix on the PP extractability [1], [24], [51], [52]. Therefore, the phenomenon that elevated fibre levels prevent the loss of extractable PPs to some extent, was possibly the net result of the retention of original PPs and the release of free PPs from phenolic-containing complexes.

Vitamin C Content

The possible relationship between the levels of vitamin C and PPs during storage was examined using a constant fibre level (7.5 g per 300 mL serve). Rapid degradation of vitamin C was observed in smoothies during a 14 day storage period at 4°C (Fig. 4). The rates of vitamin C loss for smoothies that contained the same amount of fibre but varying PP levels appeared to be different during storage. After eight days, vitamin C was not detectable in the smoothie containing 750 mg PPs, while ~33% of the original vitamin C level was retained in smoothies containing either 250 or 500 mg PPs. After 14 days, the amount of vitamin C had further diminished and was only detectable in the smoothie containing the least amount of PPs (i.e. 250 mg).

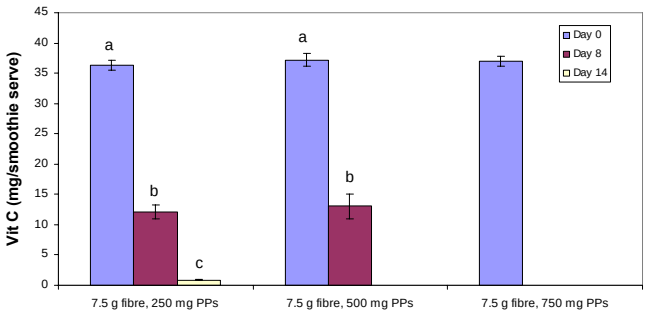

Fig. 4 Vitamin C content of smoothies (250, 500 or 750 mg polyphenols, and 7.5 g fibre) during storage at 4°C for 14 days. For each smoothie, columns with different letters (a, b, c) are significantly different (α = 0.05). Error bars are the standard deviation of the mean

The experiments here have used a constant fibre level for examining the effect of added PPs on vitamin C, because different fibres have been found to preserve vitamin C to varied extent [30], [31]. The degradation of vitamin C seemed to be associated with the concentration of PPs. It is well known that vitamin C is susceptible to degradation even during refrigerated storage, and the degradation could be affected by the matrix environment e.g. the soluble solids content [53], [54]. Vitamin C may retard PP degradation [55] and be used to regenerate PPs [56]. Therefore, the vitamin C initially present in the smoothie might be used up more quickly when the smoothie contained greater amounts of PPs, as it would be used to slow degradation of these PPs in the smoothie during storage.

Colour

It was noted that the levels of both PPs and fibre affected the smoothie colour and different degrees of dark red colour that were derived mainly from the BPE ingredient, dominated the smoothies. In the presence of a constant level of fibre, different amounts of BPE and APE ingredients, especially BPE, substantively altered the smoothie colour: positive L^* and a^* values decreased and increased, considerably and respectively, with a 250 mg PP incremental increase (data not shown).

The effect of fibre level on the smoothie colour over the 14-day storage at 4°C can be seen in Fig. 5. At a constant PP concentration (250 mg), the amount of added fibre influenced the colour observed. In general, L^*, a^* and b^* values (all positive) increased in smoothies with lower fibre content. Greater changes were found in the a^* and b^* values. This suggests that less fibre would lead to a smoothie with lighter colour, and with more yellowness and redness. Fig. 5 shows the colours of the smoothies containing 7.5 and 4.5 g fibre were closer in value than those of the smoothies containing 4.5 and 2.5 g fibre. Discolouration in redness occurred but at least 75% of the initial redness was retained after 14 days. An 8-day storage caused a smaller change in redness than a 14-day storage. These results suggest the degradation of anthocyanin compounds, which was possibly associated with the differences in the total extractable PP (Fig. 3) and vitamin C (Fig. 4) contents between the 8-day and 14-day storages.

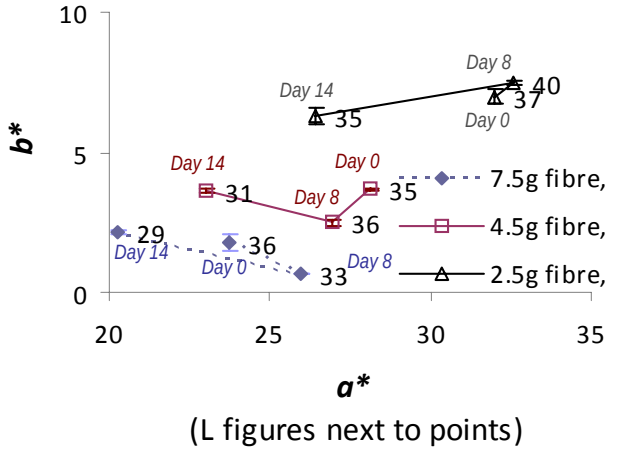

Fig. 5 Colour changes of smoothies containing 250 mg polyphenols (PPs) and 2.5, 4.5 or 7.5 g fibre during storage at 4°C for 14 days. Error bars are the standard deviation of the mean

The colours of the smoothies were initially due to the added ingredients, especially the anthocyanin pigments from BPE. With storage, the degradation of these pigments was possible, and other colours might also be generated during processing and storage due to the degradation of PPs and vitamin C,

and/or other non-enzymatic browning reactions such as Maillard reaction and/or caramelisation [55], [57], [58]. There were possibly different mechanisms of action of anthocyanin colour change in the smoothie system. One might be associated with the change in vitamin C content during storage (Fig. 4). Previous studies reported that the colours of anthocyanins are pH-dependent [28], and degradation of ascorbic acid can accelerate anthocyanin degradation with storage time via unknown kinetics [59]–[61].

E. Effect of Heat Treatments on PP Stability and Extractability in Absence or Presence of Apple Fibre

The effects of two heat treatments that are routinely used during smoothie production on the individual phenolic compounds or total phenolic content were examined. Different PPs have been found to possess various biological activities [5], [33], [36], [37] and processing stability [26]–[28]. Functional foods or beverages are developed to deliver one or more targeted phenolic compounds to the consumers. Therefore, it is a prerequisite to understand the stability of individual PPs in the smoothie format and the quantitative change of total PP content in the final product after a heat treatment.

The heat treatments resulted in different levels of increases in total extractable PPs for all the model systems except for that containing BPE (Fig. 6). The magnitudes of these changes were different between the systems with and without fibre.

In the absence of apple fibre (Fig. 6), heating at 75°C for 45 min led to approximately 4, 32, 131 and 16% increases in extractable PPs for the models containing chlorogenic acid, *p*-coumaric acid, phloridzin and APE, respectively, whereas heating at 85°C for 1 min resulted in approximately 3, 4, 25 and 66% increases in extractable PPs for the same models. For the BPE-containing system, heating at 85°C for 1 min led to a smaller decrease (~3%) in extractable PPs than heating at 75°C for 45 min (~28%). Therefore, the two heat treatments influenced the total extractable PPs of various phenolic-containing systems to different extents, depending on the type of PPs present. The combination of elevated heating temperature and reduced heating time (e.g. 85°C for 1 min) may be more suitable for the systems that contain non-purified fruit PP ingredients such as APE and BPE. This may be because of the matrix effect derived from the non-phenolic compounds in the APE and BPE (APE and BPE contain ~21.5 and 71% of non-phenolic compounds that have been co-extracted during manufacturing).

In the presence of apple fibre (Fig. 6), heating at 75°C for 45 min led to approximately 65, 47, 135 and 3% increases in extractable PPs for the models containing chlorogenic acid, *p*-coumaric acid, phloridzin and APE, respectively, whereas heating at 85°C for 1 min resulted in approximately 5, 26, 43 and 29% increases in extractable PPs for the same models. Therefore, the effects of added fibre on the extractable PP contents were different for the two heat treatments. The presence of fibre resulted in a particularly large increase in the extractable PPs, for the model systems containing *p*-coumaric acid, phloridzin, chlorogenic acid (at 75°C), and APE (at 85°C).

It is not surprising that similar trends were observed between the model containing APE and those containing purified phenolic chemicals (*p*-coumaric acid, phloridzin and chlorogenic acid), because these purified compounds are the major PPs in APE. The fact that the BPE-apple fibre model systems had substantively higher extractable PP contents than those containing BPE only, with or without heating, further confirmed that fibre assists in the stability and extractability of PPs, or alternatively provides an additional source of extractable PPs.

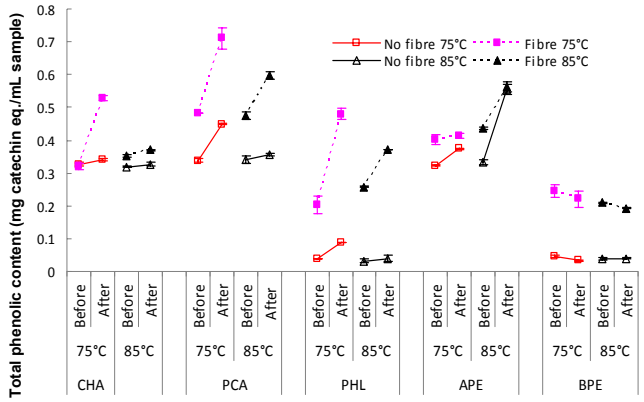

Fig. 6 Total phenolic content as a function of heat treatment (75°C, 45 min or 85°C, 1 min) of model systems with/without apple fibre. CHA=Chlorogenic acid; PCA=*p*-Coumaric acid; PHL=Phloridzin; APE=Apple polyphenol (PP) extract; BPE=Blackcurrant PP extract. Error bars are the standard deviation of the mean

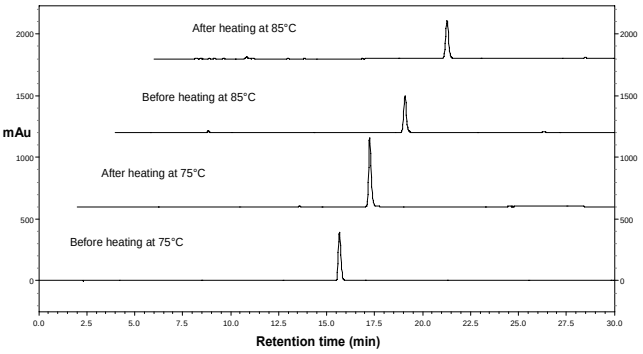

Fig. 7 HPLC profiles of a model system containing *p*-coumaric acid and without apple fibre, before and after heating at 75°C for 45 min or 85°C for 1 min

HPLC analyses further examined the effects of heat exposure on the concentration of one targeted phenolic compound. Fig. 7 shows the HPLC chromatograms of the model systems containing *p*-coumaric acid but in the absence of added apple fibre. Heating at 75°C for 45 min resulted in ~46% increase in *p*-coumaric acid while heating at 85°C for 1 min caused no significant changes ($P > 0.05$) in the quantity of *p*-coumaric acid, a trend in agreement with that of the total

PP contents (Fig. 6. detected by the Folin–Ciocalteu assay). Given the fact that this model system only contained *p*-coumaric acid, citric acid and apple pectin, it can be concluded that the two heat treatments did not cause the breakdown of *p*-coumaric acid; it was *p*-coumaric acid itself that was responsible for the increased total extractable PP content of the model system after heating. Thus, the two heat treatments were suitable for the systems that carry *p*-coumaric acid.

In summary, the effects of heat treatments on the stability and extractability of PP depend on the type of phenolic compounds, method of heating, and absence/presence of fibre. Heating might cause the degradation of some PPs that initially occurred in a food system [62], but conversely might simultaneously facilitate the release of bound PPs from the food matrix (including from fibre). An increase in the total extractable PP content of a product suggests either good stability and extractability of PPs, or compensation of the loss of initial PPs due to degradation by released PPs from the food matrix [31], [50], [51], [63]. The release of a phenolic compound is associated with its polarity and ultimately its chemical structure [64]–[69]. The more hydrophobic the added phenolic compound, the greater the amount of this phenolic compound that can be released into the food system upon mild heating e.g. at 75°C. Theoretically, phenolic compounds have a pK_a range of 8–12, possessing more than one hydroxyl group that can conjugate with sugars, acids or alkyl groups [64], [65]. Complexation between phenolics and polysaccharides and/or proteins can occur reversibly via hydrogen bonding between hydroxyl groups of PPs and the unshared electron pairs of oxygen atoms on the carbonyl group or the undissociated carboxyl groups in the pectic polysaccharides or peptide residues of proteins [44], [66], [67]. The acidic and phenolic compounds (hydrogen-bonding donors) might contribute to the strength of hydrogen bonding as a function of acid strength (pK_a) and the number of carboxyl and hydroxyl groups [68], [69]. Thus, it is not surprising that chlorogenic acid, *p*-coumaric acid and phloridzin, which have two carboxyl and six hydroxyl groups, one carboxyl and one hydroxyl group, and one carboxyl and three hydroxyl groups, respectively, behaved differently in this study.

IV. Conclusions

The addition of phenolic antioxidants as well as DF as ingredients would offer the potential of health-promoting properties in food products. This study has shown other advantages and characteristics of fibre addition. It was found that the presence of apple fibre improved the stability of phenolic compounds during heat treatment and storage. In the presence of a high level of fibre, vitamin C was preserved better when a lower level of PPs was added. Furthermore, addition of apple fibre could influence the smoothie colour.

There are challenges in developing a smoothie-type product carrying the level of fibre required for label claims. The incorporation of high levels of fibre and PPs led to reduced consumer acceptability. Consumer acceptability of the smoothies in this study was rated not more than 7 (on a 9-point hedonic scale), and acceptance decreased as the fibre and PP contents increased. Although this sensory evaluation was conducted without disclosing the health benefits associated with the product, and using smoothies that were produced in the absence of extensive efforts for product optimisation, the indicative information gained, such as the maximum fibre and/or PP concentrations for an acceptable smoothie beverage, is still valuable. Providing information on a products health benefits may improve consumer acceptance [70], [71]. Optimising the interactions among phenolics, anthocyanins, vitamin C and fibre polysaccharides during formulation, processing and storage, is a feasible approach to deliver high fibre and high PPs in a smoothie format. Other approaches, such as increasing sweetness (to counteract sourness and/or bitterness), and the use of masking techniques [72], may also improve the flavour profile to some extent.

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