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Comparative analysis of fruit-based functional snack bars

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ABSTRACT

The aim was to develop snack bars with high dietary fibre (DF) and polyphenol contents. Snack bar base was formulated without (control bar) or with fibre (inulin or apple DF bar). Snack bar filling was formulated with or without apple polyphenol extract (APE). Nutritional assessment of snack bars was based on the total DF, phenolics, protein, fat, uronic acid (UA) and moisture contents, water activity, Hunter $L^*a^*b^*$ colour, hardness, and phenolic composition. Results showed that snack bars with added apple DFs gave the highest amount of total DF (~5.3% w/w). Good quantities of phenolics were also retained in the snack bars with added apple DF and APE after baking. With APE in the bar filling, the snack bars enhanced with apple DF or inulin had higher levels of extracted phenolics (2.87 and 2.22 mg catechin equivalent (CtE)/g bar) than the control bars (1.45 mg CtE/g bar). The phenolic profiles of snack bars obtained by HPLC resembled that of the APE ingredient. The Hunter $L^*a^*b^*$ colour and water activity values suggested that the addition of APE did not cause extra browning and the resultant snack bars would possess a good shelf life. Therefore, snack bars enhanced with apple DF and APE may be a convenient functional food, offering a good source of DF and apple polyphenols.

1. Introduction

Epidemiological studies suggest that regular or increased consumption of fruits and vegetables may reduce the risk of chronic diseases (Block, Patterson, & Subar, 1992; Joshipura et al., 2001; Rimm et al., 1996), and these health benefits are thought to be mainly attributable to their natural antioxidant and dietary fibre (DF) content (Bravo, Abia, & Saura-Calixto, 1994; Lee, Kim, Kim, Lee, & Lee, 2003; Middleton, Kandaswami, & Theoharides, 2000; Pelucchi et al., 2004; Seeram et al., 2005; Williamson, Day, Plumb, & Couteau, 2000). Because of the growing consumer demand for healthy, natural and convenient foods, attempts are being made to improve snack foods' nutritional values *via* modifying their nutritive composition (Bhaskaran & Hardley, 2002; Gray, Armstrong, & Farley, 2003). Snack bars are a popular and convenient food and, therefore, would be an ideal food format to deliver fruit-derived phenolic antioxidants and DF.

The positive roles of fibre in health and disease particularly in digestive health, energy balance, cancer, heart and diabetes problems justify the demand of increasing DF content in the daily diet (Alexiou & Franck, 2008; Elia & Cummings, 2007; Lunn & Buttriss, 2007; Prosky, 2000; Schulze et al., 2004; Scott, Duncan, & Flint, 2008; Slavin & Green, 2007). DF is a collective term for a group of substances with varied chemical composition, structure, physical properties and physiological effects. The definition of DF has evolved since 1976 (AFSSA, 2002; EFSA, 2007; European Commission, 2008; Ferguson, Chavan, & Harris, 2001; FSANZ, 2001; Health Council of the Netherlands, 2006; IOM, 2001; Prosky, 2000; Trowell et al., 1976). A final agreement was only reached in November 2008 on a global DF definition for the Codex Alimentarius (ALINORM 09/32/26, 2009). Now the Codex defines DF as carbohydrate polymers with 10 or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: (1) edible carbohydrate polymers naturally occurring in the food as consumed; (2) carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; (3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health, as demonstrated by generally accepted scientific evidence to competent authorities. Such an agreed definition facilitates consistent application of labelling and health claims that bring consumers clarity and confidence. In the past, the debate was not only on the DF definition but also on a DF analysis method. A globally integrated method for determining the new Codex DF is still underway. Currently, the DF analysis methods used widely include the approved AOAC DF methods (such as the enzymatic–gravimetric method) and the Englyst method (Englyst & Cummings, 1988).

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Different sources of fibre are increasingly being explored as an ingredient to provide desirable properties, such as water-holding capacity in food applications (Alminger & Eklund-Jonsson, 2008; Chaudhary & Awasthi, 2009; Frutos, Guilabert-Anton, Tomas-Bellido, & Hernandez-Herrero, 2008), including whole plant cell walls, and non-starch oligo- and polysaccharides, such as pectins and inulin (Ferguson et al., 2001; Prosky, 2000). Fruit cell walls with complex polysaccharide network consist of cellulose and non-cellulose polysaccharides, glycoproteins, water, and other smaller molecules, such as phenolic acids (Fry, 1988; O'Neill & York, 2003; Sun-Waterhouse, Melton, O'Connor, Kilmartin, & Smith, 2008; Sun-Waterhouse, Smith, O'Connor, & Melton, 2008). Fruit fibres have balanced soluble and insoluble DF contents, which may be suitable for DF enrichment (Grigelmo-Miguel, Gorinstein, & Martín-Belloso, 1999; López et al., 1996; McKee & Latner, 2000).

Apples are always part of a healthy diet because of their high DF content (Sun-Waterhouse, Farr, Wibisono, & Saleh, 2008; Sun-Waterhouse, Melton, et al., 2008; Sun-Waterhouse, Smith, et al., 2008) and the presence of various health beneficial polyphenols, such as procyanidin, catechin, epicatechin, chlorogenic acid, phloridzin, quercetin and their conjugates (Amiot, Fleuriet, Cheynier, & Nicolas, 1997; Gardner, White, McPhail, & Duthie, 2000; Lee et al., 2003; McGhie, Hunt, & Barnett, 2005). Apple phenolic compounds have exhibited anti-inflammatory and anticarcinogenic properties, and the ability to prevent a variety of chronic diseases (Boyer & Liu, 2004).

It is a challenge to develop a snack bar in manageable portions with high levels of polyphenols and/or fibre. Technical challenges include the retention of polyphenol and fibre contents, and amelioration of desired sensory attributes of finished foods after food processing. This study examined the effects of added fibres and/or polyphenols on the physicochemical properties of snack bars. Chemical composition, colour, hardness and water activity of the formulated snack bars were analysed to evaluate the product's appearance, nutritional value, quality and shelf life.

2. Materials and methods

2.1. Materials and chemicals

Granny Smith apples (*Malus domestica* Borkh 'Granny Smith') were purchased from a local supermarket. Apple polyphenol extract (APE) was purchased from Penglai Marine Biochemicals Ltd., Shandong, China, containing 770 mg oligoproanthocyanidins complex/g dried weight.

Catechin, phloridzin, *p*-coumaric acid, protocatechuic acid, epicatichin, syringic acid, phloretin, quercetin, *p*-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, syringic acid, ferulic acid, salicylic acid, D-galacturonic acid, *m*-hydroxydiphenyl, Folin–Ciocalteu phenol reagent, bovine serum albumin (Fraction *V*, ~99%) were purchased from Sigma–Aldrich, St. Louis, MO. Hydrochloric acid, methanol, acetone, sulphamic acid, hexane and sulphuric acid were from Ajax Chemical Ltd., Sydney. Ethanol, sodium hydroxide and phosphate buffer were from Merck, Darmstadt, Germany. Total dietary fibre test kit (including α-amylase, protease and amyloglucosidase enzymes) was from Megazyme International, Wicklow, Ireland. Bradford reagent was from BioRad, Hercules, CA. Celite™ was from Johns Manville Corporation, Denver, Co. Milli-Q^{PLUS} water was used for all reagent preparation.

2.2. Natural apple dietary fibre preparation

Natural apple DF was prepared using the aqueous method of Sun-Waterhouse, Farr, et al. (2008). Fresh apples were peeled, cored, ground, mechanically ruptured and washed in an aqueous system. No solvent was introduced to the fibre preparation process. The resultant apple fibre was freeze-dried using a freeze drier (Telstar Cryodos-80, Telstar Industrial, SL, Terrassa, Spain) before addition to the snack bar formulations.

2.3. Apple purée preparation

Apple purée was prepared at Plant and Food Research Palmerston North. Canned diced apple (Pams®) was purchased from a local supermarket, and then puréed in a food processor (Hallde VCS-61 Commercial Food Preparing Machine, Kista, Sweden) prior to snack bar preparation.

2.4. Snack bar preparation

2.4.1. Snack bar base preparation

Ingredients of the control snack bar base include rice crisps (from supermarket, Sanitarium, Auckland, New Zealand), glucose syrup (Avonsweet, Penfords, Auckland, New Zealand), honey (from supermarket, Pams Creamed Clover Honey, Auckland, New Zealand), vegetable oil (from supermarket, Alpha One Rice Bran Oil, Old Fashioned Foods Group Ltd., Auckland, New Zealand), quick-cook rolled oats (Davis Trading, Petone, New Zealand), glycerol (Bronson & Jacobs Pty, Auckland, New Zealand), whey protein concentrate (WPC 80 CHS 4501, NZMP, Fonterra, Auckland, New Zealand), maltodextrin (Dridex 10, Penfords, Auckland, New Zealand), and pectin (CF 120, Grinsted (R), Danisco NZ Ltd., Auckland, New Zealand). For the apple DF and inulin-enhanced bars, apple DF prepared in this study or inulin (Frutafit® IQ, Sensus Asia Pacific, Kuala Lumpur, Malaysia) were added to replace the same amount of quick-cook rolled oats (2.7%).

The wet ingredients were mixed, and then poured into the premixed dry ingredients. The resultant mixture was mixed thoroughly by hand, and 37.5 g were weighed out and moulded into a rectangular bar (45 mm × 95 mm × 12 mm). The moulded bars were baked at 130 °C for 15 min and cooled at room temperature.

2.4.2. Snack bar filling making

Ingredients of the snack bar filling include pectin (CF 120), sugar (sucrose, White Sugar, Chelsea, Auckland, New Zealand), citric acid (Hawkins Watts, Auckland, New Zealand), glucose syrup (dextrose monohydrate, Penfords, Lane Cove, Australia), honey, vegetable shortening (from supermarket, Kremelta Vegetable Shortening, Epping, Australia), glycerol, and apple purée. For the filling containing added APE, APE (1.2%) was added to replace apple purée (0.3%), sugar (sucrose, 0.1%), glucose syrup (0.2%), honey (0.5%) and vegetable shortening (0.1%).

All ingredients, except for APE, were weighed into a pan and heated on the stove with constant stirring. The mixture was simmered until the final soluble solids content ranged from 84% to 86%. For the filling enhanced with added APE, the APE was added last when the mixture was still warm.

2.4.3. Snack bar assembly

Warm filling mixture (25 g) was applied over one side of a snack bar base. Another bar base was then placed on top of the filling mixture, i.e., the filling was sandwiched between two bar bases. Once cooled to room temperature, the snack bars were packed in a foil wrapper. The final snack bar product weighed 85 g in total.

2.5. Uronic acid content determination

The snack bars of the same formulation were blended using a Sunbeam multi-blender (Model PB7600, Mt Wellington, Auckland, New Zealand). The uronic acid content of the snack bars

or apple DF was determined following the procedures of Sun-Waterhouse, Melton, et al. (2008). Uronic acid was measured at 525 nm using the method of Filisetti-Cozzi and Carpita (1991), after hydrolysis of the freeze-dried snack bar by concentrated sulphuric acid and reaction of the hydrolysate with *m*-hydroxydiphenyl. Potassium sulphamate and sodium tetraborate were added to prevent interference from neutral monosaccharides. A calibration curve for the standard, D-galacturonic acid, was also established.

2.6. Neutral monosaccharide content of apple DF

The neutral monosaccharide composition (as their alditol acetates) of the apple DF were determined *via* hydrolysis by trifluoroacetic acid (2 M, 121 °C, 1 h) and H_2SO_4 (72%, 30 °C for 2 h, and 1 M, 100 °C for 3 h) for cellulose (Sun-Waterhouse, Smith, et al., 2008).

2.7. Preparation of polyphenol extracts from snack bar by accelerated solvent extraction

The freeze-dried snack bar (5 g) was mixed with Celite™ (diatomaceous earth) at a ratio of 1:1 w/w and then transferred into the Dionex standard 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: 40 °C and 1500 psi with 5 min heating and 10 min static time). Three extraction cycles were performed using 95% methanol. The polyphenol extracts obtained were concentrated (to remove methanol) using the Centrivap® (Model 78100–01, Ultra-Low Cold Trap, Labconco Corp., Kansas City, MO) followed by freeze-drying. The extracts were kept at −80 °C for Folin–Ciocalteu assay and HPLC analysis.

2.8. Preparation of polyphenol extracts from apple DF

Polyphenol extracts from the apple DF were prepared for the total phenolic content and individual phenolic analysis following the procedure of Sun-Waterhouse, Farr, et al. (2008). Freeze-dried apple fibre (100 mg) was mixed with 0.1 M NaOH (at a concentration of 10 mg/ml, under N_2), vortexed, acidified to pH 3 with 1 M HCl, and then extracted with ethyl acetate (1:1) three times. The upper ethyl acetate fraction was collected after centrifugation (3000*g*, 5 min). The three ethyl acetate extracts were combined and dried (under N_2).

2.9. Total phenolic content determination

Total phenolic content of snack bar or apple DF was analysed followed the method of Singleton, Orthofer, and Lamuela-Raventos (1997) and expressed as catechin equivalents (CtE). The absorbance at 760 nm was detected using a microplate reader (SpectraMax Plus 384, Sunnyvale, CA). A calibration curve (absorbance vs. catechin concentration mg CtE/ml) was established for the extraction medium.

2.10. Individual phenolic analysis

The dried polyphenol extracts from Sections 2.7 or 2.8 were then reconstituted with 25% methanol to give a concentration of 50 mg/ml, and analysed by HPLC following the method of Stevenson, Wibisono, Jensen, Stanley, and Cooney (2006). The HPLC analysis was run 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 × 4.6 mm, 4 μm particle size, 80 Å ether-linked column, Phenomenex, Auckland, New Zealand). 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 45 °C. The injection volume was 40 μl, and three wavelengths (280, 320 and 370 nm) were used. Individual phenolic compounds were identified based on their retention time and the absorbance maximum (λ_{max}).

External standards were used to aid the identification of phenolics (including catechin, protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, 2,4-dihydroxybenzoic acid, epicatichin, syringic acid, ferulic acid, phloridzin, phloretin, quercetin, and salicylic acid).

2.11. Total protein, fat, fibre and ash content determination

2.11.1. Total dietary fibre and ash contents

Total DF content of the snack bars or apple DF was determined using the Megazyme International total DF assay (adopted from AACC method 32–05 and AOAC method 985.29).

The snack bar or apple DF samples were blended, ground, and de-fatted through mixing a portion of the dried sample with petroleum ether (at a ratio of 25 ml petroleum ether per gram dried sample) and centrifuging at 2000*g* for 5 min at room temperature. This step was repeated three times for each snack bar sample. The residual petroleum ether was removed through flushing N_2 in the RapidVap® concentrator unit (model 79100-01, LabConco Corporation). The de-fatted snack bar samples were accurately weighed (~1 g) in duplicate into beakers. Phosphate buffer (50 ml, pH 6.0) was then added, followed by the α-amylase solution (50 μl). The mixture was heated to boiling for 15 min with gentle shaking at 5-min intervals. A blank was run in parallel.

After the mixtures were cooled to room temperature, their pH values were adjusted to 7.5 ± 0.1 using NaOH (0.275 N). After the addition of protease solution (100 μl), the mixtures were heated to 60 °C with continuous agitation for 30 min. Amyloglucosidase (200 μl) was then added followed by incubation at 60 °C for 20 min. The resultant mixtures were allowed to precipitate by adding pre-heated ethanol (60 °C, 95%, 280 ml) and incubating at room temperature for 60 min.

Pre-weighed crucibles (sintered glass, G1, Pyrex, England) containing Celite were prepared and wetted using a stream of 78% ethanol. The mixtures generated from previous steps were then filtered through the glass funnel to the crucibles. Residues in crucible were washed successively with three portions of 78% ethanol, followed by two portions of 95% ethanol (10 ml) and two portions of acetone (10 ml). One set of the replicates was dried in an air oven overnight at 105 °C before the next stage of analysis (protein measurement). The other replicate was incinerated for 5 h at 525 °C, cooled in the oven (until the oven could be opened), and further cooled in a desiccator before weighing (to obtain the ash content). The total DF is taken as the weight of the sample after correction for the protein and ash contents.

2.11.2. Total protein content

The protein content of the snack bars was determined using the Bradford colorimetric assay (Bradford, 1976). BSA solutions in water at five different concentrations (ranging from 0 to 1000 μg/ml) were used as standards to establish a calibration curve.

All the snack bar sample residues (derived from the total DF assay) were redissolved in Milli-Q water (10 mg/ml), out of which an aliquot (100 μl) was mixed with diluted Bradford reagent (3 ml). The resultant mixture was vortexed (15 s) and allowed to stand

at room temperature for 5 min before the absorbance at 595 nm was recorded.

2.11.3. Total fat content

The freeze-dried snack bar samples (5 g) were mixed with Celite™ at a ratio of 1:1 w/w and then loaded into Dionex standard 33-ml stainless steel extraction cells. A cellulose filter paper (30 mm, Whatman) was placed at the end of the thimble. Extraction was carried out under N_2 in a pressurised multiple-sample extractor (ASE 300, Dionex) (operating conditions: 60 °C and 1500 psi with 5 min heating and 10 min static time). After four extraction cycles were performed using *n*-hexane, the extracts were transferred to pre-weighed Labconco® glasses (LabConco Corporation) and dried in the RapidVap® concentrator unit at 60 °C, 60% speed for 50 min. The Labconco® glasses containing the extracted lipids were weighed. The total lipid content was calculated as a percentage of the snack bar sample.

2.12. Water activity and moisture content

Water activity (a_w) of the snack bar base and filling was measured separately at room temperature using a water activity metre (AQUA LAB CX-2, Decagon Devices Inc., Pullman, WA). Moisture content was determined using a freeze drier (Telstar Cryodos-80).

2.13. 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 (L^* value defines the lightness, a^* value the red-greenness and b^* value the blue-yellowness, respectively). Total colour difference (ΔE^*) between (inulin or apple DF) fibre-enhanced and control snack bars, in the absence or presence of added APE, was calculated using the equation:

$$\Delta E^* = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{1/2}$$

2.14. Hardness measurement

The hardness of the snack bar base was measured in triplicate using a Model 3401Universal Instron Testing Instrument (Norwood, MA). The force (N) required for a circular probe of 14 mm diameter to penetrate 5 mm into the bar was measured.

2.15. Statistical analysis

At least three replicate determinations were obtained for each datum point. Data were analysed using ANOVA (*via* Minitab 15).

3. Results and discussion

3.1. Chemical analyses of apple DF

The apple DF prepared using the aqueous method contained 2.3–2.6 mg CtE phenolics/g dried fibre and 70.9% total fibre (UA and neutral monosaccharide contents are 282 ± 0.1 and 354 ± 1.2 mg/g dried fibre, respectively). These results are comparable to those reported for other apple varieties (Renard, Baron, Guyot, & Drilleau, 2001; Sun-Waterhouse, Melton, et al., 2008). Different types of pectic polysaccharides, such as homogalacturonan, arabinan-rich rhamnogalacturonan and xylogalacturonan, were found in apple cell walls (Fry, 1988; Schols, Posthumus, & Voragen, 1990). Phenolic compounds were reported to be associated closely with cell wall polysaccharides and contribute to cell wall cross-linking (O'Neill & York, 2003; Renard et al., 2001; Sun-Waterhouse, Farr, et al., 2008). Our previous work (Sun-Waterhouse, Farr, et al., 2008; Sun-Waterhouse, Melton, et al., 2008) showed that the fibre prepared used the current aqueous method was able to retain the natural 3D polymeric network of cell wall polysaccharides. The apple fibre prepared in this study is within the newly established DF definition.

3.2. Chemical analyses

3.2.1. Uronic acid content

Uronic acid content was calculated based on the D-galacturonic acid calibration curve ($y = 0.0093x$, $r^2 = 0.999$). The UA contents in the six snack bar formulations ranged from 2.3% to 3.5%, with those in the apple DF-snack bars being the highest (Table 1). The UA content came from the apple fibre, pectin and other ingredients included in the snack bar formulations, such as rice (Shibuya, 1989). The higher UA content in the apple DF-snack bar formulations, particularly the bar enhanced with APE, indicated an increased pectic polysaccharide concentration, as a result of the addition of apple DF. The apple DF had 0.28 g uronic acid (as GalA) per g dried fibre.

The apple DF of this study was in the form of plant cell walls, which are mainly composed of cellulose and non-cellulosic polysaccharides, such as pectic polysaccharides, xyloglucans, glucomannans and galactoglucomannans (Fry, 1988). These polysaccharides possess ester, ether and cyclic acetal functional groups (particularly carboxyl groups of uronic acid) participating in various chemical reactions that influence the fibre's properties during processing (David, 2001; Fry, 1988; Kaplan, Christiaen, & Arad, 1988; Sun-Waterhouse, Farr, et al., 2008; Sun-Waterhouse, Melton, et al., 2008).

Table 1
Chemical analyses of the six types of snack bars.

Snack bar	Uronic acid (GalA, %)	Total phenolics (mg CtE/g bar)	Total DF (%)	Protein (%)	Lipid (%)	Ash (%)	a_w	Moisture content (%)
Control + APE	2.41 ± 0.06	1.45 ± 0.23	2.54 ± 0.11	2.74 ± 0.07	9.97 ± 0.24	0.89 ± 0.08	B 0.483 F 0.440	10.7 ± 0.62
Control − APE	2.30 ± 0.22	0.50 ± 0.02	2.47 ± 0.15	1.07 ± 0.06	9.85 ± 0.05	0.71 ± 0.05	B 0.483 F 0.440	5.6 ± 0.18
Inulin + APE	2.64 ± 0.16	2.22 ± 0.18	4.78 ± 0.21	2.36 ± 0.11	9.59 ± 0.03	1.33 ± 0.09	B 0.460 F 0.440	11.7 ± 0.14
Inulin − APE	2.34 ± 0.07	0.46 ± 0.02	4.95 ± 0.27	3.66 ± 0.05	8.73 ± 0.27	0.96 ± 0.05	B 0.460 F 0.440	6.7 ± 0.35
ADF + APE	3.50 ± 0.33	2.87 ± 0.02	5.42 ± 0.34	2.01 ± 0.07	8.70 ± 0.11	1.28 ± 0.09	B 0.460 F 0.440	11.5 ± 0.16
ADF − APE	2.88 ± 0.11	0.60 ± 0.03	5.29 ± 0.35	2.44 ± 0.03	8.48 ± 0.09	1.03 ± 0.05	B 0.460 F 0.440	7.6 ± 0.40

Note: values are expressed as mean ± standard deviation. "B" and "F" refer to bar base and filling, respectively. APE, apple polyphenol extract; ADF, apple dietary fibre.

3.2.2. Total phenolic analysis

The total phenolic content of snack bar was calculated based on the established calibration curve: $y = 3.315x + 0.0446$ ($r^2 = 0.9977$). In the absence of added APE, the total phenolic content of control, inulin and apple DF-snack bars were 0.50, 0.46 and 0.60 mg CtE/g bar, respectively (Table 1). In the presence of added APE, the total phenolic content of control, inulin and apple DF-snack bars were 1.45, 2.22 and 2.87 mg CtE/g bar, respectively (Table 1). Much higher phenolic contents were achieved with the incorporation of inulin or apple DF into the snack bar recipe.

The apple purée of this study contained 0.204 mg CtE phenolics per g dried purée. The added apple DF used for enhancing the snack bar's fibre content also contained bound phenolics. So the small variations in total phenolic content among the control, inulin and apple DF bars with no added APE, resulted from the phenolics present in the apple DF. Interestingly, although incorporated with the same amount of APE, the control and fibre-enhanced bars significantly differed in their extracted phenolic contents. Thus, the fibre type accounted for the difference in the extracted phenolic contents of inulin and apple DF bars. The use of inulin and apple DF had an overall positive impact on the total phenolic content.

ADF, inulin or quick-cook rolled oats might influence differently the extractability of the apple phenolic compounds (Box, 1983; Naczk & Shahidi, 2004; Peterson, 1979). The extracted phenolics of the snack bars were the net result of various reactions that occurred during baking. Baking at a temperature above 60 °C possibly induced oxidative condensation or decomposition of thermo-labile phenolic compounds (Asami, Hong, Barrett, & Mitchell, 2003). The formation of different complexes was possible during baking, *via* multiple hydrogen bonds between the hydroxyl groups of polyphenols and the carboxyl groups derived from fibre pectic polysaccharides and/or protein peptides in all the bar formulations (Table 1). The prolonged exposure of snack bars to high temperature during baking might also destroy some of the complexes formed between bound phenolics and other food components, causing an increase in extracted phenolics of some snack bars. The recovery of phenolics could be a result of their interactions with wheat protein *via* hydrogen bonding during dough preparation (Wang & Zhou, 2004). The consistency of our phenolic results could roughly be estimated and confirmed by comparing them with those of Sudha, Baskaran, and Leelavathi (2007).

3.2.3. Total DF, lipid, protein and ash contents

Significant differences ($p < 0.05$) in total DF content were detected between the control bars (2.47–2.54%) and the bars enhanced with inulin or apple DF (4.78–4.95% and 5.29–5.42%, respectively). Apple DF-snack bars contained the highest DF content. A fibre content of 4.5 g per serving, which is equal to 5.29% w/w (if the serving size is 85 g per bar), would allow claims of "a good source of fibre" according to FSANZ requirements (ANZFA, 1995; FSANZ, 2008). In this study, the snack bars with added apple DF contained 5.29 ± 0.35 or 5.42 ± 0.34 g total DF per 100 g snack bar. Such results provide a rough approximation for food labelling that the apple DF-snack bar formulations may qualify for the claim "a good source of fibre". The apple DF used in this study contained largely insoluble and some soluble fibres (Sun-Waterhouse, Farr, et al., 2008; Sun-Waterhouse, Melton, et al., 2008), whereas, the inulin fibre is a soluble DF. The biggest difference between insoluble DF and soluble DF for food formulation lies in the water-holding capacity.

The lipid content was positively correlated with the content of vegetable shortening or oil, ranging from 8.5% (in the bar with apple DF but no added APE) to 10.0% (in control bar with added APE). No vegetable shortening or oil was observed flowing out of the bar during baking. In the absence or presence of added APE, total lipid content decreased in the order of control, inulin and apple DF-snack bar. Total lipid content was significantly reduced in the apple DF-enhanced bars in which quick-cook rolled oats were partly substituted with apple DF. The addition of APE to the same type of snack bar (control, inulin or apple DF) slightly increased the total lipid content. The control snack bars with the highest level of quick-cook rolled oats had the highest fat content (Table 1), probably because of their lower moisture content and higher oil-binding ability. Extraction of lipids using an accelerated solvent extractor was found to be advantageous over the conventional Soxhlet extraction, with reduced extraction time, the ability to use a variety of solvents and temperature programming, and reduced solvent usage.

For control snack bars, the total protein content in the formulation with added APE was much higher than that without APE. The formation of protein–polyphenol complexes (Rawel, Kroll, & Hohl, 2001; Rohn, Rawel, & Kroll, 2004) possibly reduced the heat-induced breakdown of proteins during baking. With the addition of inulin and apple DF, however, the trend reversed, and the total protein content was lower in the presence of added APE. The percentage of protein would have been diluted when dry ingredients were added. Processing removes moisture, resulting in higher protein concentration in the final products. For the fibre-enhanced snack bars, greater protein content was associated with lower moisture content (Table 1). For the formulations with added APE, the use of inulin fibre or apple DF reduced the total protein content, which possibly resulted from the reduced amount of protein available for the formation of protein–polyphenol complexes, due to the complexation of polysaccharide-polyphenol (Chao & Chiang, 1999) and other protein-related interactions, such as the Maillard reaction (Damodaran, 1996).

The presence of APE increased the ash contents of the control, inulin and apple DF-snack bars. Ash content was found to be similar in bars enhanced with inulin and apple DF, but the control bars had the lowest ash contents.

3.2.4. Moisture content and water activity

The moisture contents of the control, inulin and apple DF-snack bars with added APE were 10.7%, 11.7% and 11.5%, respectively. Control, inulin and apple DF-snack bars with no added APE had a moisture content of 5.6%, 6.7% and 7.6%, respectively. The moisture contents of the snack bars without added APE were lower (5.6–7.6%) than those (10.7–11.7%) with added APE. The moisture content of the control bars was the lowest, which might be associated with the greatest amount of quick-cook rolled oats in the formulations. For fibre-enhanced snack bars, the reduced rate of moisture loss was possibly due to the water-holding capacity of the fibres used (Grigelmo-Miguel, Carreras-Boladeras, & Martín-Belloso, 1999). Water is a constituent of food that influences food stability, quality and physical properties. The textural characteristics of dry cereal foods greatly contribute to their high popularity and are closely related to their moisture content (Gates, Dobraszczyk, Stoddard, Sontag-Strohm, & Salovaara, 2008; Liu, Hsieh, Heymann, & Huff, 2000). Elevated water content can cause plasticising or anti-plasticising effects (Lewicki, 2004), and brittle material consequently loses crispness.

Water activity (a_w) measurements help predict food mechanical properties, stability and shelf life. The a_w of control and fibre-enhanced snack bar bases were 0.483 and 0.460, respectively. The a_w of all the snack bar fillings was 0.440 (Table 1). These a_w values were all well below 0.7, indicating low risk of microbial proliferation and pathogenic spoilage and good shelf life (Beuchat, 1981). The a_w of the bases and filling were designed to be similar to minimise moisture transfer and subsequent changes in the constituent's properties associated with product shelf life and texture. The low a_w filling would offer a good shelf life and preserve the bar dryness during storage. The variable a_w, which represents the

'water availability' in material, is defined as the ratio of vapour partial pressure of water in food to the vapour partial pressure of pure water at the same temperature and total pressure (Scott, 1957); a_w influences microbial spoilage as well as chemical reactivity and enzymatic activity (Labuza, 2000). The changes in a_w are responsible for the mechanical properties of snack bars, which are probably associated with the differences in a product's microstructure and chemical composition (Lewicki, Jakubczyk, Marzec, Cabral, & Pereira, 2004). The texture of snack bars would be affected by a_w (Katz & Labuza, 1981). A rapid decrease of crispness and complete loss of brittleness in breakfast cereals was observed when a_w reached 0.8 (Sauvageot & Blond, 1991).

The snack bar processing would have induced chemical changes upon the addition of APE or fibre in snack bars. The sugars, proteins, fibres and polyphenols present in the snack bar formulations would have been involved in chemical reactions. Ingredients like sucrose, liquid sugar, corn syrup, invert sugar, honey or a combination of these, are quite often used to impart sweetness and modify water activity in the fillings of baking products. There must be enough liquid sugar to obtain the desired hygroscopic property and a_w without making the dough too sticky to process. The higher the liquid sugar level, the stickier the dough will be. Most bar formulations contain fats to facilitate lubrication, mouthfeel, flavour release, machineability as well as provide energy. Vegetable shortening is a typical fat for this type of snack bar. Emulsifiers such as mono- and di-glycerides and lecithin help retain air and leavening gases, promote even crumb structure and modify dough texture. Hydrocolloids and specialty starches manage moisture as the bar equilibrates, contributing to tenderness and mouthfeel. Protein fortification enables an improved nutritional value and functional properties. The added protein functions to hold ingredients together, sets the structure, increases its strength, and/or contributes to water-binding, gelation and Maillard browning.

3.3. Effect of added polyphenols and fibres on the appearance and hardness of snack bars

The force required to penetrate 5 mm in the control, apple DF or inulin snack bars was 62.22 N ± 2.18, 61.72 N ± 8.79, and 44.05 N ± 5.72, respectively. No detectable difference was found between the same type of snack bars (control or each type of fibre-enhanced bars) in the absence or presence of added APE. The snack bars with added inulin were much softer than both control bars and the snack bars with added apple DF.

Apple DF or inulin was added to replace the same amount of quick-cook rolled oats in the bar base. APE was added to replace the same amount of apple purée, sugar, glucose syrup, honey and vegetable shortening mixture. Therefore, the control bars with no added fibre containing the greatest amount of quick-cook oats possessed the hardest texture. Snack bars with added apple DF possessed comparable hardness to control bars. The presence of inulin facilitated the reduction of bar hardness. The small difference in honey content and the absence/presence of APE did not have detectable effects on snack bar hardness.

During the production, it was observed that the bars made without fibre (control) and those containing inulin did not hold their structure very well, unlike the bars made with the apple DF. Premixing the apple DF with the liquid ingredient affected the hardness of snack bars in a noticeable way. The apple DF was observed to absorb the liquid ingredient (i.e., honey) and swell up very quickly, while the other ingredients still stayed relatively dry, resulting in a longer mixing time for the formulation containing apple DF. Control bars and those made with inulin had the same consistency when mixed with honey, and appeared to be wetter and possessed a thinner bar structure during moulding, compared with the bars containing apple DF. The snack bar containing apple DF was easier to mould into a bar shape, compared with the control bars and those containing inulin.

The shelf life of a snack bar is determined substantially by its texture. Protein ingredients such as whey protein that has significant viscosity, gel strength and water-binding properties, would positively influence bar texture *via* a hardening effect over shelf life (Ortiz, Martín-Martínez, & Padilla, 2008; Shaun, 2008; Uthayakumaran, Newberry, Keentok, Stoddard, & Bekes, 2000). Such a hardening effect might be associated with water migration between the carbohydrate fraction (such as pectins, maltodextrin, starches and sugars) and the protein fraction (Shaun, 2008). Carbohydrate ingredients added to hold moisture and modify texture, could prevent moisture loss to the environment but failed to prevent moisture transfer from and/or to the protein ingredients (Shaun, 2008). An increase in the amount of carbohydrates such as maltodextrin would tend to enhance the hardening effect during shelf life (Shaun, 2008). Addition of a highly branched carbohydrate, a soluble fibre, and/or indigestible or poorly digestible carbohydrate to the bar formulation can increase the shelf life of a snack bar. Inulins, pectins, maltodextrins are among the softeners used to increase initial softness of snack bars after formulation. Moreover, the water absorption capacity of insoluble fibre, e.g. apple DF, would also facilitate an increase in shelf life, evident partly by the decreased water activity.

3.4. HPLC phenolic profiling

Figs. 1–3 show that the same type of phenolic compounds appeared in the snack bars with and without added APE. The amounts of the identified phenolic compounds, catechin, chlorogenic acid, epicatechin, phloretin derivative, phloridzin and *p*-coumaric acid, were found to increase in the order of control, inulin and apple DF bars with added APE (Table 2). Phloridzin, chlorogenic acid and epicatechin were the dominant phenolic acids. For the three snack bars containing added APE, the HPLC chromatograms have little difference in terms of the type of phenolic compounds, featuring a typical apple polyphenol profile, i.e., the presence of phenolic compounds, such as chlorogenic acid, epicatechin, *p*-coumaric acid, *p*-hydroxybenzoic acid, rutin/quercetin derivative (Wegrzyn et al., 2008). Although the addition of apple DF might introduce additional polyphenol oxidase (PPO) to the apple DF-snack bar, little difference in the type and amount of phenolic compounds was detected, possibly due to heat inactivation of PPO during snack bar making (Erat, Sakiroglu, & Kufrevioglu, 2006; Martinez & Whitaker, 1995; Yemenivioğlu, Özkan, & Cemeroğlu, 1997). For the three snack bars without added APE, the phenolic compounds that were present in the HPLC chromatograms were those present in the apple DF and apple purée ingredients, including chlorogenic acid, epicatechin, rutin/quercetin derivative, phloridzin, *p*-hydroxybenzoic acid (Sun-Waterhouse, Farr, et al., 2008). Fig. 4 shows the HPLC chromatograms of the replicate apple purée (an ingredient for the fillings of snack bars with and without APE). The apple purée used in this study also contained phenolic antioxidants, such as catechin, chlorogenic acid, phloretin derivative and phloridzin, which agrees with the study of Oszmiański, Wolniak, Wojdyło, and Wawer (2008). During the bar filling preparation, APE was added to replace part of the apple purée (0.3%). Therefore, it was not surprising that a similar background pattern was observed in the HPLC chromatograms of Figs. 1–4, although the peaks present in Fig. 4 would be smaller than those in Figs. 1–3, due to the low polyphenol concentration in apple purée and low concentration of apple purée in the snack bar formulation.

Interestingly, quercetin and particularly phloretin appeared at a higher level in control bars than inulin bars, and neither was detected in apple DF bars under the same analysis conditions. This suggests that these two phenolic compounds were either lost or

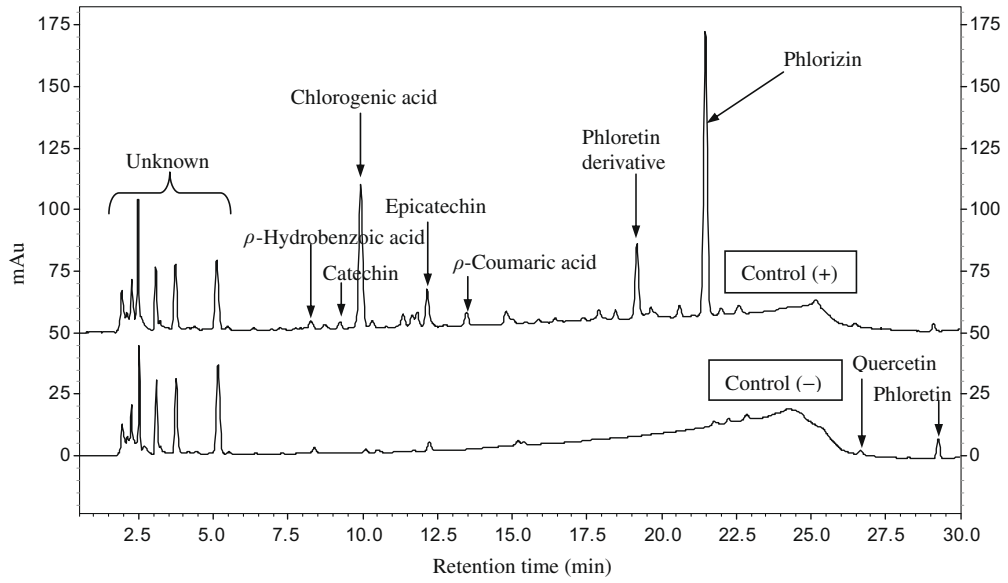

Fig. 1. HPLC chromatograms of control snack bars with ("Control + ") and without ("Control −") added apple polyphenol extract (APE).

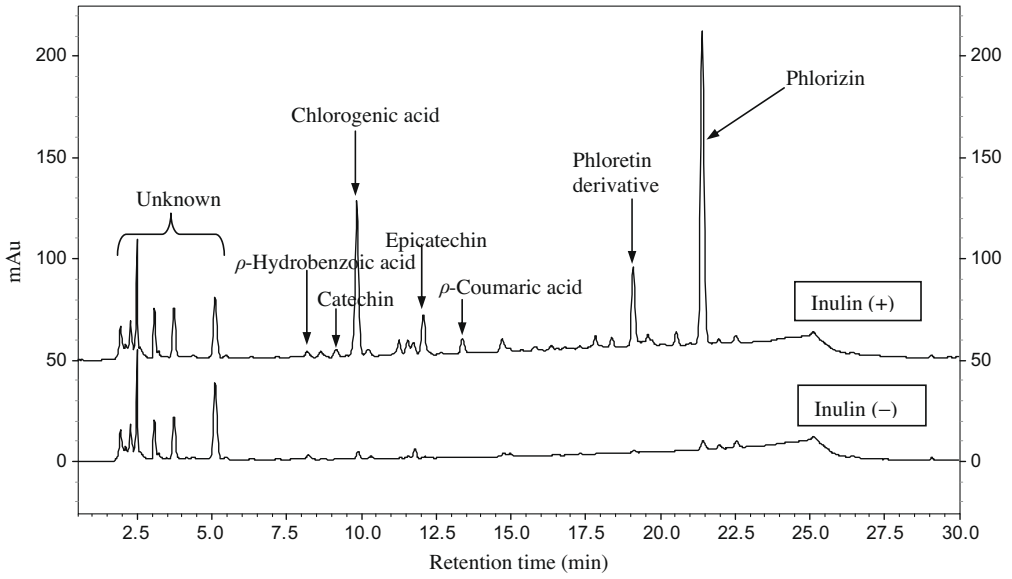

Fig. 2. HPLC chromatograms of inulin-enhanced snack bars with ("Inulin + ") and without ("Inulin −") added apple polyphenol extract (APE).

cross-linked with some components in the fibre-enhanced bars (in a complex form) after processing. The extent of loss or cross-linking might be more severe in apple DF formulation than in inulin formulation, possibly due to the distinct nature and properties of apple DF and inulin.

3.5. Colour determination

An important appearance feature of snack bars to consumers is the surface colour of the base and the filling, which indicates the magnitude of thermal energy applied during baking, as a prolonged exposure to high temperature would have seriously influenced the colour of the baked foods. Comparisons between the colour of a control bar and that enhanced with apple polyphenols and/or fibres, indicate not only the quality of different snack bar formulations, but also the interactions among the food components included in the ingredients. Both process and ingredient variables influence the physical properties and sensory attributes of snack products (Liu et al., 2000).

For the snack bar base (Fig. 5), the L^* values (positive) of the two apple DF formulations appeared to be the highest, with little difference being detected among the other four formulations ($p < 0.05$), suggesting the lightest colour of the snack bar base occurred when it was enhanced with apple DF. The a^* and b^* values of all the formulated bars were positive, indicating that redness and yellowness in the colour of all the snack bar bases. The b^* values (positive) decreased in the order of control, apple DF and inulin bars, although the magnitude of difference in the b^* values was relatively small. This suggests that the control formulations had the greatest yellowness in colour.

For the snack bar filling (Fig. 5), the L^* values (positive) of the formulations without added APE were higher than those with added APE ($p < 0.05$), suggesting a darkening effect with the addition of APE. In the absence of APE, the apple DF formulation had the

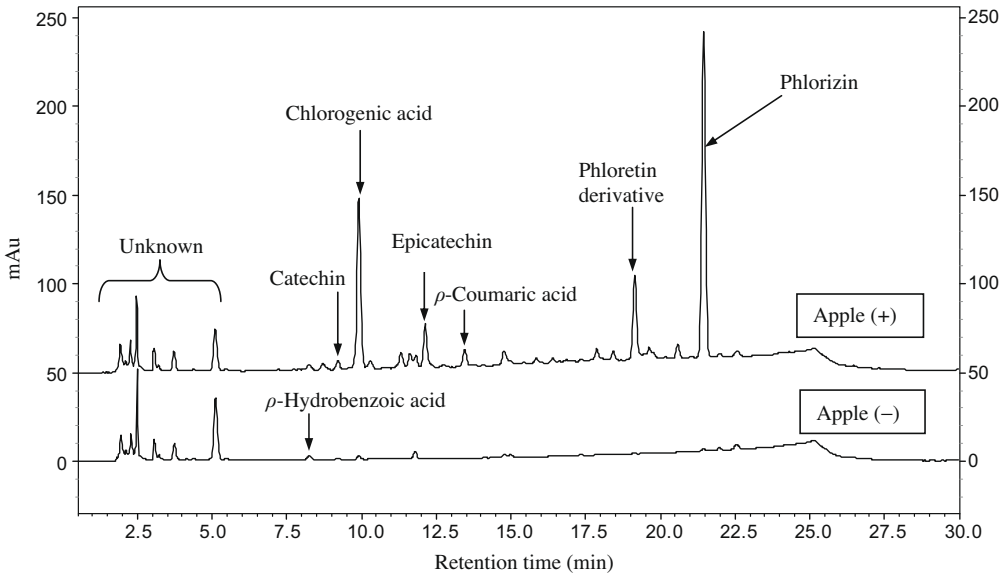

Fig. 3. HPLC chromatograms of ADF-enhanced snack bars with ("Apple + ") and without ("Apple −") added apple polyphenol extract (APE).

Table 2
Phenolic compounds identified in HPLC chromatograms of snack bars with added apple polyphenol extract.

Peak	Possible compound identified	Concentrations of identified phenolic compounds (μg/g dried extract)		
		Control + APE	Inulin + APE	ADF + APE
1	Catechin	37.3 ± 1.12	41.1 ± 0.96	42.1 ± 1.48
2	Chlorogenic acid	244 ± 12.4	327 ± 13.8	411 ± 16.4
3	Epicatechin	160 ± 1.03	196 ± 4.15	228 ± 3.52
4	Phloretin derivative	47.0 ± 0.84	60.8 ± 10.7	75.6 ± 1.08
5	Phloridzin	371 ± 9.53	480 ± 12.4	581 ± 0.01
6	*p*-Coumaric acid	7.8 ± 0.07	10.2 ± 0.53	12.7 ± 0.21

Data are expressed as (mean ± standard deviations) of duplicate measurements. APE, apple polyphenol extract; ADF, apple dietary fibre.

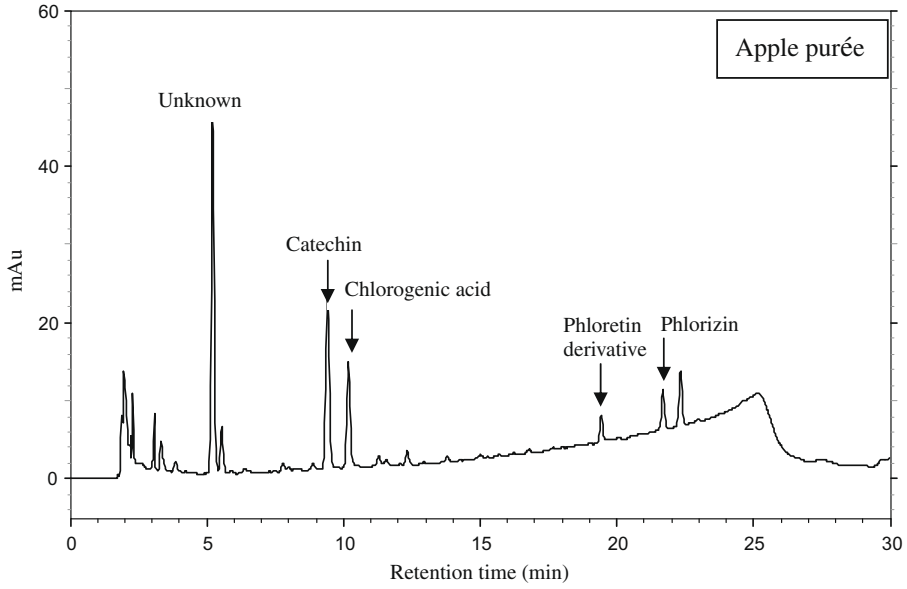

Fig. 4. HPLC chromatogram of apple purée.

highest L^* value. In the presence of APE, the control formulation had the highest L^* value. This suggests that different snack bar base formulation might influence the colour of the filling with/without APE in different ways. This may be associated with the difference in moisture migration and heat transfer, resulting from the varied fibre contents in the bar base, in addition to the difference in polyphenol and sugar contents in the snack bar filling. The bar filling without added APE had lower a^* value (positive) and higher b^* val-

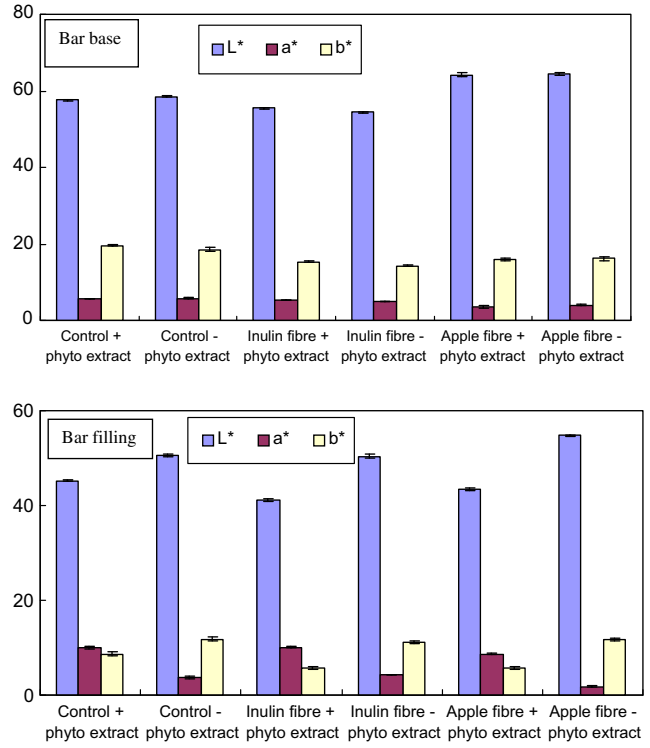

Fig. 5. Colour measurement (Hunter $L^*a^*b^*$ values) of snack bars.

ues (positive) than that with added APE ($p < 0.05$), suggesting increased redness and decreased yellowness in colour caused by the APE. In the presence of APE, the control bar had a higher b^* value than the inulin and apple DF bars (the latter two had the same b^* values). Addition of fibres (inulin or apple DF) to bar base resulted in reduced yellowness in colour of the bar filling, suggesting the bar composition and structure would have influenced heat and moisture transfer to bar filling during baking (Shaun, 2008).

The ΔE^* value listed in Table 3 is an overall measure of the change in colour that resulted in the snack bar formulation, comparing fibre-enhanced and control snack bars either with or without added APE. In this study, the colour difference (ΔE^* value) is set at 3.7 units since most studies fix the proposed acceptance limit for colour matching at this level (Eliades, Gioka, Heim, Eliades, & Makou, 2004; Eliades, Kakaboura, Eliades, & Bradley, 2001; Johnston & Kao, 1989; Seghi, Hewlett, & Kim, 1989). Based on the ΔE^* values in Table 3, the greatest ΔE^* value (7.82) was observed in bar base colour (between the control bar with added APE and the bar enhanced with both apple DF and APE). There were differences in bar base colour between the inulin/apple DF-enhanced bars and the control bars, both with or without added APE. For the formulations with added APE, much greater difference ($\Delta E^* = 7.82$) was detected between the apple DF-enhanced bar base and the control bar base, compared with that ($\Delta E^* = 4.46$) between the inulin-enhanced bar base and the control bar base. For the formulations with no added APE, same ΔE^* values (6.6) were obtained when the apple DF-enhanced or the inulin-enhanced bar base was compared with the control bar base. Whey protein and reducing sugars such as honey have been used to make the current snack bar base formulations. The use of reducing sugars (e.g., from honey and inulin ingredients) would lead to the non-enzymatic Maillard browning reaction (Damodaran, 1996) and/or caramelisation (Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008; Kroh, 1994) at an elevated temperature, which explains the light brown colour detected in the bar base. Apple DF or inulin (2.7%) was added to replace the same amount of quick-cook rolled oats. Such a change in the formulation was responsible for the varied Hunter $L^*a^*b^*$ colour (Fig. 5) and ΔE^* values (Table 3). Inulin would have participated in the Maillard browning reaction, thereby reducing the L^* values. For a high baking temperature (>120 °C), the darkening rate constant would have strongly depended on water content (Broyart, Trystram, & Duquenoy, 1998). Difference in the water absorption between the rolled oats and the natural apple DF might be associated with the variation in the base darkness of control and apple DF-enhanced snack bars. The plant cell wall layers of commercial oat products are usually collapsed, resulting in ineffective water absorption (Dreher, 1999). The apple DF used in this study largely retained the native 3D plant cell wall structure, which, in return, may enable good water absorption (Sun-Waterhouse, Farr, et al., 2008; Sun-Waterhouse, Melton, et al., 2008). The PPO possibly included in the apple DF ingredient did not cause enzymatic browning, due to heat inactivation during snack bar making (Erat et al., 2006; Martinez & Whitaker, 1995; Yemenivioğlu et al., 1997).

For the bar filling, there was colour difference between the inulin-enhanced bar and the control bar when APE was added into the filling ($\Delta E^* = 5.00$), and between the apple DF-enhanced bar and the control bar when no APE was applied ($\Delta E^* = 4.58$). There was no difference in filling colour between the snack bar enhanced with inulin fibre only and the control bar with no added APE ($\Delta E^* = 0.90$). Some studies described ΔE^* value in the range of one unit as a colour match (Eliades et al., 2001, 2004). Similarly, there was no difference in filling colour between the snack bar enhanced with apple DF with APE, and the control bar with added APE ($\Delta E^* = 3.71$).

The change of colour in snack bars was a dynamic process in which certain colour transitions occurred as the baking proceeded. The fortified and control snack bars were baked similarly but the ΔE^* values mostly ranged from 3.71 to 7.82 (except for the bar filling with inulin and no APE). This suggests that the colour characteristics were also influenced by formulations. It has been shown previously that the colour of cereal products is affected by product formulation (e.g., raw ingredients and water content) and processing conditions (Baardseth, Kvaal, Lea, Ellekjaer, & Faergestad, 2000; Hatcher, Dexter, & Fu, 2008; Miskelly, 1996; Poinot et al., 2008; Pozo-Bayón, Guichard, & Cayot, 2006).

The APE ingredient was a brown powder with a bitter taste. Therefore, the snack bars with added APE were more dark brown in colour, whereas the bars without APE were more yellow in colour. The small increment in honey content did not cause a significant increase in brown colour for the bars without added APE, as the APE was added to replace apple purée, sugar, glucose syrup, honey and vegetable shortening. Theoretically, darkening (a brown colour developing) in a product would increase with the use of reducing sugars (e.g., liquid honey), due to an increase in the amount of reducing sugars available to participate in the Maillard reaction (Damodaran, 1996; Yilmaz & Toledo, 2005) and/or caramelisation (Ameur et al., 2008; Kroh, 1994) at an elevated temperature. However, for the bar filling containing added APE, although less reducing sugars were used, a small increase in darkness and redness was detected instead. This suggests that other types of

Table 3
Colour difference (ΔE^*) of snack bars with enhanced fibre, compared with equivalent (+/− APE) control snack bars.

Snack bar	ΔE^*	
	Bar base	Bar filling
Inulin + APE	4.46 ± 0.21	5.00 ± 0.33
Inulin − APE	6.62 ± 0.38	0.96 ± 0.28
ADF + APE	7.82 ± 0.45	3.71 ± 0.32
ADF − APE	6.64 ± 0.48	4.58 ± 0.39

APE, apple polyphenol extract; ADF, apple dietary fibre.

reactions, such as polyphenol non-enzymatic browning reactions including non-enzymatic polyphenol oxidation and autoxidative phenolic browning (Cilliers & Singleton, 1989; Gvozdenoviü & Curakoviü, 1995; Kyi et al., 2005), might be a more dominant contributor to the colour darkening. The colour was the net result of all these reactions. For the snack bar base formulations, the use of inulin has introduced some more reducing sugars, causing additional browning effects. The different impact of inulin (soluble fibre) and apple DF (a mixture of soluble or insoluble fibres) on snack bar processing (e.g., moisture transfer, stress formation and heat transfer) might be associated with a difference in the structural arrangement of the bar base (Itaya, Kobayashi, & Hayakawa, 1995; Zanoni & Peri, 1993).

4. Conclusions

It is feasible and beneficial to incorporate two types of apple bioactive components (i.e., polyphenols and DF) into snack bars. Adding apple DF did not adversely affect snack bar texture compared with the control bar. Both inulin (soluble fibre) and apple DF (a mixture of soluble or insoluble fibres) have influenced differently the snack bar's texture, appearance, composition and nutritional values. Bars containing apple fibre performed better than those containing inulin. Increased phenolic content was also achieved in all modified snack bars. Significantly high amounts of phloridzin and chlorogenic acid have been retained, which may enable the health claims associated with these phenolic compounds. The amount of fibre in the apple DF-snack bar was calculated to be no less than 4.5 g per serving (if the serving size is 85 g per bar), which may also enable a fibre content claim of "a good source of fibre".

The apple DF used in this study (mainly in the form of plant cell walls) has demonstrated gut health benefits in *in vitro* faecal fermentation and bacteria attachment studies, and ability to enhance antioxidant bioavailability in a rat model study (New Zealand Plant and Food Research 2009, unpublished results). A more challenging question is whether such a combined use of apple polyphenols and apple DF in a snack bar format would enhance the bioavailability of apple polyphenols and the gut health functionality of apple fibre.

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