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Original article

Apple pomace as a potential ingredient for the development of new functional foods

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Summary Extruded snacks and baked scones were formulated with increasing levels (0–30%) of apple pomace (AP). The incorporation of up to 20% of AP in extruded snacks and in baked scones does not change significantly ($P < 0.05$) the proximate composition of the final products, except for the content of starch of baked scones. At this level of incorporation, the fibre content, phenolic content and antioxidant capacity (DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) and β -carotene/linoleic acid system) increased when compared to the products to which no AP was added. Chlorogenic acid and quercetin were the major phenolic compounds found in the products. Loss of phenolic compounds during heat treatment occurred; however, the DPPH radical scavenging activity of final products was not affected.

Keywords Antioxidants, apple pomace, baking, dietary fibre, extrusion, LC-ESI/MS.

Introduction

Apple pomace (AP) is the main by-product of cider processing and pose a serious environmental problem due to the large amounts (millions of tonnes in EU alone) produced every year. AP is composed mainly of carbohydrates and dietary fibre, small amounts of protein, fat and ash (Wang & Thomas, 1989; Carson *et al.*, 1994; Sudha *et al.*, 2007). AP is also a good source of phytochemicals primarily phenolic acids and flavonoids (Lu & Foo, 2000; Schieber *et al.*, 2003; Sanchez-Rabaneda *et al.*, 2004; Cetkovic *et al.*, 2008; Diñeiro García *et al.*, 2009). Some of the phenolic compounds identified in AP have been correlated with antioxidant capacities using various methods (DPPH, hydroxyl and superoxide anion radical scavenging activity, FRAP) and thereby confirming that the AP is a potential source of antioxidants (Lu & Foo, 2000; Cetkovic *et al.*, 2008; Diñeiro García *et al.*, 2009). The common applications of this by-product are the direct disposal to soil in a landfill and for the recovery of pectins (gelling agent, stabiliser and source of dietary fibre). The very large volumes produced each year exceed existing uses and new applications for AP are required, and it may find use as a valuable food additive to the high levels of fibre and antioxidants it contains.

Functional foods reflect recent consumer interest in convenient food and healthy eating. The US Food and Drug Administration (FDA) suggests that functional foods are a food or food ingredients that may provide a health benefit beyond their nutritional content. Among the target compounds considered to be health benefiting for functional foods are dietary fibre and antioxidants (Goldberg, 1994). AP rich in fibre with significant amounts of antioxidants can be incorporated in human food-chain, thus generating new potential functional foods.

Extruded and baked snack products are predominantly made from cereal flour or starches and tend to be low in protein and thus with low nutritional value. The incorporation of enriched fibre flours with significant antioxidants is one way to improve the nutritional value of these snacks, which have been reflected in several previous studies using food by-products (Ainsworth *et al.*, 2007; Stojceska *et al.*, 2008a,b; Ajila *et al.*, 2010). Apple by-products have been investigated as a source of dietary fibre in baked muffins and cookies (Wang & Thomas, 1989) and also as a source of polyphenols in cakes (Sudha *et al.*, 2007) and muffins (Rupasinghe *et al.*, 2008). However, the few studies on baked products lack information regarding antioxidants and in addition, research on AP incorporation in extruded products has not been reported yet. In this study, we present

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the chemical composition and the antioxidant capacity of extruded and baked products that incorporate AP.

Materials and methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (Arklow, Ireland) except for hydrochloric acid that was purchased from AlfaAesar (Karlsruhe, Germany), sodium citrate tribasic dehydrate from AnalaR NORMAPUR® (VWR, Ballycoolin, Ireland) and sodium hydroxide from Applichem (Darmstadt, Germany). The solvents used for sample preparation for LC-MS studies were HPLC grade.

Apple pomace

Apple pomace (AP) was provided by Bulmers Limited (Clonmel, Ireland). On arrival, the samples were packed under vacuum to prevent oxidation and fermentation and stored at $-20\text{ }^{\circ}\text{C}$ until being freeze-dried. The freeze-dried AP was coarsely ground and passed through $250\text{ }\mu\text{m}$ sieve and stored in polyethylene bags at $-20\text{ }^{\circ}\text{C}$.

Incorporation in extruded products

Extruded products were prepared from blends of rice flour and wheat semolina in a ratio of (2:1) with different proportions (0, 10, 20 and 30%) of AP on a dry weight basis. The samples were then conditioned to 15–20% moisture by spraying with a calculated amount of water and were mixed continuously at medium speed in a mixer (Hobart Mixer, Model F-50; HOBART, Troy, OH, USA), followed by storage at $4\text{ }^{\circ}\text{C}$ overnight. Extrusion was performed in a single-screw, laboratory model extruder (Brabender, Duisburg, Germany) with a DCE 330 attachment consisting of three independent zones of controlled temperature in the barrel. The screw speed used was 60 rpm and the length to diameter (L:D) ratio for the extruder was 20:1. The temperature profiles in the feed and compression metering zones were kept constant at 110 and $150\text{ }^{\circ}\text{C}$, respectively, and the die head temperature was $190\text{ }^{\circ}\text{C}$. After stable conditions were established, the extrudates were collected and dried in air oven at $60\text{ }^{\circ}\text{C}$ for 1 h. Extruded material was coarsely ground and passed through $250\text{ }\mu\text{m}$ sieve stored at $4\text{ }^{\circ}\text{C}$ in polyethylene bags.

Incorporation in baked products

Baked products were formulated as scones with Odlums Cream flour (Odlum Group, Dublin, Ireland)

composed of 11% protein, 13% fat, 3.1% fibre and 13% moisture. In addition to Odlums Cream flour, the formulation also included baking powder (4.5%), salt (0.25%), caster sugar (6.0%), margarine (17%), eggs medium (32%) and milk (41%). Odlums Cream flour was replaced with 10, 20 and 30% of AP for the different incorporated products. Baking was performed at $220\text{ }^{\circ}\text{C}$ for 16 min. The baked products were coarsely ground and passed through $250\text{ }\mu\text{m}$ sieve, freeze-dried and stored in polyethylene bags at $4\text{ }^{\circ}\text{C}$ for further analysis.

Chemical characterisation

Moisture and ash contents were determined according to AOAC 925.10 and 923.03 methods, respectively. Fat was determined by weight difference after extraction of the sample with diethyl ether (1:20; w/v) in a Soxhlet system for 8 h at $60\text{ }^{\circ}\text{C}$. Protein was determined as total nitrogen content ($\text{N}\times 6.25$) using the Kjeldahl method following the AOAC 920.87 method. Starch was determined by amyloglucosidase/ α -amylase method using Megazyme analysis kit (Megazyme International Ltd, Bray, Ireland) based on AOAC 996.11 method. Total dietary fibre (TDF) was determined using a Sigma-Aldrich analysis kit based on AOAC 991.43 method. Protein and starch digestibility was determined according to methods previously described (Onyango *et al.*, 2004). All measurements were carried out in triplicate.

Extraction of phenolic compounds

The extraction of phenolic compounds followed the method described previously by the same authors (Reis *et al.*, 2012). Briefly, the freeze-dried and ground samples (3 g) were stirred with 40 mL of water three times (40, 40, 10 min) at room temperature. In each extraction, the water extracts were filtered and the three supernatants obtained were combined. Solid-phase extraction (SPE) with C_{18} cartridges (DSC-18; Supelco, Sigma-Aldrich) was performed to select the organic compounds from the crude extract and to remove the sugars from each extract, which will otherwise interfere with the Folin assay. The sugars were eluted from the SPE cartridges with 300 mL of 2% acetic acid and the phenolic-rich fraction was eluted with methanol containing 0.1% HCl. The phenolic-rich fraction was concentrated (by evaporation at $40\text{ }^{\circ}\text{C}$), frozen ($-70\text{ }^{\circ}\text{C}$) and freeze-dried.

Determination of phenolic compounds

Total phenolic content (TPC), total flavonoid content (TFC) and proanthocyanidins content (PAC) were determined according to Reis *et al.* (2012).

Antioxidant capacity evaluation

The antioxidant capacity was evaluated by the DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) and β -carotene/linoleic acid system (BCLAS) according to Reis *et al.* (2012).

Liquid chromatography – electrospray ionisation mass spectrometry (LC-ESI/MS)

LC-ESI/MS was performed on a Q-ToF Premier mass spectrometer (Waters Corp., Micromass MS Technologies, Manchester, UK) coupled to an Alliance 2695 HPLC system (Waters Corp., Milford, MA, USA) as described previously by Reis *et al.* (2012).

Statistical analysis

All TPC, TFC, PAC, DPPH, FRAP and BCLAS measurements were carried out in three independent extractions and performed in triplicate for each extraction. All data were reported as mean \pm standard deviation (SD). ANOVA one-way statistical analysis was carried out to determine significant differences between means. To assess which means were different from which means, Tukey's method was used as an additional test to ANOVA. Differences were considered to be statistically significant at $P \leq 0.05$.

Results and discussion

Chemical characterisation

The significant differences observed in extruded and baked products with AP incorporation were the decrease in protein and starch contents while the TDF increased (Table 1). The decrease in protein and starch can be attributed to the partial replacement of the flour mixture by AP, due to the flour mixture in both extruded and baked products is richer in protein and starch than AP. TDF of AP-incorporated extruded products increased 1.8-fold with 30% AP incorporation. However, the recovery of TDF in final products was 0.64 for 10 and 30% AP addition and 0.76 for 20% AP addition (Table S1). It is likely that extrusion is promoting polymerisation reactions between polysaccharides and proteins or phenolic compounds as suggested by Onyango *et al.* (2004), Stojceska *et al.* (2008a) and Ajila *et al.* (2010) thus affecting the recovery of TDF. For 30% AP incorporation in baked products, a 2.3-fold increase was observed in TDF and total recovery was obtained for all levels of AP incorporation. Wang & Thomas (1989) also observed 1.6-fold increase in TDF in moon cookies where the quick-cooking oats used in the filling were substituted by 40% AP. Interestingly, Sudha *et al.* (2007) observed a

substantially high (30-fold increase) TDF upon 25% AP incorporation in cakes. This high increase is probably due to variations in the size of the ground powder of AP used to mix in with the flour, because reducing particle size increases surface area thus enhancing nutrients extraction. Other possible causes for Sudha *et al.* (2007) result can also be due to differences in AP fibre composition and processing parameters used in the formulation of the baked products; however, as far as we know, it was never reported such a high difference due to these parameters.

In vitro soluble (IVSP) and insoluble (IVISP) protein digestibility in extruded products showed a significant decrease upon 20% incorporation of AP (Table 1). A reduction of 50% in IVSP and 57% in IVISP was observed with the incorporation of 30% of AP in comparison with the control sample (0% AP). The low protein digestibility has been attributed to possible changes in the proteins during cooking such as the formation of enzyme-resistant complexes due to the interaction between the proteins and nonstarch polysaccharides, polyphenols, starch and antinutrients (Onyango *et al.*, 2004; Stojceska *et al.*, 2008a). It is possible that adding AP with high contents of dietary fibre and polyphenols has promoted the formation of complexes resistant to pepsin action as indicated earlier for the TDF recoveries. Baked products showed no significant differences for IVSP and IVISP between control and AP-incorporated products (Table 1). Nitrogen solubility index (NSI) indicates the level of protein solubility in water which gives an indication of the extent of protein denaturation (Onyango *et al.*, 2004). In extruded products, NSI decreased significantly by 23% with the addition of 30% AP (Table 1). It implies that polymerisation, cross-linking and reorientation of the proteins to form new fibrous structures must have occurred as postulated by Onyango *et al.* (2004). In baked products, NSI results showed a significant increase of up to 16% with the addition of 30% AP (Table 1), indicating that protein denaturation has actually occurred. Starch digestibility showed a significant decrease upon the addition of 30% AP in extruded products; however, no significant changes were recorded for the additions of AP in baked products (data not shown).

The incorporation of AP in extruded and baked products does not compromise the nutritional value of the final products but improves it by increasing the overall fibre content. However, for the 30% AP incorporation in extruded products, the results indicate the formation of enzyme-resistant complexes, which contributes to up to 50% decrease in protein digestibility.

Determination of phenolic compounds

The incorporation of AP in extruded and baked products significantly increased TPC, TFC and PAC when

Table 1 Proximate composition of AP, incorporated AP extruded and baked products and protein digestibility of incorporated AP products

	Moisture %	Ash %	Fat %	Protein %	Starch %	TDF %	IVSP %	NSI %	IVISP %
AP	8 ± 0.4	1 ± 0.2	2 ± 0.1	3 ± 0.1	8 ± 0.5	42 ± 1.4	–	–	–
Extruded products									
0%	8 ± 0.01	0.4 ± 0.1	tr	11 ± 0.04	72 ± 5.4	7 ± 0.3	71 ± 3.1	10 ± 0.9	67 ± 3.6
10%	8 ± 0.1	0.4 ± 0.02	0.1 ± 0.02	10 ± 0.02	70 ± 2.2	9 ± 0.7	60 ± 6.8	9 ± 0.8	55 ± 7.8
20%	6 ± 0.1	0.4 ± 0.01	0.2 ± 0.02	10 ± 1.2	63 ± 1.3	12 ± 0.5	51 ± 9.0	5 ± 0.3	47 ± 9.3
30%	6 ± 0.1	0.5 ± 0.1	0.2 ± 0.02	9 ± 0.2	56 ± 0.8	13 ± 1.0	35 ± 3.5	8 ± 0.4	29 ± 5.2
Baked products									
0%	2 ± 0.4	3 ± 0.03	13 ± 0.02	14 ± 1.8	61 ± 2.7	8 ± 0.05	87 ± 7.7	11 ± 2.3	85 ± 13
10%	2 ± 0.1	3 ± 0.02	13 ± 0.1	12 ± 0.02	50 ± 3.0	12 ± 0.1	83 ± 6.3	12 ± 0.3	81 ± 10
20%	3 ± 0.1	3 ± 0.2	13 ± 0.5	12 ± 0.2	27 ± 2.2	15 ± 0.1	81 ± 5.3	13 ± 1.1	76 ± 6.0
30%	3 ± 0.1	3 ± 0.1	13 ± 0.8	11 ± 0.2	34 ± 1.8	18 ± 0.2	74 ± 5.5	13 ± 0.2	73 ± 4.7

TDF, total dietary fibre; tr, traces; IVSP, *in vitro* soluble protein digestibility; NSI, nitrogen solubility index; IVISP, *in vitro* insoluble protein digestibility.

Table 2 Quantities of TPC, TFC, PAC, DPPH, FRAP and BCLAS expected and observed in incorporated AP extruded and baked products and their respective recoveries

	TPC			TFC			PAC		
	Expected µg gallic acid/g DW	Observed	Recovery %	Expected µg quercetin/g DW	Observed	Recovery %	Expected µg catechin/g DW	Observed	Recovery %
Extruded products									
10%	170	185	109	480	460	96	43	37	86
20%	340	287	84	960	487	51	86	26	30
30%	510	413	81	1440	671	47	129	53	41
Baked products									
10%	170	90	53	480	278	58	43	7	17
20%	340	249	73	960	475	49	86	35	41
30%	510	274	54	1440	550	38	129	22	17

	DPPH			FRAP			BCLAS		
	Expected % inhibition	Observed	Recovery %	Expected µg ascorbic acid/g DW	Observed	Recovery %	Expected % preservation	Observed	Recovery %
Extruded products									
10%	10	34	341	120	144	120	6	4	67
20%	20	54	268	240	216	90	12	7	58
30%	30	66	219	360	256	71	18	6	33
Baked products									
10%	10	12	125	120	46	38	6	6	102
20%	20	26	131	240	156	65	12	6	52
30%	30	33	110	360	182	51	18	9	52

TPC, total phenolic content; TFC, total flavonoid content; PAC, proanthocyanidins content; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; FRAP, ferric reducing antioxidant power; BCLAS, β-carotene/linoleic acid system.

compared to the control (Fig. 1). The best results in extruded products were obtained for 30% AP incorporation resulting in 2.8-fold, 4-fold and 1.8-fold increase in TPC, TFC and PAC, respectively. In baked products, the best results were obtained for 20% AP incorporation with an increase of 3.3-fold, 4-fold and 3.1-fold for TPC, TFC and PAC, respectively.

The quantities expected of TPC, TFC and PAC for the respective percentage of AP incorporation observed and their respective recoveries are shown in Table 2. The expected results were calculated considering the TPC, TFC and PAC results obtained for AP reported by Reis *et al.* (2012) and the respective proportion of AP incorporated. Overall, the recoveries

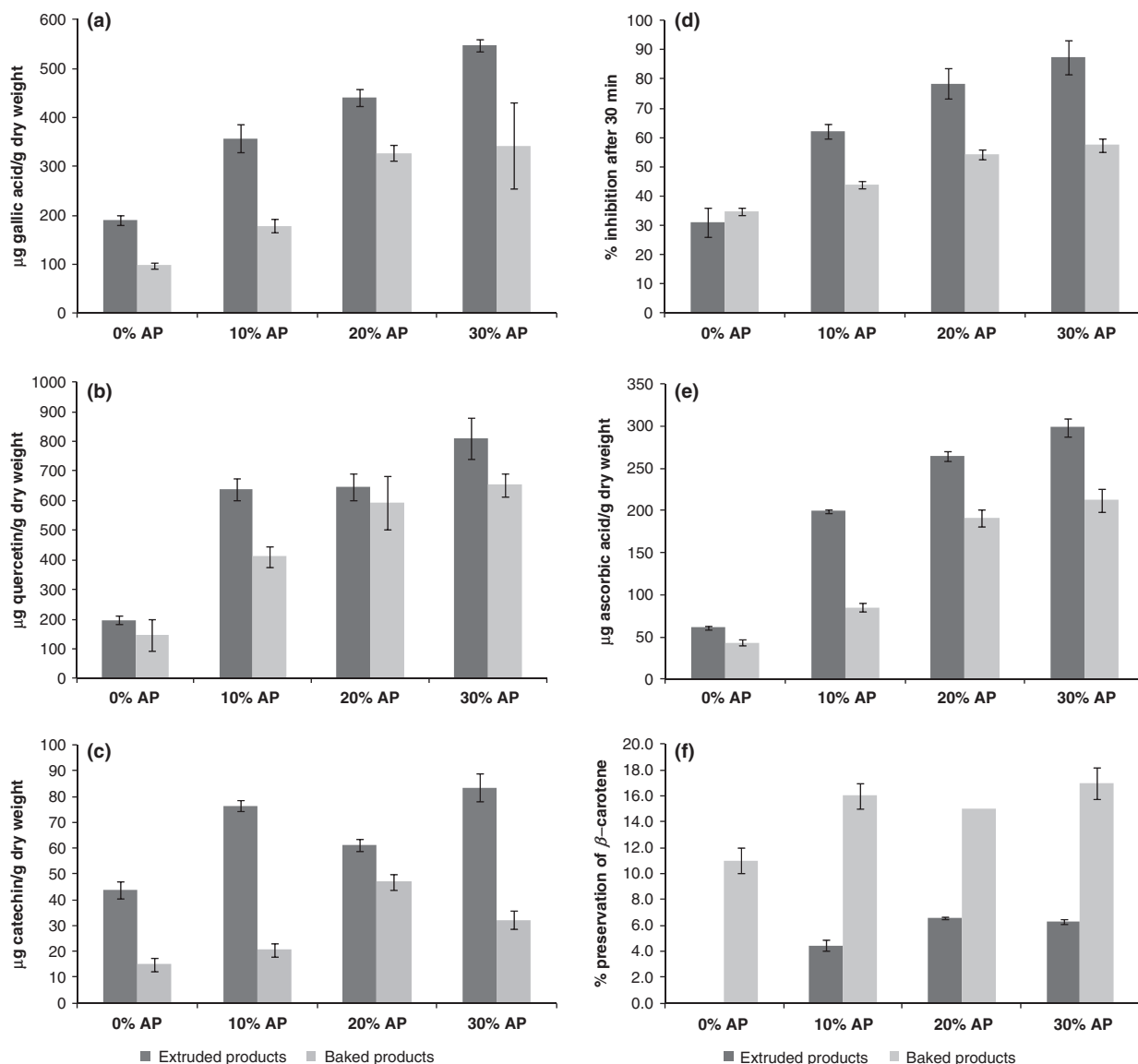


Figure 1 Results of (a) TPC, (b) TFC, (c) PAC, (d) DPPH radical scavenging activity, (e) FRAP and (f) β -carotene/linoleic acid system in AP incorporated extruded and baked products.

obtained showed a decreasing trend with the increase in AP incorporation. It is likely that polymerisation was promoted by heat treatment, as also seen in the previous section, which affected the extractability of such compounds, and the more phenolic compounds were incorporated, the higher was the polymerisation. Moreover, it was observed that TFC and PAC recoveries were lower than TPC recoveries for all the levels of incorporation. For 30% AP incorporation in extruded products, TPC recovery was double of TFC and PAC recoveries. This observation suggests that losses of flavonoids have occurred due to heat processing, and that other compounds were compensating for

TPC from the losses of flavonols and flavanols, probably thermally induced degradative products of phenolic origin. Rupasinghe *et al.* (2008) observed losses of flavonoids after the incorporation of 32% of apple skin powder in muffins, while the TPC was threefold higher than the control, and suggested that quercetin glycosides were partially converted to quercetin aglycone due to thermohydrolysis or deglycosilation.

Antioxidant capacity evaluation

The incorporation of AP significantly improved the antioxidant properties of the extruded and baked

products when compared to the control (Fig. 1). Incorporation of 30% AP increased 2.8-fold and 1.6-fold the DPPH radical scavenging activity, fivefold FRAP and sixfold and 1.5-fold the preservation of β -carotene in a linoleic acid system in extruded and baked products, respectively.

The recoveries obtained for DPPH, up to 300% in extruded products (Table 2), together with the loss observed in phenolic compounds clearly show that other compounds than phenolics affected the DPPH radical scavenging activity. This is a strong evidence that these compounds are probably the well-known Maillard reaction products, already known as a result of the extrusion process (Singh *et al.*, 2007) and often related with high scavenging activities (Amarowicz, 2009). A slight decrease in FRAP recoveries in extruded products was observed upon AP incorporation, and comparing with TPC results, it seems that FRAP is reflecting the antioxidant capacity from the recovered phenolic compounds. FRAP recoveries in baked products increased with the addition of AP but

declined upon 30% AP incorporation, following the trend of TPC and PAC recoveries thus suggesting, as in extruded products, that the antioxidant activity measured by FRAP is influenced only by the recovered phenolic compounds. BCLAS recoveries in extruded products were the lowest; however, they were very close to the recoveries obtained for TFC and PAC. In baked products, BCLAS recoveries decreased up to 50% upon addition of 20% AP. A study by Lu & Foo (2000) had shown that there is a direct correlation between the lower molecular weight procyanidins and quercetin glycosides and the excellent activity measured by the β -carotene/linoleic acid assay.

The incorporation of AP in extruded and baked products significantly increased the antioxidant activity measured by the DPPH radical scavenging activity, FRAP and β -carotene/linoleic acid system when compared to the control. However, these properties are not only influenced by the phenolic content but by new products generated by heat treatment, and from the high scavenging activity observed in extruded

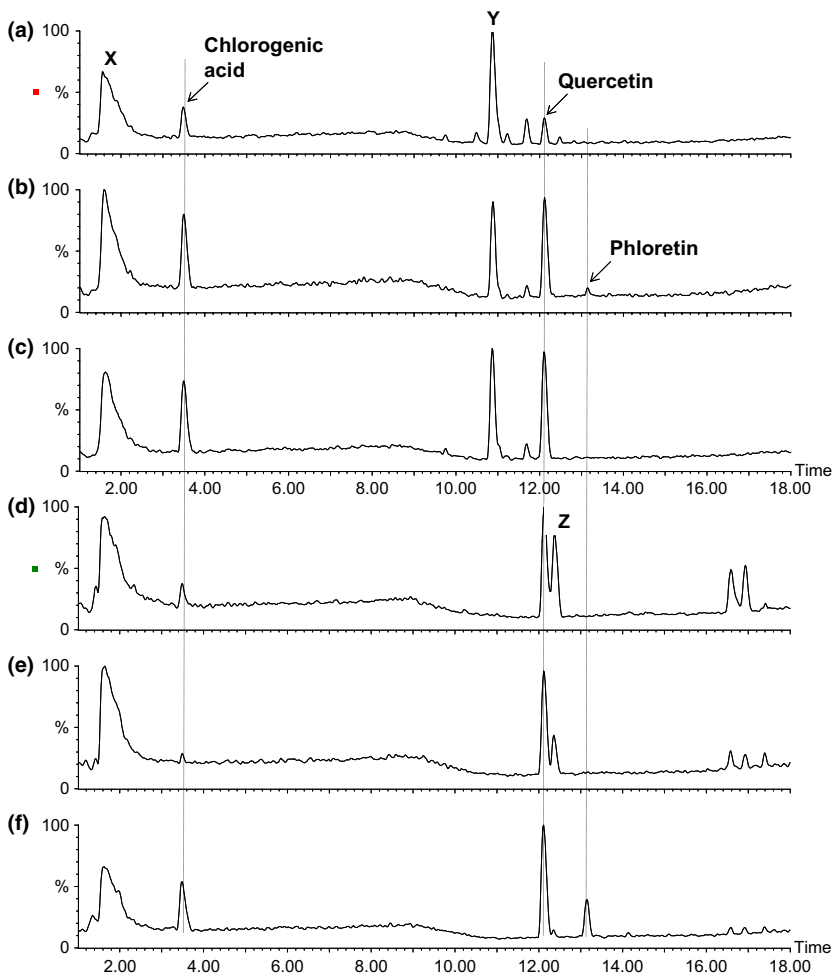


Figure 2 LC-MS chromatograms of (a) 10%, (b) 20% and (c) 30% AP incorporation in extruded products and (d) 10%, (e) 20% and (f) 30% AP incorporation in baked products.

products, they are most likely to be Maillard reaction products (Amarowicz, 2009).

Identification of phenolic compounds by LC-ESI/MS

For all levels of AP incorporated in extruded and baked products, three polyphenols namely chlorogenic acid, quercetin and phloretin were identified (Fig. 2). With the increasing percentage of AP incorporation, the intensity of the chlorogenic acid and quercetin peaks also increased. Phloretin in extruded products declined with 30% AP incorporation, but in baked products, it increased with the different levels of AP incorporation. There were three other major peaks, which could not be identified. One peak containing very polar compounds (peak X) most likely dominated by sugar molecules was present in all incorporated products. Unknown peak Y exclusively present in the extruded products with the exact mass of 188.0968 Da gave a molecular formula of $C_9H_{16}O_4$, while the unknown peak Z exclusively in the baked products with a molecular weight of 742 Da was prominent at 10% AP incorporation and its intensity decreased with increasing levels of AP incorporation. These three unidentified peaks must have been originated from non-AP matrix or from the chemical interaction between the AP matrix and other ingredients used in extrusion and baking products. On close scrutiny of the LC-MS data, other polyphenols such as feruloylquinic acid [(M-H)⁻ at m/z 367.118], isorhamnetin [(M-H)⁻ at m/z 315.067], phloridzin [(M-H)⁻ at m/z 435.178] and kaempferol [(M-H)⁻ at m/z 285.06] were also observed (Fig. S1). With respect to the identified compounds in the AP of the same extract (Reis *et al.*, 2012), in general the AP-incorporated extruded and baked products showed very little presence of the glycosidic forms of quercetin and phloretin, and all flavanols as illustrated by the extracted ion chromatogram for quercetin-*O*-glucoside [(M-H)⁻ at m/z 463.09] in Fig. S1. This fact proves the loss of phenolic compounds observed in previous sections, and the intensities of chlorogenic acid, quercetin and phloretin explain the thermally induced degradative products of phenolic origin compensating that loss. Similar observation was made by Rupasinghe *et al.* (2008), as poor recovery of individual polyphenols was observed upon heat treatment, suggested that thermohydrolysis or deglycosylation of the glycosides of quercetin and phloretin could result in significant increase in quercetin and phloretin aglycones.

The LC-MS is in agreement with the results obtained for TFC, PAC and BCLAS described earlier. The poor presence of flavanols and quercetin glycosides was reflected in TFC, PAC and β -carotene/lipoic acid system recoveries.

Conclusion

Apple pomace from a chemical and nutritional point of view can be incorporated in extruded and baked products increasing the fibre content and the antioxidant properties of the final products. Acceptability studies should be performed in the extruded snacks and in the scones up to 20% addition of AP. Epidemiological studies would be required to prove that the fibre and antioxidant properties conferred by the AP incorporation can confer health promoting properties, thus proving AP as an ingredient for the development of functional foods.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Quantities of TDF expected and observed in incorporated AP extruded and baked products and their respective recoveries.

Figure S1. Extracted ion chromatograms of feruloyl-quinic acid (368 Da), isorhamnetin (316 Da), phloretin (274 Da), phloridzin (436 Da), kaempferol (286 Da) and quercetin-*O*-glucoside (464 Da) in (a) extruded and (b) baked products with 30% AP incorporation.