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Valorisation of Apple Peels

Laura Massini[1*], Daniel Rico[2], Ana Belen Martín Diana[2] and Catherine Barry-Ryan[1]

[1]School of Food Science and Environmental Health, Dublin Institute of Technology, Cathal Brugha Street, D1, Dublin, Ireland.
[2]Agro Technological Institute of Castilla y Leon (ITACYL), Government of Castilla y Leon, Finca Zamadueñas, Valladolid, Spain.

Authors' contributions

The experimental study was designed in collaboration between all authors. Author LM managed the literature searches, the analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Research Article

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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

**Corresponding author: Email: laura.massini@dit.ie;*

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 [1] or natural preservatives [2] and antioxidants [3,4,5]. Various agri-food waste and by-products have been screened for the recovery of natural phenolic antioxidants [6]. The recovery of valuable materials is a strategy of waste minimisation [7]. Some nutraceutical products have been developed from grape waste or apple peels, and marketed for the functional markets of Japan and USA [8,9]. 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.) [1]; however, the functional applications of many botanicals have not yet received the scientific opinions of the European Food Safety Authority (EFSA) [10].

Apples are important dietary sources of phenolic compounds and have strong antioxidant capacity compared to other fruits [11]. Apple polyphenols have various *in vitro* bioactivities, possibly in combination with dietary fibre (i.e. reduced risk of coronary heart disease) [12]. Higher amounts of polyphenols, in particular flavonol glycosides, are generally found in the skin of the fruit, compared to the pulp [13].

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 of 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.

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 [14]. For example, the extracts from apple peels developed in [15] 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 [16]. 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 [17].

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	[18]
	Water blanching;	Oven-drying (60ºC, with air circulation)	Methanol	Fibre formulation/ Functional foods	Dietary fibre and phenolic compounds	[19]
	Water blanching; ascorbic acid dip	Freeze-drying; air-drying; oven-drying (at 40/60/80ºC, no air circulation)	80% Acetone or 80% ethanol (v/v)	Nutraceuticals	Phenolic compounds	[20]
Antioxidant peel extracts	N/A	Freeze-drying	Methanol	Functional foods	Phenolic compounds	[15]
	N/A	N/A	N/A	Functional foods	Phenolic compounds	[21][a]
	N/A	N/A	Ethanol or methanol	Nutraceuticals	Phenolic compounds	[22]
	N/A	Freeze-drying	80% Acetone (v/v)	Nutraceuticals	Phenolic compounds	[23]

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

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 [24]; the same trend has occurred in Ireland, with the consequent overproduction at low farm gate prices [25]. 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 [26], 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.[1]

The peels and/or pulp of cooking apples were assessed for their phenolic content in order to establish their dietary significance [27,28]. However, few studies have investigated their recovery for valuable applications. Polyphenols were extracted from the pomace as potential nutraceutical compounds [29]. 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 [30].

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.

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).

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

The purchased apples were stored at 4ºC 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 ºC (OD) on stainless steel trays in a ventilated oven (BS Oven 250, Weiss Gallenkamp, 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 -20ºC 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 [2]. 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 [31]: 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 [32]. 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 [33]. 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 [31] (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 [20]. 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 40ºC 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 -20ºC 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 [34]. 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 [35]. 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 [36]. 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 37ºC 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 37ºC 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 was measured against the synthetic radical compound $DPPH^{\bullet}$ [37]. 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.

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

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

The % Reduced values were expressed as AEAC (mg AAE/g peels, FW) by comparison with a standard calibration curve with ascorbic acid. The IC_{50} value (i.e. concentration of plant extract that reduces by 50% the initial concentration of the radical form of $DPPH^{\bullet}$ in the reaction mixture) was calculated from the curves of sample concentration (as mg/mL, FW) vs. % Reduced $DPPH^{\bullet}$. The values were expressed as Antiradical Power (ARP) = $1/IC_{50}$ (mL/g sample, FW) according to [38]. For the preparation of plant extracts with reference antiradical power, fresh leaves of oregano (OR) and rosemary (ROS) were purchased from a local store (Dublin, Ireland) and oven-dried at 60ºC ± 2ºC in a ventilated air oven (Weiss Gallenkamp BS Oven 250, UK) until constant weight was achieved, in the dark. The samples were pulverised using a mortar and a pestle. Rosemary (5 g) and oregano (2 g) leaf powders were extracted with 95% ethanol (v/v) homogenising for 2 minutes [39]. The resulting ROS and OR extracts were filtered through Nº6 Whatman filter paper using a Büchner funnel, under vacuum. The filtrates were collected and further evaporated in a rotary evaporator at 40ºC under vacuum, until 20% of the original volume remained. The extracts were stored in amber glass bottles at -20ºC 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 [18], cv Northern Spy or cv Ida Red [19], 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); values between 0.7–3.4 mg/g were reported in the peels of various dessert apples [40].

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 for various plants [6]. Natural antioxidants are normally accumulated in the skin in order to supply their antioxidant protection [40]. Phenolics could be regenerated by non-enzymatic reactions with ascorbate in the apple fruit [41]. The TPC values of the Bramley apple peels were in agreement with results already reported for this variety [27].

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} ± 0.74	7.63^{a} ± 0.17
	Ethanol	6.35^{b} ± 0.76	5.86^{b} ± 0.35
TFC (as CE)	Acetone	5.34^{a} ± 0.48	4.51^{b} ± 0.10
	Ethanol	4.76^{b} ± 0.47	4.03^{c} ± 0.06
FRAP (as AEAC)	Acetone	13.26^{a} ± 0.88	13.92^{a} ± 0.29
	Ethanol	9.88^{b} ± 1.66	10.43^{b} ± 1.34
Radical scavenging capacity (DPPH) (as AEAC)	Acetone	12.11^{a} ± 1.22	10.43^{b} ± 1.34
	Ethanol	9.15^{c} ± 0.61	7.27^{d} ± 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 [42].

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 [43].

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 [20]. 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 [44], 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 [45]. The two antioxidant assays, FRAP and DPPH, could respond differently to the antioxidant mixtures as they are based on different antioxidant mechanisms [46,47]. 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 dessert apples [13]. 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 (Fig. 1).

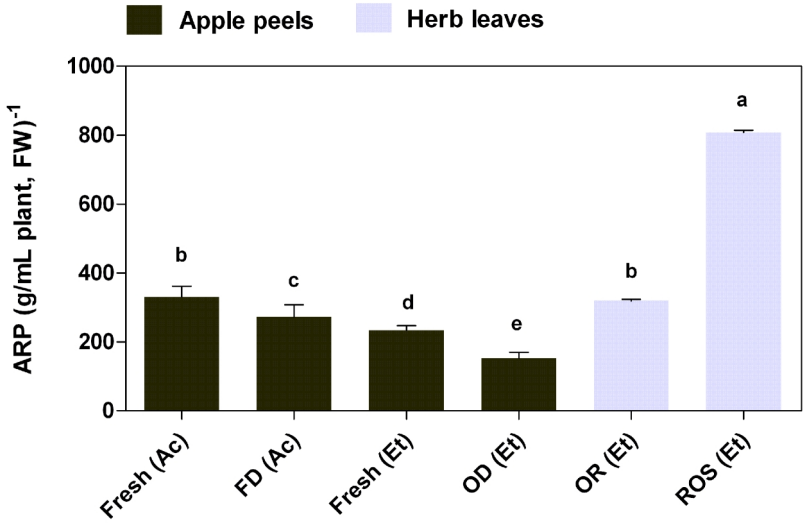

Fig. 1. Antiradical power of apple peel and herb leaf extracts.

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. [48] 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 [49].

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*<0.66) 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 [27] 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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