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Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder

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Abstract

Apple fruit skin, a rich source of dietary fibre and phenolics, is a by-product of apple processing. The effect of baking on the dietary fibre, phenolics, and total antioxidant capacity was investigated using a model system of muffins incorporated with dried apple skin powder (ASP) as a value-added food ingredient. The blanched, dehydrated, and ground ASP contained approximately 41% total dietary fibre and oxygen radical absorption capacity (ORAC) of 52 mg Trolox equivalents g^{-1} dry weight. The total dietary fibre content, total phenolic content, and total antioxidant capacity of muffins were positively correlated to the amount of ASP incorporated into muffins. The mean percent recovery of quercetin glycosides, catechins, chlorogenic acid, phloridzin, and cyanidin galactoside after baking were 61%, 57%, 53%, 44%, and 20%.
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Keywords: *Malus domestica*; Apple skin; Food ingredient; Dietary fibre; Polyphenolics; Antioxidant; Baking

1. Introduction

Interest in nutrition and disease prevention is driving consumer demand for value-added foods or functional foods with higher levels of antioxidants and dietary fibre (de Roos, 2004). It is widely accepted that increased consumption of fruits and vegetables can reduce the risk of cancer, heart disease, and stroke (Liu, 2003). Phenolic compounds present in fruits have been demonstrated to possess anti-inflammatory and anticarcinogenic properties and the ability to prevent a variety of chronic diseases (Boyer & Liu, 2004). Apples are a good source of phenolic compounds (Eberhardt, Lee, & Liu, 2000), especially the skin, is rich in many health-enhancing phytonutrients including flavonoids and phenolic acids (Boyer & Liu, 2004). Constituents of apple skins inhibited the HepG2 (human hepatocellular liver carcinoma) cell proliferation significantly greater than that of whole apple (Wolfe, Wu, & Liu, 2003). Apple skin has three- to six-fold more flavonoids than apple flesh and has unique flavonoids, such as quercetin glycosides, not found in the flesh (Wolfe et al., 2003; Wolfe & Liu, 2003). Epidemiological studies have suggested that apple consumption has been inversely associated with lung cancer (Le Marchand, Murphy, Hankin, Wilkens, & Kolonel, 2000), cardiovascular disease (Knekt, Jarvinen, Reunanen, & Maatela, 1996), and chronic obstructive pulmonary disease (Tabak, Arts, Smit, Heederik, & Kromhout, 2001).

Dietary fibre is defined as lignin and various polysaccharide components of plants, which are indigestible by enzymes in the human gastrointestinal tract (Bermink, 1994). Epidemiological studies have suggested a relationship between a decrease in the consumption of dietary fibre and an increase in gastrointestinal disease (Mendeloff, 1987), hypercholesterolaemia (Tinker, Schneeman, Davis, Gallaher, & Waggoner, 1991), and colorectal cancer (Cassidy, Bingham, & Cummings, 1994). Apples are a good source of dietary fibre with a well-balanced proportion of

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soluble and insoluble fibre fractions (McKee & Latner, 2000).

Identification of ways to incorporate apple skins, one of the by-products of apple pie and sauce manufacturing, as a health food ingredient in human diet could provide many health benefits. It has been estimated that 2–3 million kg of apple skins are generated as a result of apple processing in Nova Scotia, Canada (Rupasinghe, 2003), and use of apple skins as a value-added food ingredient has been suggested (Wolfe & Liu, 2003). However, there is limited information on the potential for incorporation of apple skin in bakery products such as muffins and its contribution to total antioxidant capacity (TAC) and dietary fibre levels of the final product. Therefore, the objective of this research was to evaluate the impact of baking on dietary fibre, phenolics, and total antioxidant capacity of muffins incorporated with different levels of apple skin powder (ASP).

2. Materials and methods

2.1. Chemicals and apparatus

Gallic acid, sodium carbonate, sodium acetate trihydrate, 2,4,6-tris(2-pyridyl)-*S*-triazine (TPTZ), Trolox, fluorescin, Folin–Ciocalteau reagent, ferric chloride, phosphate buffer were obtained from Sigma–Aldrich (St. Louis, MO, USA). 2,2′-Azobis(2-amidinopropane)dihydrocholride (AAPH) was purchased from Walco Chemical Products Co Inc., Buffalo, NY, USA. Acetic acid, hydrochloric acid, and 96-well microplates were purchased from Fisher Scientific (Ottawa, ON, Canada). Ingredients for muffin preparation were purchased from the local market. HPLC-grade methanol, acetonitrile and formic acid were purchased from Sigma–Aldrich Canada. The liquid chromatography standards used for the study were obtained as follows: quercetin-3-rhamnoside and quercetin-3-galactoside were from Indofine Chemical Company (Hillsborough, NJ, USA); quercetin, phloridzin, and chlorogenic acid were from Sigma–Aldrich Canada, cyanidin-3-galactoside and phloretin from Extrasynthese (Paris, France); and catechin, epicatechin, quercetin-3-glucoside, and quercetin-3-rutinoside were from ChromaDex (Santa Ana, CA, USA).

2.2. Apple skin powder (ASP) preparation

The apple skins of two apple cultivars; 'Idared' and 'Northern Spy' were collected from a commercial pie manufacturer, Apple Valley Foods Inc., Kentville, NS, Canada in 2005. Immediately after peeling, the skins were blanched with boiling potable water for 30 s to prevent enzymatic browning. After draining the excess water and within 3 h of blanching treatment, the treated peels were transported in plastic containers to the Nova Scotia Agricultural College (NSAC). The apple skins were dried in clean plastic trays at 60 ± 2 °C for 48 h using a convection oven with air circulation (Milner Agincourt, ON, Canada). The dried skins were ground into a fine powder using a Willey mill with 1 mm sieve screen (Model Laboratory Heavy Duty, Arthur Thomas Co., Philadelphia, PA, USA).

2.3. Proximate analysis

ASP and muffin samples were subjected to proximate analysis using methods of the AOAC (2000): moisture (Method 925.09), crude fat (Method 969.24), protein (Method 950.48), and ash (Method 923.03).

2.4. Dietary fibre analysis

The soluble and insoluble dietary fibre analysis of ASP and muffins was performed using the method (Method 991.42) of AOAC (2000) at an ISO 17025 registered analytical laboratory, Maxxam Analytics Inc., Mississauga, ON, Canada.

2.5. Sugar and vitamin C analysis

Sugar and vitamin C analyses were conducted using methods of the AOAC (Method 977.20 and 984.26, respectively) (2000).

2.6. Muffin preparation

Muffin formulation contained the following ingredients: 34.05% wheat flour; 32.13% water; 15.42% sugar; 13.88% vegetable oil; 2.57% skim milk powder; 1.29% baking powder; 0.45% egg powder; and 0.13% salt (weight basis). Before the current experiment, a pilot baking trial was conducted to determine the maximum level of ASP that could be incorporated in the muffins. It was found that the muffins were not baked properly if the ASP content exceeded 32% (w/w) flour replacement. Apple skin powder was incorporated into muffins at 6 levels (0%, 4%, 8%, 16%, 24%, and 32% w/w) by replacing equivalent amount of wheat flour of the muffin mixture. The batter was massed (80 g) into paper muffin cups (Dorval, QC, Canada) and baked in a preheated oven (General Electricals, Louisville, KY, USA) at 175 °C for 20 min. Muffins for chemical analysis were frozen, freeze-dried and subsequently ground into a fine powder using a coffee grinder and stored at −18 °C in airtight vials.

2.7. Preparation of extracts for phenolics and antioxidant assays

Methanol (100%; 15 mL) was added to 0.3 g of dehydrated ASP or muffin powder in 40 mL capacity amber glass vials. The mixtures were then subjected to sonication for 15 min $\times$ 3 times, with 10 min intervals in between sonication cycles to keep the temperature below 30 °C during the extraction. The crude extract was centrifuge at 3000*g* for 15 min. Samples of each treatment were extracted and analyzed in triplicate immediately or stored in amber vials at −76 °C until used for analysis.

2.8. Determination of total phenolic content (TPC)

TPC in extracts were determined according to the Folin–Ciocalteu assay as described by Singleton, Orthofer, and Lamuela-Raventos (1999) with some modifications. Gallic acid was used for the generation of a standard curve using the extraction solvent (100% methanol) and diluted to 8.82, 5.88, 4.70, 3.53, 2.35, and 1.18 μM concentrations. The solutions were made fresh under reduced light conditions and the reaction was carried out under dark conditions. Twenty micro liters of the diluted extract, or gallic acid standard was mixed with 100 μL of 0.2 N the Folin–Ciocalteu's phenol reagent in 96-well, clear, polystyrene microplates (COSTAR 9017) and gently mixed. After 6 min, 80 μL of 7.5% (w/v) sodium carbonate was added to each well and mixed. The mixture was incubated for 2 h at ambient temperature before absorption was measured at 760 nm using the FLUOstar OPTIMA plate reader (BMG Labtech, Durham, NC, USA). Results were expressed as μg of gallic acid equivalent (GAE) per g dry weight (μg GAE g^{-1} DW).

2.9. The ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie and Strain (1996) with some modifications. The reaction reagent (FRAP solution) was made immediately before the assay by mixing 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ solution, and 20 mmol L^{-1} ferric chloride solution in the ratio of 10:1:1. The TPTZ solution was prepared the same day as the analysis. The Trolox standard solution was prepared by dissolving 0.025 g of Trolox in 100 mL extraction solvent (methanol) to make 1 mmol L^{-1} Trolox and this stock solution was stored in small aliquots in a freezer (−70 °C) until needed. For development of the calibration curve, the Trolox stock solution was diluted appropriately with methanol to make 300, 150, 75, 25, 10, and 5 μM Trolox concentrations. The FRAP analysis was performed by reacting 20 μL of blank, standard or sample with 180 μL FRAP solution in 96-well clear polystyrene plates (COSTAR 9017). The FLUOstar OPTIMA plate reader with an incubator and injection pump (BMG Labtech, Durham, NC, USA) was programmed using the BMG Labtech software to take an absorbance reading at 595 nm, 6 min after the injection of the FRAP solution and a shaking time of 3 s. Both the FRAP solution and the samples in the microplate were warmed to 37 °C prior to assay. FRAP values were expressed as μg Trolox equivalents (TE) per g sample dry weight.

2.10. The oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was performed as described by Prior et al. (2003) with some modifications. Solutions required for the assay include: 75 mM phosphate buffer (K_2HPO_4/NaH_2PO_4) with a pH of 7; a fluorescein solution at 5.98 μM with a working solution made daily at 0.957 μM; the Trolox standard solution; and 150 mM AAPH which was also prepared daily, immediately before the assay. Both the flourescein and AAPH solutions were diluted with the phosphate buffer (75 mM, pH 7). The Trolox standard solution was made using the phosphate buffer and diluted the day of analysis for creation of the calibration curve consisting of 75, 50, 25, 10, and 5 μM Trolox. The measurements were carried out on a FLUOstar OPTIMA plate reader (BMG Labtech, Durham, NC). The temperature of the incubator was set to 37 °C and the fluorescence filters were set to an excitation of 490 nm and emission of 510 nm. The buffer, standard, or sample (30 μL) and 0.957 fluorescein (120 μL) solutions as well as extra buffer (30 μL) were placed in the 96-well plates (COSTAR 3915). The mixture was preincubated at 37 °C for 10 min using the plate reader. The fluorescence was recorded every 42 s up to 598 s, then every 2 min up to 2878 s after injection of 35 μL pre-warmed (37 °C) AAPH to each well. The microplate was shaken for 3 s after injection of AAPH and prior to each reading. All measurements were expressed relative to the initial reading. Final results were calculated using the differences of areas under the fluorescence decay curves between the blank and each sample and were expressed as μg Trolox equivalents (TE) per g sample dry weight (μg TE/g DW).

2.11. Liquid chromatography mass spectrometry analysis of phenolics

Analyses of all individual phenolic compounds were performed with a Waters Alliance 2695 separations module (Waters, Milford, MA, USA) coupled with a Micromass Quattro *micro* API MS/MS system and controlled with Masslynx V4.0 data analysis system (Micromass, Cary, USA). The column used was a Phenomenex Luna C_{18} (150 mm × 2.1 mm, 5 μm) with a Waters X-Terra MS C_{18} guard column. A previously reported method (Sanchez-Rabaneda et al., 2003) was modified and used for the separation of the flavonol, flavan-3-ol, phenolic acid and dihydrochalcone compounds. Gradient elution was carried out with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.35 mL/min. A linear gradient profile was used with the following proportions of solvent A applied at time t (min); $(t, A\%)$: (0, 94%), (9, 83.5%), (11.5, 83%), (14, 82.5%), (16, 82.5%), (18, 81.5%), (21, 80%), (29, 0%), (31, 94%), (40, 94%). The separation of the anthocyanin compounds was carried out using the same HPLC system with different mobile phases (Vrhovsek, Rigo, Tonon, & Mattivi, 2004). The mobile phases used were 5% formic acid in water (solvent A) and 5% formic acid in methanol (solvent B) at a flow rate of 0.35 mL/min. The linear gradient profile used was as follows; $(t, A\%)$: (0, 90%), (10, 70%), (17, 60%), (21, 48.8%), (26, 36%), (30, 10%), (31, 90%), (37, 90%).

Electrospray ionization in negative ion mode (ESI-) was used for the analysis of the flavonol, flavan-3-ol, phenolic acid and dihydrochalcone compounds. The following conditions were used: capillary voltage −3000 V, nebulizer gas (N_2) temperature 375 °C at a flow rate of 0.35 mL/min. For the analysis of the anthocyanins compounds, electrospray ionization in positive ion mode (ESI+) was used. The settings for the positive ion experiments were as follows: capillary voltage −3500 V, nebulizer gas 375 °C at a flow rate of 0.35 mL/min. The cone voltage (25–50 V) was optimized for each individual compound. Multiple reaction-monitoring (MRM) mode using specific precursor/product ion transitions was employed for quantification in comparison with standards: m/z 301 → 105 for Quercetin (Q), m/z 609 → 301 for Q-3-*O*-rutinoside, m/z 463 → 301 for Q-3-*O*-glucosides and Q-3-*O*-galactoside, m/z 448 → 301 for Q-3-*O*-rhamnoside, m/z 273 → 167 for phloritin, m/z 435 → 273 for phloridzin, m/z 353 → 191 for chlorogenic acid, m/z 449 → 287 for cyanidin-3-*O*-galactoside, m/z 289 → 109 for catechin, and m/z 290 → 109 for epicatechin. In MRM experiments, both quadrupoles were operated at unit resolution.

The % recovery of phenolics from ASP incorporated muffin was calculated based on:

$$\%\text{recovery} = \frac{\text{mg of compound detected in g of muffin on dry matter (DM) basis}}{\text{mg of compound estimated for g of muffin on DM basis}} \times 100$$

The estimated amount was calculated based on:

$$\text{estimated amount} = \text{concentration of the compound} \times \%\text{ASP in muffin in ASP on DM basis.}$$

2.12. Statistical analysis

One way ANOVA was performed on all the variables and each cultivar was analyzed separately using SAS general linear model (SAS Institute, Inc. 1999). When there were significant differences at $P \leqslant 0.05$, the means were compared using Tukey's Honestly Significant Difference (HSD) test. All the analyses were performed in triplicate.

3. Results and discussion

3.1. Characteristics of ASP

The chemical composition, TPC and TAC of ASP prepared from 'Idared' and 'Northern Spy', the two major apple cultivars used for pie manufacturing, are presented in Table 1. Total protein content of ASP was about 3% and total fat was about 10% that could be mainly due to sesquiterpene α-farnesene and wax compounds present in apple skin (Rupasinghe, Paliyath, & Murr, 2000). Ash content of ASP of 'Idared' and 'Northern Spy' were 4.4% and 4.9%, respectively and the dietary fibre content were 43% and 39%, respectively. Dried apple pomace, comprised skin, as well as flesh, core and seeds, contained 37% dietary fibre (Carson, Collins, & Penfield, 1994), which supports the present findings. Highest TPC and TAC (FRAP and ORAC) were found to be in the peel of the 'Northern Spy' cultivar than that of 'Idared' (Table 1). The major polyphenolic compounds detected from apple skins belong

Table 1
Chemical composition, total phenolic content and total antioxidant capacity of ASP

Characteristics		Apple cultivar	
		'Idared'	'Northern Spy'
Chemical composition (dry weight basis)	Crude protein (%)	3.16 ± 0.16	3.23 ± 0.11
	Crude fat (%)	10.23 ± 0.28	11.10 ± 0.64
	Ash (%)	4.86 ± 0.06	4.46 ± 0.08
	Total carbohydrate (%)	80.25	79.91
	Insoluble fibre (%)	11.2 ± 0.7	8.1 ± 0.8
	Soluble fibre (%)	32.1 ± 1.8	30.5 ± 1.3
	Fructose (%)	19.9 ± 1.4	21.8 ± 1.7
	Glucose (%)	6.3 ± 0.6	9.2 ± 0.8
	Sucrose (%)	13.1 ± 0.9	12.3 ± 0.4
	Vitamin C (mg kg^{-1})	510 ± 40	680 ± 60
Total phenolic content	Folin–Ciocalteu assay (μg GAE/g DW)	4628 ± 616	5588 ± 644
Total antioxidant	FRAP assay (μg TE/g DW)	7157.9 ± 623.7	8080.0 ± 617.4
Capacity	ORAC assay (μg TE/g DW)	46459 ± 2012	58361 ± 5400

Values are means ± standard deviation of triplicate determination or mean of duplicate analysis.

Table 2
The concentration of polyphenolic compounds of dried apple skin powder prepared from 'Idared' and 'Northern Spy'' apples

Polyphenols category	Compound	Polyphenolic content[a] (mg/100 g dry matter)	
		'Idared'	'Northern Spy'
Flavonols	Quercetin-3-galactoside	75.6 ± 4.4	94.4 ± 3.2
	Quercetin-3-glucoside	16.2 ± 1.3	21.4 ± 0.4
	Quercetin-3-rhamnoside	28.8 ± 3.1	62.6 ± 2.8
	Quercetin-3-rutinoside	2.7 ± 0.4	10.2 ± 0.6
	Quercetin	0.2 ± 0.9	0.2 ± 0.3
Dihydrochalcones	Phloridzin	54.9 ± 2.7	37.1 ± 1.9
	Phloretin	1.3 ± 0.1	0.8 ± 0.1
Phenolic acids	Chlorogenic acid	33.0 ± 4.0	62.0 ± 8.5
Anthocyanins	Cyanidin-3-galactoside	127.5 ± 11.9	51.0 ± 8.2
Flavan-3-ols	Catechin	7.8 ± 0.4	8.6 ± 0.3
	Epicatechin	33.4 ± 1.8	49.4 ± 2.7

[a] Mean ± standard deviation of two cultivars × three replicates.

to sub-classes of flavonols, dihydrochalcones, flavan-3-ols, phenolic acids, and anthocyanins (Table 2). Similar results of phenolic content differences of these two apple cultivars have been reported (Tsao, Yang, Young, & Zhu, 2003).

3.2. Proximate composition

There was no evidence that increasing ASP significantly affected the level of moisture retained in the product immediately after baking (Table 3). The incorporation of ASP did not influence the crude fat content of muffins (Table 3). However, the crude protein content decreased in muffins when the incorporation level of ASP increased (Table 3). The protein content in control muffins is in agreement with the values reported by Grigelmo-Miguel, Carreras-Boladeras and Matin-Belloso (1999). Since wheat flour is richer in protein (7–9%) than ASP (3.2%; Table 1), the higher the level of replacement of wheat flour by ASP would be expected to lower the content of the protein content in muffins (Table 3). Ash content in control muffins (Table 3) are also in agreement with the values reported by Grigelmo et al. (1999).

3.3. Dietary fibre content

The total dietary fibre content of muffins significantly increased with the level (0%, 8%, 16%, and 24%) of incorporation of ASP of 'Idared' (Table 4). For example, muffins of 24% ASP (7.6% DW basis) contained 5.8-fold greater total dietary fibre when compared that of muffins without ASP (1.3% DW basis). The soluble fibre content of ASP incorporated muffins was two- to three-fold higher than insoluble fibre (Table 4) in agreement with that was observed in ASP (Table 1). In apple, soluble fibre content mainly consists of pectins and insoluble fibre consists of cellulose, hemicellulose and lignins (McKee & Latner, 2000). The results indicate that ASP could be considered as an alternative dietary fibre source or specialty food ingredient for muffins and other bakery product.

Table 4
Dietary fibre content (% dry weight) of muffins incorporated with ASP of 'Idared'

Apple skin powder (% w/w incorporation)	Insoluble fibre	Soluble fibre	Total dietary fibre
0	0.9 ± 0.10^{b}	0.5 ± 0.07^{c}	1.3 ± 0.09^{d}
8	1.2 ± 0.10^{ab}	2.3 ± 0.60^{bc}	3.5 ± 0.56^{c}
16	1.8 ± 0.28^{a}	3.9 ± 0.72^{ab}	5.8 ± 0.80^{b}
24	2.0 ± 0.66^{a}	$5.5 \pm 0.1.51^{a}$	7.6 ± 0.89^{a}
Statistics	T^{*}	T^{***}	T^{***}
SEM, d.f	0.1054, 11	0.2560, 11	0.1906, 11

*Values are means ± standard deviation; $n = 3$; means in columns without letters in common differ significantly ($P < 0.05$).

3.4. Effect of baking on the phenolic compounds

A critical question to be addressed was to understand the impact of baking on the phenolic constituents present in ASP. Based on the recovery of original polyphenols of ASP from the baked muffins, the baking process affected all polyphenolic compounds (Table 5). In general, the anthocyanin in apple skin, cyanidin-3-*O*-galactoside was relatively the most affected in comparison to flavonols, dihydrochalcones, phenolic acids, and flavan-3-ols. One of the very interesting finding of this study was that the mean percent loss of the quercetin glycosides was least (38.6%) among other sub-classes of phenolics while there was a significant increase in free quercetin. Phloridzin values were found to be 56% less than expected and there was a 784% increase in phloretin levels. However, quercetin and phloretin aglycones were very low in ASP, thus, the

Table 3
Proximate composition of the muffins incorporated with ASP

Apple skin powder (% w/w incorporation)	Moisture (%)	Crude fat (%)	Crude protein (%)	Ash (%)
'Idared'				
0	31.39 ± 0.68	27.01 ± 0.52	7.29 ± 0.08^{a}	1.39 ± 0.05
4	30.18 ± 0.60	27.16 ± 0.30	7.13 ± 0.00^{a}	1.38 ± 0.06
8	30.94 ± 0.31	26.71 ± 0.61	6.85 ± 0.12^{ab}	1.38 ± 0.06
16	30.52 ± 0.52	26.36 ± 0.23	6.27 ± 0.04^{bc}	1.48 ± 0.05
24	30.99 ± 0.42	26.65 ± 0.32	6.10 ± 0.04^{cd}	1.47 ± 0.09
32	30.93 ± 0.63	27.16 ± 1.24	5.55 ± 0.59^{d}	1.54 ± 0.07
Statistics	T^{NS}	T^{NS}	T^{***}	T^{NS}
SEM, d.f	0.1285, 17	0.1507, 17	0.0597, 17	0.0702, 17
'Northern Spy'				
0	30.71 ± 0.16	27.53 ± 0.77	7.32 ± 0.14^{a}	1.42 ± 0.02
4	31.73 ± 1.11	28.74 ± 0.98	7.13 ± 0.16^{a}	1.39 ± 0.07
8	30.56 ± 0.31	27.76 ± 0.46	6.93 ± 0.13^{a}	1.40 ± 0.15
16	32.09 ± 0.84	27.36 ± 0.77	6.36 ± 0.17^{b}	1.46 ± 0.07
24	30.98 ± 0.59	27.89 ± 1.18	6.19 ± 0.04^{b}	1.52 ± 0.13
32	31.76 ± 0.33	27.35 ± 1.26	5.60 ± 0.32^{c}	1.52 ± 0.12
Statistics	T^{NS}	T^{NS}	T^{***}	T^{NS}
SEM, d.f	0.1534, 17	0.2231, 17	0.0434, 17	0.0258, 17

Means ± standard deviation followed by different letters within a column for each cultivar are significantly different at $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$.

Table 5
The mean percent recovery of polyphenolic compounds of dried apple skin powder after baking in a model muffin system

Polyphenols category	Compound	Polyphenolic content[a] (mg/100 g dry matter)		Recovery of polyphenols (%)
		Estimated amount[b]	Detected amount	
'Idared'				
Flavonols	Quercetin-3-galactoside	12.21 ± 0.42	7.82 ± 0.32	64.05
	Quercetin-3-glucoside	2.63 ± 0.12	1.54 ± 0.16	58.6
	Quercetin-3-rhamnoside	4.65 ± 0.28	2.60 ± 0.30	55.9
	Quercetin-3-rutinoside	0.44 ± 0.12	0.32 ± 0.11	73.4
	Quercetin	0.04 ± 0.13	1.60 ± 0.65	4503.2
Dihydrochalcones	Phloridzin	8.86 ± 0.25	3.99 ± 0.08	45.0
	Phloretin	0.20 ± 0.43	1.68 ± 0.76	825.6
Phenolic acids	Chlorogenic acid	5.33 ± 0.98	2.70 ± 0.06	50.7
Anthocyanins	Cyanidin-3-galactoside	20.59 ± 0.41	3.23 ± 0.89	15.7
Flavan-3-ols	Catechin	1.26 ± 0.05	0.80 ± 0.06	63.5
	Epicatechin	5.39 ± 0.12	3.00 ± 0.32	55.6
'Northern Spy'				
Flavonols	Quercetin-3-galactoside	15.25 ± 1.10	8.80 ± 0.32	57.7
	Quercetin-3-glucoside	3.46 ± 0.24	1.90 ± 0.16	55.0
	Quercetin-3-rhamnoside	10.11 ± 0.54	6.20 ± 0.30	61.3
	Quercetin-3-rutinoside	1.65 ± 0.11	1.08 ± 0.10	65.6
	Quercetin	0.03 ± 0.12	0.30 ± 0.71	1160.9
Dihydrochalcones	Phloridzin	5.99 ± 0.21	2.56 ± 0.09	42.8
	Phloretin	0.14 ± 0.26	1.01 ± 0.76	743.0
Phenolic acids	Chlorogenic acid	10.01 ± 1.51	5.60 ± 0.09	55.9
Anthocyanins	Cyanidin-3-galactoside	8.24 ± 0.40	2.04 ± 0.82	24.8
Flavan-3-ols	Catechin	1.39 ± 0.02	0.76 ± 0.06	54.7
	Epicatechin	7.98 ± 0.17	4.40 ± 0.33	55.2

[a] Mean ± standard deviation of two cultivars × three replicates.
[b] Estimated amount is based on the amount of specific polyphenol in the ASP added to the muffin mixture.

compounds seem to be produced during the baking process due to the thermohydrolysis or deglycosylation of the glycosides of quercetin and phloretin. Therefore, it is apparent that baking process of quercetin glycosides, which are predominantly present in ASP, has partially converted to quercetin aglycone, the more bioavalable form (Erlund, 2004). Frying of onion for 15 min resulted in about 25% loss of the major favonol glucosides, quercetin 3,4'-*O*-diglucoside and quercetin-4'-*O*-monoglucoside (Price, Bacon, & Rhodes, 1997). Similar to present findings, recently, Rohn, Buchner, Driemel, Rauser, and Kroh (2007) found that main product of thermal degradation of onion quercetin glucosides is their aglycone quercetin, which remained stable during roasting at 180 °C.

Overall, when changes of quercetin and phloretin levels are not taken in to consideration, the recovery of major phenolics of ASP after baking process seems to be highly dependent on individual compounds and their structure dependent thermal stability and ranged between 16% and 73%. A major interesting finding is that flavonols and favan-3-ol present in ASP are relatively greater in resistant to thermal degradation during baking when compared with that of anthocyanins and phenolic acids. When a green tea extract was incorporated into the bread dough, the recovery of catechins from freshly baked bread was about 84% (Wang & Zhou, 2004). The losses could be due to the combined effect of oxidation, isomerization/epimerization and degradation of catechin as a result of various bread making stages including baking. Anthocyanins are not stable polyphenolics, and they tend to be decolorized or degraded during processing (Markakis, 1974). At high temperatures, such as cooking, cyanidin glycosides are hydrolyzed to cyanidin aglycone, which undergoes spontaneous degradation to various products (Seeram, Bourquin, & Nair, 2001). The poor recovery of original polyphenolics of ASP in this study could also be a result of their interactions with wheat protein via hydrogen bonding during dough preparation (Wang & Zhou, 2004). The stability of polyphenolics can also be affected by various other ingredients of muffin mixture.

3.5. TPC and TAC

Despite the poor recovery of the original phenolic compounds from muffins incorporated with ASP, a positive linear relationship between both the TPC ($r^2 = 0.90$) and TAC measured by FRAP ($r^2 = 0.99$) and ORAC ($r^2 = 0.94$) assays of muffins and the level of ASP incorporation was observed (Fig. 1). At each level of ASP incorporation, the TPC and TAC of muffins were higher for 'Northern Spy' than 'Idared' due to the higher phenolic content in the ASP of 'Northern Spy' than that of 'Idared' (Table 1). Interestingly, TPC and TAC was also detected in the muffins without ASP incorporation indicating that wheat flour contains phenolic acids, mainly ferulic acid (Beta, Nam, Dexter, & Sapirstein, 2005), contributing to total antioxidant capacity. Furthermore, a strong positive liner correlation was also found between TPC and TAC

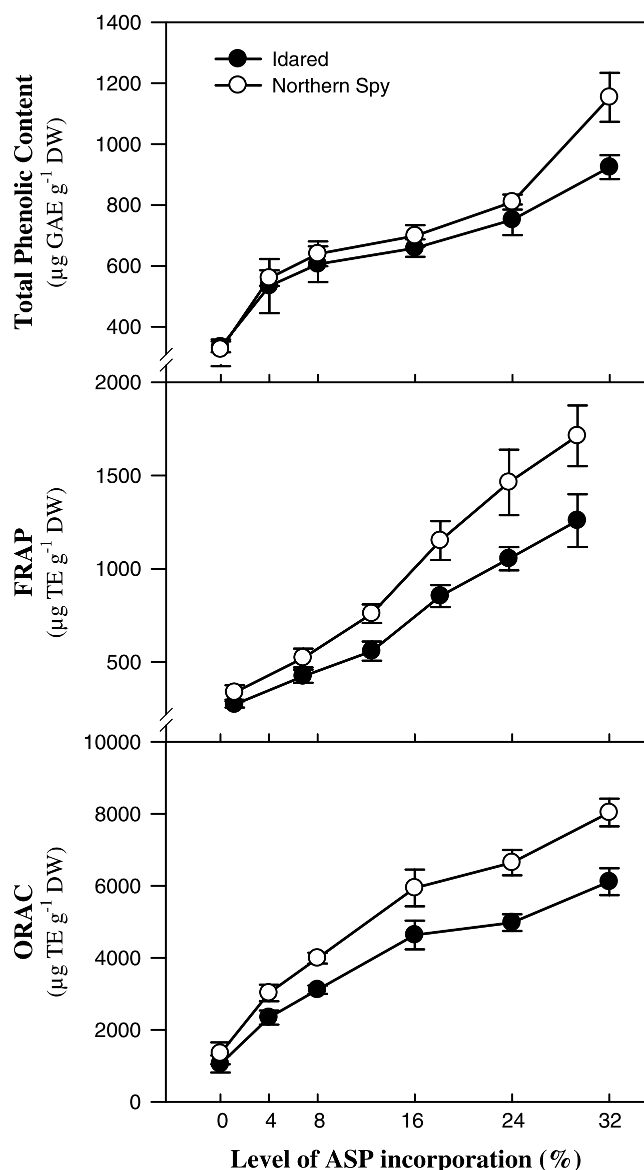

Fig. 1. Total phenolic content and total antioxidant capacity (FRAP and ORAC) of muffins incorporated with ASP of 'Idared' and 'Northern Spy'.

(FRAP; $r^2 = 0.89$ and ORAC, $r^2 = 0.91$) of muffins with different levels of ASP of both cultivars.

The TAC estimated by FRAP and ORAC of the methanolic extract of the muffins prepared with apple skin powder were 4.9 and 5.8-fold, respectively, greater than that of muffins without ASP incorporation (Fig. 1). Based on the expected values of TPC by Folin–Ciocalteu, FRAP and ORAC in 32% ASP incorporated muffins estimated based on the respective values of ASP, were 127%, 107%, and 80% respectively. Therefore, despite the significant loss of original polyphenolic compounds of ASP during the baking, antioxidant capacity of muffins remains as expected perhaps due to the antioxidative properties of thermal-induced degradative products of original phenolics of ASP. Recently, Buchner, Krumbein, Rohn, and Kroh (2007) reported that thermal treatment of quercetin and quercetin-3-O-rutinoside in an aqueous system could produce degradation products such as protocatechuic acid, which possess radical scavenging activity. Similarly, Seeram et al. (2001) identified three benzoic acid derivatives from the thermal degradation products of tart cherry anthocyanins (cyanidin glycosides). The thermal stability of many polyphenolic in ASP has not been well documented and further investigations are being carried out to identify the various degradation products of apple polyphenols during the baking process.

4. Conclusions

The majority of phenolics of apples are present in the skin, which is an underutilized food-processing by-product. To our knowledge, apple skin as a food ingredient in a bakery product has not been previously reported. Muffins incorporated with ASP were higher in dietary fibre, TPC, and TAC than the control. The enhanced potential health promoting indicators such as higher dietary fibre, TPC, and TAC coupled with enhanced food preparation properties such as greater water holding capacity provide new insight for use of apple skin as a value-added food ingredient. Recovery of individual polyphenolics of ASP after incorporation in muffin dough and baking was poor and the % recoveries were ranged between 16% and 64%. However, less or no impact of thermal processing on TAC of muffins incorporated with ASP was observed. Furthermore, better use of the by-product will also provide benefits to the apple industry as well as solutions for environment concerns associated with disposal. The results indicate that ASP could be considered as an alternative dietary fibre source or specialty food ingredient for muffins, other bakery products, or selected functional foods and nutraceuticals.

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