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VALORISATION OF APPLE PEELS

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Abstract

The peels of processed apples can be recovered for further food applications. Limited information on the valorisation of this type of waste is available for cooking varieties, e.g. cv Bramley's Seedling. Extracts from fresh or dried (oven-dried or freeze-dried) peels were obtained with solvents of different polarity (aqueous acetone or ethanol) and assayed for their total phenolic content and antioxidant capacity; their antiradical power was compared to herb extracts. The dried peels were also characterised as bulk powders by assessing their nutritional value and total phenolic content. High amounts of ascorbic acid (up to 4 mg/g, dry weight) and polyphenols (up to 27 mg gallic acid equivalents/g, dry weight) were found in the peels, with the latter contributing significantly to the antioxidant capacity; the nutrient profile was low in

protein (less than 10%, w/w) and total dietary fibre content (less than 40%, w/w). Higher yields of phenolic antioxidants were recovered with acetone from freeze-dried peels; the resulting extracts had equivalent antioxidant power to oregano leaves (*Origanum vulgare* L.). The combination of oven-drying/ethanol led to lower recovery yields of phenolic antioxidants; however, these conditions could increase the feasibility of the extraction process, leading to antioxidant extracts with lower energy or cost input, and higher suitability for further food use. The recovery of phenolic antioxidants from the peels of processed apples could be a valuable alternative to traditional disposal routes (including landfill), in particular for cooking varieties. The recycling process could enhance the growth of traditional culinary apple markets in UK and Ireland thanks to the new business opportunities for the peel-derived materials.

Keywords: waste valorisation; cooking apples; peel polyphenols; antioxidant value.

1 INTRODUCTION

There is an increasing interest about natural plant extracts (i.e. botanicals) in novel food applications, as nutraceutical ingredients or natural preservatives and antioxidants (Coppens et al., 2006; Decker et al., 2010; Medina et al., 2003; Naidu et al., 2000; Pazos et al., 2005). Various agri-food waste and by-products have been screened for the recovery of natural phenolic antioxidants (Moure et al., 2001). The recovery of valuable materials is a strategy of waste minimisation (Bates, Phillips, 1999). Some nutraceutical products have been developed from grape waste or apple peels, and marketed for the functional markets of Japan and U.S.A. (Shoji et al., 2004; Yamakoshi et al., 2002). In Europe, the use of botanicals such as vegetable

and fruits, herbs and spices, herbal teas and infusions, and herbs is allowed in food and beverages for taste or functional purposes (e.g. guarana, gentian, etc.) (Coppens et al., 2006); however, the functional applications of many botanicals have not yet received the scientific opinions of the European Food Safety Authority (EFSA) (Gilsenan, 2011).

Apples are important dietary sources of phenolic compounds and have strong antioxidant capacity compared to other fruits (Sun et al., 2002). Apple polyphenols have various *in vitro* bioactivities, possibly in combination with dietary fibre (i.e. reduced risk of coronary heart disease) (Boyer, Liu, 2004). Higher amounts of polyphenols, in particular flavonol glycosides, are generally found in the skin of the fruit, compared to the pulp (Khanizadeh et al., 2008). Some studies have reported about the recycling of apple peels as a source of phenolic compounds and/or dietary fibre; depending on the compounds, different peel waste-derived materials were developed (Table 1).

The apple peels were preferably processed into a dried and pulverised bulk material for fibre formulation or nutraceutical use. Phenolics were extracted with organic solvents (or aqueous mixtures thereof) and then characterised for their potential health benefits. The second recycling option involved the preparation crude or purified mixtures of phenolic antioxidants and/or their formulation in nutraceutical or functional food applications. To the best of our knowledge, the preparation and characterisation of apple peel extracts for food stabilisation or preservation has not been studied.

Table 1 **Recycling of apple peel-derived materials: processing conditions (drying; extraction solvent); target compounds; and further applications.**

Peel-derived materials	***Preservation conditions*** *(peel material)*		***Extraction solvent*** *(phenolic compounds)*	***Applications***	***Target compounds***	***References***
	Pre-drying treatments	*Drying*				
Bulk peel powders	N/A	Drum-drying;	70% Acetone (v/v)	Fibre formulation/ Functional foods	Dietary fibre and phenolic compounds	(Henríquez et al., 2010)
	Water blanching;	Oven-drying (60°C, with air circulation)	Methanol	Fibre formulation/ Functional foods	Dietary fibre and phenolic compounds	(Rupasinghe et al., 2008)
	Water blanching; ascorbic acid dip	Freeze-drying; air-drying; oven-drying (at 40/60/80°C, no air circulation)	80% Acetone or 80% ethanol (v/v)	Nutraceuticals	Phenolic compounds	(Wolfe, Liu, 2003)
Antioxidant peel extracts	N/A	Freeze-drying	Methanol	Functional foods	Phenolic compounds	(Huber, Rupasinghe, 2009)
	N/A	N/A	N/A	Functional foods	Phenolic compounds	(Wegrzyn et al., 2008) [a]
	N/A	N/A	Ethanol or methanol	Nutraceuticals	Phenolic compounds	(Tanabe et al., 1994)
	N/A	Freeze-drying	80% Acetone (v/v)	Nutraceuticals	Phenolic compounds	(Wolfe et al., 2003)

[a] In this study, the apple peel extract was commercially available; the conditions used for its preparation were not described. N/A: not applicable.

In the preparation and characterisation of plant waste-derived materials, conditions such as the drying and the liquid extraction of phenolic compounds have an impact onto the feasibility of the recycling process (i.e. energy consumption and cost input), and further applications of the recovered ingredient (Peschel et al., 2006). For example, the extracts from apple peels developed by Huber, Rupasinghe (2009) were obtained with methanol; therefore they could not be tested in food systems. Ethanol and water should be preferred over methanol in view of food applications (Spigno et al., 2007). Freeze-drying, which is advantageous for heat sensitive materials, also requires higher energy consumption and initial and maintenance costs than oven-drying or air-drying, therefore its use could be limited in the industry (Ciurzyńska, Lenart, 2011).

The diversion of the peel waste from traditional disposal routes (landfertilising, feedstock, or landfill) towards more valuable food applications could favour the sustainable development of the culinary apple markets in the British Isles that are primarily based on cv Bramley's Seedling. This variety is known for the sole purpose of cooking, i.e. processed into sauce or puree, or used for home baking. Due to changes in the lifestyle, at the end of the 90's the fresh sector has narrowed in UK (Carter, Shaw, 1993); the same trend has occurred in Ireland, with the consequent overproduction at low farm gate prices (Bord Glas, 2003). In the absence of official statistics about the waste generated, it was estimated that 300 tonnes of peels could be discarded annually by processing lines in Ireland (Bord Bia, 2008), assuming a yield of 11%

(w/w) of peels from the whole apple. Another 5,000 tonnes of peels could be generated from the amount of processed lines in UK.[I]

The peels and/or pulp of cooking apples were assessed for their phenolic content in order to establish their dietary significance (Imeh, Khokhar, 2002; Price et al., 1999). However, few studies have investigated their recovery for valuable applications. Polyphenols were extracted from the pomace as potential nutraceutical compounds (McCann et al., 2007). The contribution of the skin to the extractable phenolics from the pomace was studied in comparison to the peeled fruit, distinguishing among soluble and insoluble bound components in view of further applications (Massini et al., 2010).

In the present study, different approaches for the preparation of peel-derived materials (bulk powders or extracts) with nutritional and/or antioxidant value from cv Bramley's Seedling apple (origin: Ireland) were investigated with the aim of establishing an optimal recovery process for further food use. The recycling value of these materials was compared to other plant-based products already developed for food applications (i.e. from the peels of different apple varieties; or herb leaves). Processing conditions (drying and/or extraction solvent) with different energetic or cost input were compared with the aim of defining a feasible recycling process with increased industrial applications. This valorisation approach could be applied to other processed apples in order to increase the type of waste-derived products recovered from solid fruit waste.

[I] http://www.bramleyapples.co.uk

2 MATERIALS and METHODS

2.1 CHEMICALS

Chemicals were purchased from Sigma-Aldrich (Ireland) and included: sodium nitrite; sodium carbonate; ferric chloride; aluminium chloride hexahydrate; 2.0 N Folin-Ciocalteu's phenol reagent; 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ); 2,2-diphenyl-1-picrylhydrazyl (DPPH); Celite, acid-washed; enzymes for the digestion of the dietary fibre: amyloglucosidase from *Aspergillus niger*; protease from *Bacillus licheniformis*; α-amylase (heat stable) from *Bacillus licheniformis*; and the standards: (+)-catechin hydrate; gallic acid and L-ascorbic acid.

2.2 PLANT MATERIAL

Two batches of apples (i.e. 3-5 kg per batch) (*Malus domestica* Borkh. cv. Bramley's Seedling) were purchased from a local store (Dublin, Ireland) between October 2007 and April 2008. According to the information provided by the retailer, the apples were grown in Co. Armagh, Northern Ireland, harvested in late August/September and made available throughout the year thanks to storage facilities (under controlled atmosphere).

The purchased apples were stored at 4°C in a polyethylene film, until processing. The apples were washed under tap water, dried by patting on a paper cloth and weighed. The peels were manually removed with a hand-peeler. Five grams of fresh peels were collected in triplicate from each batch of apples and immediately assayed. The remaining peels were oven-dried at 60 ± 2 °C (OD) on stainless steel trays in a ventilated oven (Weiss Gallenkamp BS Oven 250, UK) or freeze-dried (FD) in a Micro Modulyo E-C Apparatus (Davidson & Hardy, USA) until

a constant weight was achieved, in the dark. After drying, the samples were pulverised in a coffee grinder and the resulting powders were stored in amber bottles at -20°C until analysis.

2.3 EXPERIMENTAL DESIGN

The experimental design included the preparation of peel extracts from oven-dried samples with 80% ethanol, or freeze-dried peels with 80% acetone. The drying and solvent systems were studied under these combinations (i.e. freeze-drying/acetone; and oven-drying/ethanol) with the purpose of comparing conditions with less or more favourable impact onto the feasibility of the recovery process. The resulting extracts were compared to fresh samples extracted under similar conditions in order to assess the effect of processing onto the phenolic content and antioxidant capacity of the peels. Oregano and rosemary leaf extracts were prepared from herbs purchased from a local store and used as reference plant extracts with established food applications (Naidu, 2000).

The dried and pulverised peels were also characterised as bulk materials (i.e. nutritional value and total phenolic content). Soluble phenolic compounds were extracted with acetone or ethanol from dried peels (oven-dried or freeze-dried) and further quantified. The colour and free acidity of the powders were assessed because of their potential sensorial impact in further food formulation.

2.4 CHARACTERISATION OF BULK PEEL POWDERS

2.4.1 Proximate analysis

The proximate analysis was carried out according to official methods (AOAC, 2000): moisture content (Method 930.04); ash content (Method 930.05); protein content (Method 920.152); fat content (Method 983.23, with petroleum ether); ascorbic acid content (Method 967.21). The total dietary fibre (TDF) was determined according to Prosky et al. (Prosky et al., 1985). Sugars were extracted from the plant matrix using 80% ethanol (v/v) under boiling conditions and quantified as glucose equivalents (g/100 g) using the phenol-sulphur method by Dubois et al. (Dubois et al., 1956). The analyses were done in triplicate and expressed on a dry weight basis (DW).

2.4.2 Free titratable acidity

For the free titratable acidity, 1 g of peel powder was boiled for 10 mins in 20 mL of distilled water and filtered through a Büchner funnel. The free titratable acidity was measured according to AOAC (2000) (Method 942.15.b).

2.4.3 Colour

The CIELAB* colour (L*; a*; b* values) of the powders was measured in triplicate using ColorQuest®Xe (HunterLab, USA) applying the reflectance method: 10° observer; D65 illuminant. The instrument was calibrated with standard white and black tiles. The colour values were expressed as: L* = lightness (from 0 to 100); a* = redness/greenness (from +a* to –a*); b* = yellowness/blueness (from +b* to –b*).

2.5 CHARACTERISATION OF PEEL EXTRACTS

2.5.1 Extraction of phenolic compounds

Crude mixtures of soluble polyphenols were obtained in triplicate from fresh or dried peels, using a procedure previously described with minor modifications (Wolfe, Liu, 2003). For the dried peels, ~1 gram of powder was homogenised (ULTRA-TURRAX T25, IKA Laborteck, Germany) with 40 g of chilled aqueous 80% ethanol or 80% acetone (v/v) at 9500-13500 min^{-1} for 5 min. The obtained slurry was filtered under vacuum. The remaining solids were added to 15 mL solvent and extracted again, homogenising for 1 min. For the fresh peels, 5 g of sample was blended in a portable mini blender (dj2000 Illico Mini Chopper, Moulinex, France) with 40 g of solvent for 3 min, and then filtered through N.6 Whatman paper in a Büchner funnel. In the last filtration step, for both fresh and dried samples, another 15 mL of solvent was poured onto the filter cake. During the extraction, the extracts were kept chilled in an ice bath, in the dark. Homogenisation was stopped after one minute, waiting at least another minute before resuming. The filtrates were collected and the organic solvent was removed at 40°C using a Büchi rotavapor, until the aqueous phase remained. The concentrated extracts were brought to the volume of 25 mL with distilled water, filtered through N.1 Whatman paper, and stored at -20°C in the dark. Before analysis, they were thawed, centrifuged at 8,000 rpm for 15 min, filtered through 0.45 μm PTFE (Acrodisc, Pall, UK) membrane disc filter, and brought up to the volume of 50 mL with distilled water.

2.5.2 Total phenolic content

The total phenolic content (TPC) was assessed using Folin-Ciocalteu assay (Singleton et al., 1999). Volumes of 0.5 mL of distilled water and 0.125 mL of sample were added to a test tube. A volume of 0.125 mL of 2.0 N Folin-Ciocalteu reagent was added and allowed to react for 6 min. Then, 1.25 mL of a 7% sodium carbonate solution (v/v) was added to the mixture and allowed to stand for 90 min in the dark, for colour development. Before reading the absorbance at 760 nm in a spectrophotometer (Spectronic 1201, Milton Roy, USA), the mixture was diluted up to 3 mL with distilled water. Gallic acid solutions were used for the standard calibration curve and the total phenolic content was expressed as mg gallic acid equivalents (GAE)/g or 100 g peels (dry weight or fresh weight basis, DW or FW). All measurements were carried out in triplicate.

2.5.3 Total flavonoid content

The total flavonoid content (TFC) was assessed using aluminium-chloride assay (Zhishen et al., 1999). A volume of 0.25 mL of sample was added to a test tube containing 1.25 mL of distilled water. An aliquot of 0.075 mL of 5% sodium nitrite solution (w/v) was added to the mixture and allowed to stand for 5 min. Then, the addition of 0.15 mL of 10% aluminium chloride (w/v) developed a yellow flavonoid-aluminium complex. After 6 min, 0.5 mL of 4.3% NaOH (w/v) was added. The absorbance was measured immediately in a spectrophotometer (Spectronic 1201, Milton Roy, USA) at 510 nm and compared to a standard curve of (+)-catechin solutions. The flavonoid content was expressed as mg catechin equivalents (CE)/g peels (FW). All measurements were carried out in triplicate.

2.5.4 Ferric reducing antioxidant power

The antioxidant capacity was evaluated using a modified FRAP assay procedure based on a previously published protocol (Stratil et al., 2006). A freshly prepared FRAP-reagent (25 mL acetate buffer, 300 mM, pH 3.6 + 2.5 mL 10 mM TPTZ (2,4,6-tripyridyl-5-triazine) in 40 mM HCl + 2.5 mL 20 mM FeCl3·6 H2O) was heated in water bath at 37°C for 5 min before being transferred (0.9 mL) into tubes containing 0.1 mL of plant extracts. The tubes were left in water bath at 37°C for 40 minutes. The absorbance was then measured at 593 nm in a spectrophotometer (Spectronic 1201, Milton Roy, USA). The antioxidant capacity was compared to standard L-ascorbic acid through a calibration curve, and expressed as mg ascorbic acid equivalents (AAE)/g peels (FW), which was also referred to as AEAC (Ascorbic acid Equivalent Antioxidant Capacity). All measurements were carried out in triplicate.

2.5.5 Radical scavenging capacity

The radical scavenging capacity against a synthetic radical compound ($DPPH^{\bullet}$) was measured according to Makris et al. (2007), with some modifications. A volume of 0.1 mL of diluted extracts (bulk; 1:2; 1:5; 1:10; 1:20; 1:50) was added in a reaction vessel containing 0.9 mL of a freshly prepared $DPPH^{\bullet}$ solution (0.08 mM in 96% ethanol, v/v); the reaction was allowed to run for at least 30 minutes. The decrease in absorbance of the samples was read at 515 nm against a blank of distilled water in a spectrophotometer (Spectronic 1201, Milton Roy, USA) and compared to that of a control solution of $DPPH^{\bullet}$ prepared with 0.1 mL of distilled water.

159 The % Reduced $DPPH^{\bullet}$ was calculated using the following equation:

160

$$\% \text{ Reduced } DPPH^{\bullet} = [(1 - \text{Abs sample})/\text{Abs control})] * 100$$

161

162 The % Reduced values were expressed as AEAC (mg AAE/g peels, FW) by comparison with
163 a standard calibration curve with ascorbic acid. The IC_{50} value (i.e. concentration of plant
164 extract that reduces by 50% the initial concentration of the radical form of $DPPH^{\bullet}$ in the
165 reaction mixture) was calculated from the curves of sample concentration (as mg/mL, FW) vs.
166 % Reduced $DPPH^{\bullet}$. The values were expressed as Antiradical Power (ARP) = $1/IC_{50}$ (mL/g
167 sample, FW) according to Brand-Williams et al. (1995). For the preparation of plant extracts
168 with reference antiradical power, fresh leaves of oregano (OR) and rosemary (ROS) were
169 purchased from a local store (Dublin, Ireland) and oven-dried at 60°C ± 2°C in a ventilated air
170 oven (Weiss Gallenkamp BS Oven 250, UK) until constant weight was achieved, in the dark.
171 The samples were pulverised using a mortar and a pestle. Rosemary (5 g) and oregano (2 g)
172 leaf powders were extracted with 95% ethanol (v/v) homogenising for 2 minutes, according to
173 the method described by Almeida-Doria, Regitano-d'Arce (2000). The resulting ROS and OR
174 extracts were filtered through N°6 Whatman filter paper using a Büchner funnel, under
175 vacuum. The filtrates were collected and further evaporated in a rotary evaporator at 40°C
176 under vacuum, until 20% of the original volume remained. The extracts were stored in amber
177 glass bottles at -20°C until analysis.

2.6 STATISTICAL ANALYSIS

Statistical analysis was conducted using StatGraphics Centurion XV (Statpoint Technologies Inc., USA) and GraphPad v. 5.01 for Windows (GraphPad Software Inc., USA). Normal data was tested for significance using the one-way ANOVA (LSD post-hoc test), and F-test as appropriate. A regression analysis was also carried out. For all the statistical tests, the significance level taken was $p<0.05$.

3 RESULTS and DISCUSSION

3.1 BULK PEEL POWDERS

The characteristics of the powders obtained under different drying conditions were studied and further compared (Table 2). Regardless of the drying method, the powders generally had reduced protein content (less than 5%), making them a poor animal feed. They had high content of total carbohydrates (up to 80%, w/w). When compared to peel materials already developed from dessert varieties, e.g. cv Granny Smith (Henríquez et al., 2010), cv Northern Spy or cv Ida Red (Rupasinghe et al., 2008), the powders from Bramley apple peels had lower total dietary fibre (less than 40%, w/w, DW). They also had high acidity (almost 4-fold higher than in the peels of cv Granny Smith), which could negatively impact the sensorial characteristics in further food formulations. The ascorbic acid content was high, with values ranging from 3.0 to 4.4 (mg/g, DW); Łata (2007) reported values of 0.7–3.4 mg/g in the peels of various dessert apples.

Table 2 **Physical and chemical characteristics of bulk peel powders as affected by the drying method**

Parameter	***Drying method***	
(%, w/w)	***OD***	***FD***
Total ash	$2.23^{a} \pm 0.10$	$2.49^{a} \pm 0.44$
Total fat	$3.83^{b} \pm 0.23$	$6.61^{a} \pm 0.82$
Total protein	$5.07^{a} \pm 0.32$	$5.36^{a} \pm 0.19$
Total dietary fibre	$35.38^{a} \pm 2.22$	$32.49^{a} \pm 0.10$
Total sugars (as glucose)	$46.00^{a} \pm 8.27$	$40.36^{a} \pm 3.03$
Free titratable acidity (% malic acid, w/v)	$8.52^{a} \pm 0.11$	$8.16^{a} \pm 0.76$
Ascorbic acid (mg/g)	$3.01^{b} \pm 0.30$	$4.42^{a} \pm 0.20$
Colour		
L*	$71.3^{b} \pm 0.6$	$74.3^{a} \pm 0.2$
a*	$1.9^{a} \pm 0.2$	$-6.6^{b} \pm 0.1$
b*	$30.5^{b} \pm 0.3$	$34.6^{a} \pm 0.1$

Values were expressed as mean ± SD (n = 6) on a dry weight basis, considering an average residual moisture content of 7.5% and 9.0% for oven-dried (OD) and freeze-dried (FD) peels, respectively. Different superscript letters in each row denoted significant difference ($p<0.05$) between samples.

Some physical and chemical parameters were significantly affected by the drying system (Table 2). In particular, the thermal drying (e.g. oven-drying) produced a significant reduction of the fat and ascorbic acid content of the powders in comparison to freeze-drying. The oven-dried powders poorly retained the colour of the fresh peels in comparison to freeze-dried samples, and their colour had significant ($p<0.05$) lower greenness and yellowness values.

The drying system also influenced significantly ($p<0.001$) the yield of total phenolic compounds (calculated as TPC) in the final powders (Table 3). The yield also depended on the organic solvent used for their extraction ($p<0.001$). The thermal decomposition of the lipid substances in the skin could be associated to an increased oxidative damage of its natural antioxidants.

Table 3 Total phenolic content of oven-dried and freeze-dried bulk peel powders (extracted with different organic solvents).

Drying system	***Extraction solvent***	***Total phenolic content (mg GAE/g, DW)***
Freeze-drying (FD)	Acetone (Ac)	27.04 ± 1.76
	Ethanol (Et)	21.93 ± 0.36
Oven-drying (OD)	Acetone (Ac)	21.75 ± 0.36
	Ethanol (Et)	17.97 ± 0.42
	F-test	
Main effects	$LSD_{0.05}$ = 1.24	*Mean*
Drying system	***	24.97 (FD) 20.04 (OD)
Extraction solvent	***	24.78 (Ac) 20.23 (Et)

*** indicated a highly significant effect ($p<0.001$). TPC values were expressed as mean ± SD (n = 6). GAE: gallic acid equivalents.

The loss of phenolic compounds during oven-drying was reported in various plants by different authors (Moure et al., 2001). Natural antioxidants are normally accumulated in the skin in order to supply their antioxidant protection (Łata, 2007). According to Chinnici et al. (2004), phenolics could be regenerated by non-enzymatic reactions with ascorbate in the apple fruit. The TPC values of the Bramley apple peels were in agreement with results already reported for this variety by Imeh, Khokhar (2002).

3.2 PEEL EXTRACTS

3.2.1 Phenolic yield

The total phenolic (TPC) and flavonoid (TFC) contents of fresh and dried peels extracted with different solvents were compared (Table 4). With regard to the same solvent, dried peels had similar TPC than fresh samples, but their TFC was significantly different ($p<0.05$).

Table 4 **Phenolic content and antioxidant capacity of fresh and dried peels extracted with the same type of solvent.**

Parameter (mg/g peels, FW)	***Extraction solvent***	***Peels***	
		Fresh	*Dried* [i]
TPC (as GAE)	Acetone	$7.68^{a} \pm 0.74$	$7.63^{a} \pm 0.17$
	Ethanol	$6.35^{b} \pm 0.76$	$5.86^{b} \pm 0.35$
TFC (as CE)	Acetone	$5.34^{a} \pm 0.48$	$4.51^{b} \pm 0.10$
	Ethanol	$4.76^{b} \pm 0.47$	$4.03^{c} \pm 0.06$
FRAP (as AEAC)	Acetone	$13.26^{a} \pm 0.88$	$13.92^{a} \pm 0.29$
	Ethanol	$9.88^{b} \pm 1.66$	$10.43^{b} \pm 1.34$
Radical scavenging capacity (DPPH) (as AEAC)	Acetone	$12.11^{a} \pm 1.22$	$10.43^{b} \pm 1.34$
	Ethanol	$9.15^{c} \pm 0.61$	$7.27^{d} \pm 0.64$

[i] Freeze-dried (extracted with acetone); oven-dried (extracted with ethanol).

Values were expressed as mean ± SD (n = 6). Different superscript letters indicated significant difference ($p<0.05$) between fresh and dried samples extracted with the same type of solvent (within row). TPC: total phenolic content, expressed as gallic acid equivalents (GAE); TFC: total flavonoid content, expressed as catechin equivalents (CE); FRAP: ferric reducing antioxidant power, expressed as ascorbic acid equivalents (AEAC); Radical scavenging capacity against DPPH, expressed as ascorbic acid equivalents (AEAC).

These findings suggested that some flavonoids were lost during the processing of the peels, while other phenolics (i.e. conjugated) could be released after hydrolysis of the cell wall linkages, thus contributing to the yield of total phenolics. Most of the conjugated phenolics in apples are esters of hydroxycinammic acids (Vinson et al., 2001).

With regard to the extraction solvent, acetone extracted higher amounts of phenolic compounds than ethanol. In particular, the yield of phenolic compounds with ethanol was nearly 20% less than with acetone. The solubility of plant phenolics in solvents such as

ethanol or water is due to glycosilated forms than are more water-soluble than the related aglycones. A solvent of lower polarity, such as acetone, can favour the extraction of flavonoids of low-medium polarity (procyanidins) that remain otherwise bound to the alcohol-insoluble matrix in apples (Guyot et al., 1998).

3.2.2 Antioxidant capacity

The ascorbic acid equivalent antioxidant capacities (AEAC) of the processed samples were compared to those of fresh samples extracted under the same solvent conditions (Table 4). The radical scavenging capacity (for $DPPH^{\bullet}$) reduced significantly ($p<0.05$) after the processing of the peels, while the ferric reducing antioxidant power was not affected. These findings suggested that the redox potential (FRAP) of the fresh sample was maintained during processing because the amount of total reducing substances (including total polyphenols, TPC) remained stable possibly as a result of released hydroxycinnamic acids otherwise bound in the fresh tissue (Wolfe and Liu, 2003). On the contrary, the radical scavenging capacity of the processed mixture lowered in comparison to fresh samples, possibly in response to the loss of flavonoid compounds (TFC). In particular, it is believed that the loss of oligomeric procyanidins, i.e. indicated as the most powerful antioxidants in apples (Tsao et al., 2005), could influence significantly the radical scavenging capacity of the processed samples, as it is known that the number and substitution patterns of hydroxyl groups on the flavonoid structure is crucial for their radical scavenging capacity (Apak et al., 2007). The two antioxidant assays, FRAP and DPPH, could respond differently to the antioxidant mixtures as they are based on different antioxidant mechanisms (Prior et al., 2005; Foti et al., 2004). With regard to the

solvent, the extracts obtained with acetone showed significantly higher antioxidant capacity ($p<0.05$) than those obtained with ethanol. This was explained as due to the solubilisation of higher amounts of phenolic compounds (especially flavonoids). The FRAP capacities of fresh and dried peels from cv. Bramley's Seedling were in agreement with data reported for other dessert apples (Khanizadeh et al., 2008). To the best of our knowledge, no AEAC values measured by the DPPH assay have been reported in literature for other apple peels.

3.2.3 Antiradical power

The Antiradical Power (ARP) of apple peel extracts was compared to oregano and rosemary leaf extracts (Figure 1).

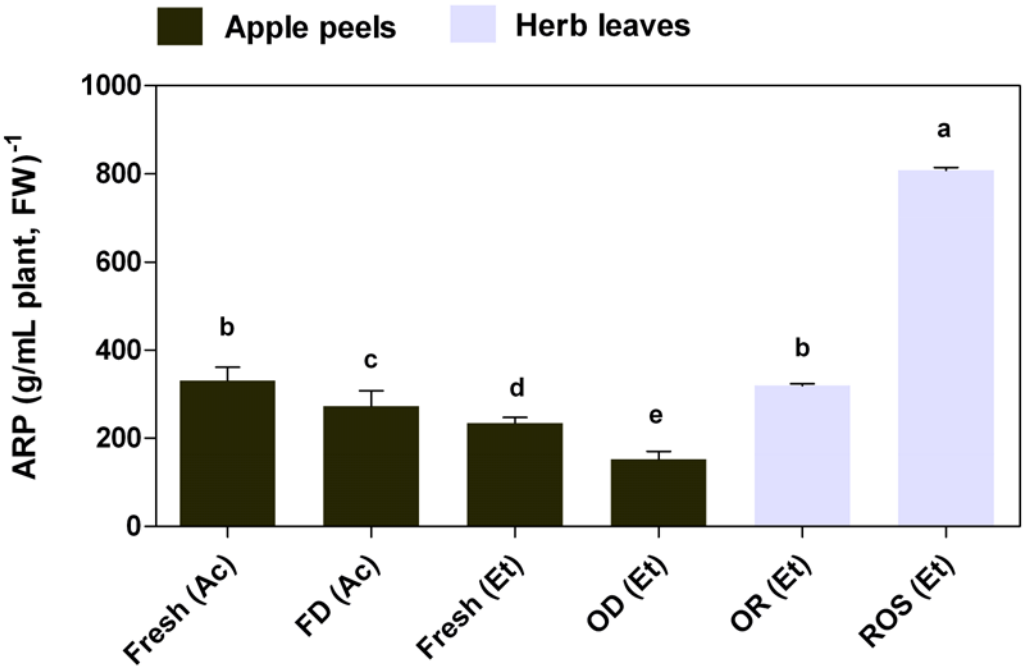

Figure 1 **Antiradical power of apple peel and herb leaf extracts. Different superscript letters denoted significant difference ($p<0.05$) among samples. Drying: oven-drying (OD); freeze-drying (FD). Extraction solvent: acetone (Ac); ethanol (Et). Herbs: oregano (OR); rosemary (ROS).**

The peel extracts obtained with acetone had similar antioxidant capacity than oregano leaf extracts. Rosemary extract had the strongest ARP ($p<0.05$) amongst the plant extracts investigated. Fresh peels had IC_{50} values of 4.28 ± 0.23 and 3.04 ± 0.27 mg peels/mL (FW) when extracted with ethanol and acetone, respectively. Dried peels had IC_{50} values of 6.51 ± 0.84 and 3.72 ± 0.48 mg peels/mL (FW), when extracted with ethanol and acetone, respectively. Kondo et al. (2002) reported for the skin of dessert and cider apples IC_{50} values lower than 5 mg peels/mL (in the reaction mixture, FW), that is ARP values higher than 200 mL/g. The ARP values for fresh peels of cv. Bramley's Seedling in this study were 234 ± 13 and 331 ± 30 mL/g peels (in the reaction mixture, FW), for the extracts obtained with ethanol and acetone, respectively.

Oregano and rosemary leaf extracts had IC_{50} values of 3.13 ± 0.04 and 1.89 ± 1.12 mg herb/mL (FW); these values were equivalent to 0.39 and 0.16 mg herb/mL on DW basis, assuming an average moisture content of 86%, w/w, which were consistent with previous data reported in literature (Koşar et al., 2005).

3.2.4 Regression analysis between antioxidant capacity and phenolic content

A regression analysis between the antioxidant capacity and the phenolic content of the peels was carried out (Table 5). The Pearson correlation coefficients were strongly significant ($p<0.01$) between the variables. However, it was observed a higher deviation from linearity in the regression values (r-square<0.6) of the whole peels (fresh + dried, n = 18) compared to dried samples (n = 12). This could indicate that reducing substances other than polyphenols

(e.g. ascorbic acid) were extracted from fresh samples and contributed to the antioxidant capacity together with phenolics. In agreement with this hypothesis, the relationship between AEAC (measured as FRAP) and the total flavonoid content (r-square<0.34) was weak; while the radical scavenging capacity was better correlated with the total flavonoid content (r-square>0.63).

Table 5 Regression analysis between antioxidant capacity and phenolic content of apple peels

Antioxidant capacity (as AEAC)	***Total phenolic content***		***Total flavonoid content***	
Fresh+Dried	*Corr.*	*r-square*	*Corr.*	*r-square*
FRAP	**	(0.66)	**	(0.34)
DPPH	**	(0.47)	**	(0.63)
Dried	*Corr.*	*r-square*	*Corr.*	*r-square*
FRAP	**	(0.76)	**	(0.48)
DPPH	**	(0.63)	**	(0.69)

** indicated a very significant correlation between the variables ($p<0.01$); the linear regression fit for the correlated data was reported in brackets (R-square). AEAC: ascorbic acid equivalent antioxidant capacity; Corr.: Pearson's correlation.

In the dried samples, the contribution of phenolic compounds to the antioxidant capacity increased above 70%, particularly for flavonoids and their radical scavenging capacity, thus indicating the possible reduction of co-extracted substances, such as ascorbic acid. Results previously reported by Imeh, Khokhar (2002) for Bramley apple indicated a weak linear correlation between the antioxidant capacity (as FRAP) and the total phenolic content (r-square<0.58).

4 CONCLUSIONS

- The recycling value of the peels from cv. Bramley's Seedling depended on its high levels of natural antioxidants, in particular phenolic compounds that contributed significantly to its antioxidant capacity.
- The recovery of target phenolic antioxidants (especially flavonoids) could be lowered by the processing, i.e. cutting; drying and pulverising; however, during the processing, phenolic compounds conjugated in the fresh plant matrix could be released with a consequent increase of the redox potential and total phenolic content of the resulting extracts.
- The drying system and the organic solvent used for the phenolic recovery affected their extraction yield, consequently their antioxidant capacity. Freeze-drying protected the antioxidant value better than oven-drying, while acetone favoured the solubilisation of higher amounts of phenolic compounds than ethanol. The resulting extracts had equivalent antioxidant power to oregano leaf extract.
- The use of oven-drying/ethanol for the phenolic recovery could lead to extracts with lower antioxidant value compared to freeze-drying/acetone but with enhanced food applications.
- Further investigation on the isolation of antioxidant phenolic compounds from the peels of Bramley's Seedling apple for future food applications is desirable.

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References

Almeida-Doria, R. F., and Regitano-D'arce, M. A. B. (2000) “Antioxidant activity of rosemary and oregano ethanol extracts in soybean oil under thermal oxidation”, *Ciência e Tecnologia de Alimentos*, 20, 197-203.

AOAC, (2000) *Official Methods of Analysis of AOAC International*, AOAC (Ed.), 17th ed., AOAC Int., Gaithersburg.

Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., Bektaşoğlu, B., Berker, K. I., and Özyurt, D. (2007) “Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay”, *Molecules*, 12, 1496-1547.

Bates, M. P., and Phillips, P. S. (1999) “Sustainable waste management in the food and drink industry”, *British Food Journal*, 101, 580-589.

Bord Bia (2008) “National Apple Orchard Census 2007”, available at: http://www.bordbia.ie (accessed 3 May 2012).

Bord Glas (2003) “National Apple Orchard Census 2002”, available at: http://www.bordglas.ie (accessed 14 March 2007).

Boyer, J., and Liu, R. H. (2004) “Apple phytochemicals and their health benefits”, *Nutrition Journal,* 3, 5.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995) “Use of a free radical method to evaluate antioxidant activity”, *Lebensmittel-Wissenschaft und Technologie*, 28, 25-30.

Carter, S., and Shaw, S. A. (1993) “The UK apple industry: production, markets and the role of differentiation”, *British Food Journal,* 95, 23-28.

Chinnici, F., Bendini, A., Gaiani, A., and Riponi, C. (2004) “Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition”, *Journal of Agricultural and Food Chemistry*, 52, 4684-4689.

Ciurzyńska, A., and Lenart, A. (2011) “Freeze-drying: application in food processing and biotechnology - A review”, *Polish Journal of Food and Nutrition Sciences,* 61, 165-171.

Coppens, P., Delmulle, L., Gulati, O., Richardson, D., Ruthsatz, M., Sievers, H., and Sidani, S. (2006) “The use of botanicals in food supplements. Regulatory scope, scientific risk assessment and claims substantiation”, *Annals of Nutrition and Metabolism,* 50, 538-554.

Decker, E. A., Elias, R. J., and Mcclements, D. J. (Eds.) (2010) *Oxidation in foods and beverages and antioxidant applications,* Woodhead Publishing, Cambridge.

Dubois, M., Gilles, K. A., Hamilton, K. A., Rebers, P. A., and Smith, F. (1956) “Colorimetric method for determination of sugars and related substances”, *Analytical Chemistry*, 28, 350-356.

Foti, M. C., Daquino, C., and Geraci, C. (2004) Electron-Transfer reaction of cinnamic acids and their methyl esters with the DPPH• radical in alcoholic solutions, *Journal of Organic Chemistry*, 69, 2309-2314.

Gilsenan, M. B. (2011) “Nutrition & health claims in the European Union: a regulatory overview”, *Trends in Food Science & Technology*, 22, 536-542.

Guyot, S., Marnet, N., Laraba, D., Sanoner, P., and Drilleau, J.-F. (1998) “Reversed-Phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien)”, *Journal of Agricultural and Food Chemistry*, 46, 1698-1705.

Henríquez, C., Speisky, H., Chiffelle, I., Valenzuela, T., Araya, M., Simpson, R., and Almonacid, S. (2010) “Development of an ingredient containing apple peel, as a source of polyphenols and dietary fiber”, *Journal of Food Science*, 75, 172-181.

411 Huber, G. M., and Rupasinghe, H. P. V. (2009) “Phenolic profiles and antioxidant properties
412 of apple skin extracts”, *Journal of Food Science,* 74, C693-C700.
413
414 Imeh, U., and Khokhar, S. (2002) “Distribution of conjugated and free phenols in fruits:
415 antioxidant activity and cultivar variations”, *Journal of Agricultural and Food Chemistry,* 50,
416 6301-6306.
417
418 Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M. T., and Rupasinghe, V. H. P.
419 (2008) “Polyphenol composition and total antioxidant capacity of selected apple genotypes for
420 processing”, *Journal of Food Composition and Analysis,* 21, 396-401.
421
422 Kondo, S., Tsuda, K., Muto, N., and Ueda, J.-E. (2002) “Antioxidative activity of apple skin
423 or flesh extracts associated with fruit development on selected apple cultivars”, *Scientia*
424 *Horticulturae,* 96, 177-185.
425
426 Koşar, M., Dorman, H. J. D., and Hiltunen, R. (2005) “Effect of an acid treatment on the
427 phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species”,
428 *Food Chemistry,* 91, 525-533.
429
430 Łata, B. (2007) “Relationship between apple peel and the whole fruit antioxidant content: year
431 and cultivar variation”, *Journal of Agricultural and Food Chemistry,* 55, 663-671.
432

Makris, D. P., Boskou, G., and Andrikopoulos, N. K. (2007) “Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts”, *Journal of Food Composition and Analysis*, 20, 125-132.

Massini, L., Martin Diana, A. B., Barry-Ryan, C., and Rico, D. (2010) “Apple (*Malus domestica* Borkh. cv Bramley's Seedling) peel waste as a valuable source of natural phenolic antioxidants”, in Waldron, K. W., Moates, G. K. & Faulds, C. B. (Eds.), *Total Food - Sustainability of the Agri-Food Chain*, RCS, Cambridge, 90-95.

Mccann, M. J., Gill, C. I. R., O' Brien, G., Rao, J. R., Mcroberts, W. C., Hughes, P., Mcentee, R., and Rowland, I. R. (2007) “Anti-cancer properties of phenolics from apple waste on colon carcinogenesis in vitro”, *Food and Chemical Toxicology*, 45, 1224-1230.

Medina, I., González, M. J., Pazos, M., Della Medaglia, D., Sacchi, R., and Gallardo, J. M. (2003) “Activity of plant extracts for preserving functional food containing *n*-3-PUFA”, *European Food Research Technology*.

Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., José Núñez, M., and Parajó, J. C. (2001) “Natural antioxidants from residual sources”, *Food Chemistry*, 72, 145-171.

Naidu, A. S. (Ed.) (2000) *Natural food and antimicrobial systems*, CRC Press, Boca Raton, Florida.

Pazos, M., Gallardo, J. M., Torres, J. L. S., and Medina, I. (2005) "Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle", *Food Chemistry*, 92, 547-557.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventós, R., Buxaderas, S., and Codina, C. (2006) "An industrial approach in the search of natural antioxidants from vegetable and fruit wastes", *Food Chemistry*, 97, 137-150.

Price, K. R., Prosser, T., Richetin, A. M. F., and Rhodes, M. J. C. (1999) "A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing", *Food Chemistry*, 66, 489-494.

Prior, R. L., Wu, X., and Schaich, K. (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, *Journal of Agricultural and Food Chemistry*, 53, 4290-4302.

Prosky, L., Asp, N. G., Furds, I., De vries, J. W., Schweizer, T. F., and Harland, B. (1985) "The determination of total dietary fiber in foods, food products: collaboratory study", *Journal of AOAC INTERNATIONAL*, 68, 677-679.

476

477 Rupasinghe, H. P. V., Wang, L., Huber, G. M., and Pitts, N. L. (2008) “Effect of baking on
478 dietary fibre and phenolics of muffins incorporated with apple skin powder”, *Food Chemistry,*
479 107, 1217-1224.

480

481 Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y., and Jiang, Y. (2005) “Extraction of
482 polyphenolics from plant material for functional foods - Engineering and technology”, *Food*
483 *Reviews International*, 21, 139-166.

484

485 Shoji, T., Akazome, Y., Kanda, T., and Ikeda, M. (2004) “The toxicology and safety of apple
486 polyphenol extract”, *Food and Chemical Toxicology,* 42, 959-967.

487

488 Singleton, V. L., Orthofer, R., and Lamuela-Raventos, R. M. (1999) “Analysis of total phenols
489 and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent”,
490 *Methods in Enzymology*, 299, 152-178.

491

492 Spigno, G., Tramelli, L., and De Faveri, D. M. (2007) “Effects of extraction time, temperature
493 and solvent on concentration and antioxidant activity of grape marc phenolics”, *Journal of*
494 *Food Engineering*, 81, 200-208.

495

Stratil, P., Klejdus, B., and Kubáň, V. (2006) “Determination of total content of phenolic compounds and their antioxidant activity in vegetables - Evaluation of spectrophotometric methods”, *Journal of Agricultural and Food Chemistry*, 54, 607-616.

Sun, J. I. E., Chu, Y.-F., Wu, X., and Liu, R. H. (2002) “Antioxidant and antiproliferative activities of common fruits”, *Journal of Agricultural and Food Chemistry*, 50, 7449-7454.

Tanabe, M., Kanda, T., and Yanagida, A. (1994) *Process for the production of fruit polyphenols from unripe Rosaceae fruit*, U.S. Patent 5,932,623.

Tsao, R., Yang, R., Xie, S., Sockovie, E., and Khanizadeh, S. (2005) “Which polyphenolic compounds contribute to the total antioxidant activities of apple?”, *Journal of Agricultural and Food Chemistry*, 53, 4989-4995.

Vinson, J. A., Su, X., Zubik, L., and Bose, P. (2001) “Phenol antioxidant quantity and quality in foods: fruits”, *Journal of Agricultural and Food Chemistry*, 49, 5315-5321.

Wegrzyn, T. F., Farr, J. M., Hunter, D. C., Au, J., Wohlers, M. W., Skinner, M. A., Stanley, R. A., and Sun-Waterhouse, D. (2008) “Stability of antioxidants in an apple polyphenol-milk model system”, *Food Chemistry*, 109, 310-318.

Wolfe, K., and Liu, R. H. (2003) “Apple peels as a value-added food ingredient”, *Journal of Agricultural and Food Chemistry*, 51, 1676-1683.

Wolfe, K., Wu, X., and Liu, R. H. (2003) “Antioxidant activity of apple peels”, *Journal of Agricultural and Food Chemistry*, 51, 609-614.

Yamakoshi, J., Saito, M., Kataoka, S., and Kikuchi, M. (2002) “Safety evaluation of proanthocyanidin-rich extract from grape seeds”, *Food and Chemical Toxicology,* 40, 599-607.

Zhishen, J., Mengcheng, T., and Jianming, W. (1999) “The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals”, *Food Chemistry,* 64, 555-559.