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Influence of apple purée preparation and storage on polyphenol contents and antioxidant activity

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Abstract

The polyphenolic components of Idared and Shampion apple purées were determined by HPLC; chlorogenic acid was the most abundant acid (20.0 mg/100 g in microwaved Idared); other polyphenols identified in high concentrations included (−)-epicatechin, procyanidin B1 and B2; quercetin and cyanidin glycosides were found in minor concentrations. The Shampion purées had higher total phenolics (142 mg/100 g) and procyanidin B2 concentrations (17.3 mg/100 g) than the Idared ones, and polymeric procyanidins represented 41% of all polyphenols. Antioxidant capacities of the samples, determined by spectrophotometric methods and EPR spectroscopy, correlated well with the concentration of polyphenols. The antioxidant properties could be better represented by EPR than by UV–vis measurements. The latter require transparent (clear) samples whereas EPR can be a method of choice in the assessment of radical-scavenging activity of dense and cloudy apple purées. Our results support the putative high antioxidant value of apple purées and define their capacity in terms of the major constituents. Apple purées are a rich source of natural antioxidants, especially of chlorogenic acid and procyanidins.
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Keywords: *Malus domestica*; Procyanidins; Ascorbic acid; Colour; Microwave; Processing; EPR; Antioxidant activity

1. Introduction

Widely consumed apples are a rich source of dietary fibre and phytochemicals, including antioxidants, such as procyanidins, quercetin, catechin, phloridzin and chlorogenic acid. Epidemiological studies have linked the consumption of apples with reduced risk of cardiovascular diseases, some cancers, asthma and diabetes. Raw apples have been found to inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol (Eberhardt, Lee, & Liu, 2000).

The health effects of apples, especially their cholesterol-lowering properties, were first ascribed to the content of pectins, soluble fraction of fibre (Aprikian et al., 2003; Cara et al., 1993; Sable-Amplis, Sicart, & Bluthe, 1983). In more recent studies, Aprikian et al. (2003) found that apple pectin and polyphenolic fractions lowered plasma, liver cholesterol and triglycerides, and are more effective together than are either apple pectin alone or apple phenolics alone. In the intact fruit, the fibres and the phenolic compounds are closely associated; they could reciprocally affect digestibility and possibly exert synergistic effects. The lyophilized apple diet lowered plasma and LDL cholesterol (22% and 70%, respectively, $P < 0.01$) in obese Zucker rats and, in parallel, reduced triglyceride accumulation in heart and liver (Aprikian et al., 2002). Many works (Aprikian et al., 2003; Cara et al., 1993; Eberhardt et al., 2000; Sable-Amplis et al., 1983) and numerous others suggest that there is a beneficial interaction between fruit fibre and polyphenolic components and also support the benefits of eating whole fruits as opposed to dietary supplements. Apples significantly lowered lipid oxidation both in humans and rats and lowered cholesterol level in humans.

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These effects, attributed to both the phenolics and the dietary fibres, may partially explain the inverse association of apple intake and the risk of cardiovascular disease (Aprikian et al., 2003).

The chemical composition varies significantly between different varieties of apples; small changes in phytochemicals occur during the maturation and ripening of the fruit (Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999). Storage has little or even no effect on apple phytochemicals, but processing can affect their profile greatly. The concentrations of fibre and phenolics in some apple products, such as clear juice and cider, are dramatically reduced after juice processing (Oszmiański, Wolniak, Wojdyło, & Wawer, 2007; Sanoner et al., 1999). Elimination of total fibre and reduction of polyphenolics are due to juice extraction (Spanos & Wrolstad, 1992; Spanos, Wrolstad, & Heatherbell, 1990). Apple phenolics, especially procyanidins, bind with cell wall material, which could lead to decreased levels of polyphenols, as found in apple juices (Renard, Baron, Guyot, & Drilleau, 2001). But procyanidins are powerful antioxidants since the corresponding oxidized forms acquire additional stabilization due to the extensive electron delocalization induced by the catechol unit on the aromatic B-ring (Rice-Evans, Miller, & Paganga, 1996). Thus special interest has been devoted to their antioxidant activity since procyanidins can protect protein and lipid for oxidation (Ivanow, Carr, & Frei, 2001). The influence of the procyanidin structure, degree of polymerization and oligomer chain length, in determining radical-scavenging properties, may also play an important role in predicting their biological activity (Hatano et al., 1989; Leite da Silva Porto, Nave Laranjinha, & Pereira de Freitas, 2003; Lotito et al., 2000). The beneficial effect of apples on human health is increased when consuming the products that contain, not only the liquid, but also the solid part of raw material, such as, e.g. the apple purée, which can be used as an intermediate product for the production of nectars, juices with solid particles, apple sauce, baby food and other products.

In industrial processes of apple purée production, the raw materials are diced and cooked at a temperature between 93 and 98 °C for about 4–5 min. This softens the fruit tissue and inactivates the polyphenoloxidase that is responsible for enzymatic browning. The duration of cooking, the temperature, and raw product input must be controlled to produce a purée of good colour and consistency (Downing, 1989). The heating rate remains one of the major limitations for the optimization of conventional thermal processes in which the heat is transferred through both conduction and convection. Microwave energy has the advantage of heating solids rapidly and uniformly, thus inactivating the enzymes more quickly and minimizing the browning (Gerard & Roberts, 2004). Apples undergo browning reactions very easily, which results in the oxidation of phenolic compounds into *o*-quinones, which in turn polymerize into complex dark-coloured pigments. The complexity of fruit by-products gives rise to a wide range of enzymatic and nonenzymatic reactions in which the oxygen supply is a key factor (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1995).

The control of purée browning has always been a challenge for the industry, the use of chemical antioxidants (e.g. ascorbic acid) and high temperature being the most common solutions (Lee & Whitaker, 1995). Unfortunately, apple purée extracts are dense and cloudy; their samples are difficult to analyze directly (without extraction). Otherwise, for spectrophotometric UV–vis measurements, background corrections for the absorbance are necessary. Chromameter readings in reflectance rather than transmittance are quite common, and measuring colour of solids is not an instrumental problem. Browning reactions, changes in chemical composition and antioxidant potential, which occur during the processing and storage of purées and juices, may have a negative effect on the quality and shelf-life of this product (Lea, 1995) and should be monitored at different stages of the production process.

Therefore, the objective of this research was to evaluate the effects of microwave heat treatment and ascorbic acid addition in apple purée production, and of storage on its colour, phenolics composition and antioxidant activity.

2. Materials and methods

2.1. Chemicals and reagents

DPPH (1,1-diphenyl-2-picrylhydrazyl radical), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'azinobis-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulfate, (−)-epicatechin, (+)-catechin, chlorogenic acid, phloridzin, isoquercitin, acetic acid, benzyl mercaptan (toluene α-thiol) and methanol were purchased from Sigma–Aldrich (Steinheim, Germany). Enzymes: β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidinase, were from Sigma Chemical Co. (Steinheim, Germany). Acetonitrile was from Merck (Darmstadt, Germany).

2.2. Plant material

Apple fruits of two varieties, Shampion and Idared, were collected at commercial maturity during the 2005 season in the experimental orchard of Agricultural University, situated near Wroclaw, Poland.

2.3. Preparation of apple purées

The scheme of technological processes is illustrated in Fig. 1. Apple fruits (whole, 1 kg of each variety) were washed, and cut manually with a knife into small irregular slices (1–2 cm thick) and immediately subjected to cooking.

Three variants of samples were prepared from Idared and Shampion varieties, one with ascorbic acid, added to the chopped fruits before cooking during 4 min at 90 °C, using a Thermomix (Vorwerk, Wuppertal, Germany). The second (control) sample was cooked under the same

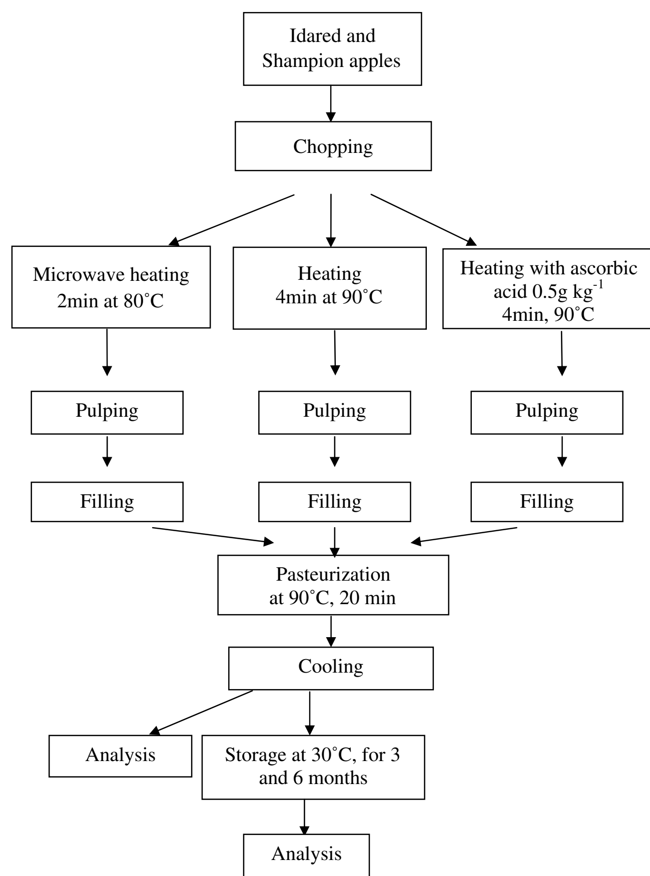

Fig. 1. Scheme of technological processes for obtaining purées from apple fruits.

conditions without ascorbic acid. The third variant was prepared by microwave heating of chopped apples during 2 min at 80 °C in a microwave oven (Amica, Wronki, Poland 2450 MHz).

Once the apples were cooked, they were passed through a laboratory pulper (Spomasz, Wroclaw, Poland) to remove coarse fibres, seeds, seed cells, stems and peel particles. Purées were filled immediately into 0.2 l jars and next heated at 90 °C for 20 min and cooled to 20 °C. Three replicates of apple purées preparation were carried out.

Oxidation of the purées during processing resulted in a significant decrease of polyphenols content; therefore the temperature and processing time were thoroughly controlled. After processing, the purées were subjected to analyses.

2.4. HPLC analysis of polyphenols

Before analysis, 20 g purées were extracted three times with 100 ml of 80% acidified (1% acetic acid) methanol and centrifuged at 15,000 rpm (20,878*g*). The analyses of flavan-3-ols, hydroxycinnamates, dihydrochalcones, and flavonol glycosides were carried out on a Merck-Hitachi L-7455 liquid chromatograph with a diode array detector (DAD) and quaternary pump L-7100 equipped with the D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). Separation was performed on a Synergi Fusion RP-80A 150 × 4.6 mm (4 μm) Phenomenex (Torrance, CA USA) column. Oven temperature was set to 30 °C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). The programme began with a linear gradient from 0% B to 36 min, 25% B, followed by washing and reconditioning of the column. The flow rate was 1.0 ml/min, and the runs were monitored at the following wavelengths: flavan-3-ols, dihydrochalcones at 280 nm, hydroxycinnamates at 320 nm, and flavonol glycosides at 360 nm. The calibration curves were made using chlorogenic acid, phloridzin, and isoquercitin as standards. Photo diode array (PAD) spectra were measured over the wavelength range 200–600 nm in steps of 2 nm. Retention times and spectra were compared with those of purée standards within the range 200–600 nm.

Additionally, an enzymatic hydrolysis of flavonol glycosides was performed. Apple purée was diluted with the citrate buffer solution at pH 5 and specific enzymes were added: β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidinase. The disappearance of single peaks in the chromatogram and formation of the corresponding aglycones was observed using HPLC after 1 h of incubation at 38 °C with a specific enzyme. Phenolics in the samples were determined by HPLC.

2.5. Procyanidins analysis by thiolysis

Direct thiolysis of freeze-dried purées was performed as described by Guyot, Marnet, Sanoner, and Drilleau (2001). Portions (0.5 g) of apple purées were precisely weighed in 1.5 ml Eppendorf vials and freeze-dried, then acidic methanol (3.3% HCl (v/v), 400 μl) and toluene α-thiol (5% in methanol, 800 μl) were added. The vials were closed and incubated at 40 °C for 30 min with agitation on a vortex every 10 min. Next, the vials were cooled in ice water and centrifuged immediately at 4 °C at 14,000 rpm (20,000*g*) during 10 min. Samples were stored at 4 °C prior to RP-HPLC analysis. All incubations were done in triplicate. Thiolysis products were separated on a Merck Purospher RP 18 end-capped column 250 × 4 mm, 5 μm (Merck, Darmstadt, Germany). The liquid chromatograph was a Waters (Milford, MA, USA) system equipped with DAD and scanning fluorescence detectors. The solvent A (aqueous acetic acid, 2.5% (v/v)) and solvent B (acetonitrile) were used as the following gradients: initial 3% B, 0–5 min, 9% B linear; 5–15 min, 16% B linear, and 15–45 min, 50% B linear, followed by washing and reconditioning of the column. Flow rate of 1 ml/min, and oven temperature of 30 °C were used. The compounds for which reference standards were available (synthesized or isolated previously), were identified on chromatograms according to their retention times and UV–vis spectra. Fluorescence was recorded at excitation wavelength of 278 nm and emission wavelength of 360 nm. Calibration curves were established using flavan-3-ol and

benzylthioether standards prepared in our laboratory. The average degree of polymerization (DP) was measured by calculating the molar ratio of all the flavan-3-ol units (thioether adducts + terminal units) to (−)-epicatechin and (+)-catechin. The calibration curves were made from (−)-epicatechin, (+)-catechin and procyanidin B2, C1, B1, as standards, using the method of Oszmianski and Bourzeix (1995).

2.6. Free radical-scavenging activity by the use of a stable ABTS radical cation

The free radical-scavenging activity was determined by ABTS radical cation decolourization assay (Re et al., 1999). ABTS was dissolved in water to a 7 μM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 μM potassium persulfate. For the study of infusion, the samples containing the $ABTS^{\cdot+}$ solution were diluted with redistilled water to an absorbance of 0.700(±0.02) at 734 nm and equilibrated at 30 °C. After addition of 3.0 ml of diluted $ABTS^{\cdot+}$ solution ($A_{734nm} = 0.700(\pm 0.02)$ to 30 μl of polyphenolic extracts, the absorbance reading was exactly 6 min after initial mixing (A_t). The results were corrected for dilution and expressed in μM trolox per 100 g dry weight (dw). All determinations were performed in triplicate.

2.7. DPPH radical-scavenging spectrophotometric assay

The DPPH radical-scavenging activity of purées was determined according to the method of Yen and Chen (1995). The centrifuged methanolic extract of purées (1 ml) was diluted with methanol. An aliquot (1 ml) of the diluted extract was added to 3 ml of absolute methanol and 1 ml of DPPH solution (0.012 g DPPH/100 ml of methanol). The mixture was shaken and left at room temperature for 10 min; the absorbance was measured at 517 nm, using a Shimadzu UV2401PC spectrophotometer (Kyoto, Japan). The reference cuvette contained all of the components except the radical, with a final volume of 1 ml. The results of the assay were expressed relative to trolox in terms of TEAC (trolox equivalent antioxidant capacity).

2.8. DPPH radical-scavenging EPR assay

EPR measurements were performed at ambient temperature (571 °C) on a Bruker ELEXSYS E 500 spectrometer (Bremen, Germany), operating in continuous wave mode at 9.4508 GHz; the SHQE – Super High Q cavity was employed. The DPPH radical-scavenging effect was determined as described by Hatano et al. (1989). The decay of DPPH signal was monitored and compared with the control sample; stock solution of the radical in methanol (4.0×10^{-3} mol/dm^3) was used. EPR spectra were recorded with the following spectrometer settings: receiver gain – 55 dB, power – 20.21 mW, centre field – 3368 G, sweep width – 200 G, sweep time – 40.96 s, time constant – 2.56 ms, modulation frequency – 100 kHz, modulation amplitude – 1 G. Apple juice (100 μl) was added to 0.5 ml DPPH solution, mixed, and a volume of 25 μl was transferred into the quartz EPR cell. DPPH radical-scavenging capacity of the juices was calculated according to the equation: scavenged DPPH (%) $= [(I_0^{\mathrm{I}} - I^{\mathrm{I}})/I_0^{\mathrm{I}}] \times 100$, where: I_0^{I} = integral intensity of DPPH signal for control sample; I^{I} = integral intensity measured after the addition of a scavenger. Signal intensity was registered as a function of magnetic induction and time. EPR spectra were recorded every 40 s. Non-linear regression analyses were carried out for $n = 30$ scans. The experiment was carried out in triplicate, and total errors (RSD) of integral intensity for I_0^{I} and I^{I} were calculated.

2.9. Colour measurement

The change of colour of apple purées was measured by a chromameter Colour Quest XE Hunter Lab (Reston, VA, USA) using the reflectance mode with Illuminanat D65 and 10° observer angle. Samples were filled in a 2 cm cell and measured against a white ceramic reference plate ($L^* = 97.43$; $a^* = -0.13$; $b^* = +1.68$), and colour parameters L^* (lightness or brightness: 0 = black, 100 = white), a^* ($-a^*$ = greenness, $+a^*$ = redness), and b^* ($-b^*$ = blueness, $+b^*$ = yellowness) and WL (dominant wavelength) values were recorded.

2.10. Statistical analyses for individual phenolics in apple samples

Statistical analysis was conducted using Statistica version 6.0 (StatSoft Poland). Tests for an association between two different variables were run using standard Pearson correlations. Multiple regression analyses were performed with scavenging activity as a dependent variable and up to 15 phenolic constituents of apple purée as the independent variables.

3. Results and discussion

The lightness (L^*), redness (a^*), and yellowness (b^*) values of apple purées, treated with and without ascorbic acid and microwave heated, are shown in Table 1.

The results showed that Shampion apple purée had higher L^* values than Idared. This pointed to a higher susceptibility of Idared apples to enzymatic browning than that of Shampion. The polyphenoloxidase activity (PPO) in Idared variety (1560 U/g) was 5.8 times higher than in Shampion (270 U/g) (Podsędek, Wilska-Jeszka, Anders, & Markowski, 2000).

The highest L^* value was obtained for Shampion sample with ascorbic acid addition, followed by microwave treated and third control. These results are in agreement with the values presented by Rababah, Ereifej, and Howard (2005) in that the addition of ascorbic acid prevents the

Table 1
Colour parameters of apple purées

Samples	L^*	a^*	b^*
1-Idared –control			
0 Months	47.99 ± 0.01f[A]	10.33 ± 0.01a	16.95 ± 0.14d
3 Months	47.88 ± 0.12e	7.59 ± 0.00a	16.90 ± 0.11d
6 Months	46.70 ± 0.01f	7.37 ± 0.04a	17.91 ± 0.15b
2-Shampion – control			
0 Months	58.92 ± 0.01c	2.10 ± 0.09f	20.16 ± 0.12b
3 Months	56.42 ± 0.15b	2.92 ± 0.11e	19.91 ± 0.14b
6 Months	55.22 ± 0.00b	3.09 ± 0.01e	17.83 ± 0.01b
3-Idared – microwave			
0 Months	56.74 ± 0.15d	5.19 ± 0.05c	14.27 ± 0.09e
3 Months	55.60 ± 0.21c	5.03 ± 0.15b	16.97 ± 0.12d
6 Months	52.41 ± 0.00d	5.00 ± 0.12c	16.96 ± 0.00e
4-Shampion – microwave			
0 Months	59.37 ± 0.11b	3.31 ± 0.02d	18.15 ± 0.01c
3 Months	56.52 ± 0.01b	4.61 ± 0.00c	18.11 ± 0.14c
6 Months	54.23 ± 0.31c	5.44 ± 0.09b	17.50 ± 0.16c
5-Idared + vitamin C			
0 Months	52.84 ± 0.11e	6.01 ± 0.12b	11.17 ± 0.01f
3 Months	52.18 ± 0.01d	4.29 ± 0.01d	16.88 ± 0.09d
6 Months	51.69 ± 0.05e	4.18 ± 0.00d	17.20 ± 0.06d
6-Shampion + vitamin C			
0 Months	60.98 ± 0.06a	2.74 ± 0.11e	20.74 ± 0.01a
3 Months	59.36 ± 0.04a	1.67 ± 0.19f	21.58 ± 0.05a
6 Months	58.22 ± 0.09a	1.04 ± 0.01f	22.19 ± 0.45a

Mean values within a column of the same colour parameters and same time of storage marked by different letters are significantly different at $P < 0.05$.
[A] Values are means ± standard deviation, $n = 3$.

decrease of lightness L^* value in apple purées. Idared purée prepared with ascorbic acid had a lower L^* value than had that prepared by microwave heating, which may be the effect of protection by ascorbic acid, whereas some natural colourants produce a darker colour. The purée colour showed a moderate degradation with time, as indicated by a slight reduction of L^* values for all the samples during 3 and 6 months of storage at 30 °C. The kinetics of sample browning showed that ascorbic acid was the most effective inhibitor of nonenzymatic browning, better than the treatment with microwaves. Parpinello, Chinnici, Versari, and Riponi (2002) showed that ascorbic acid decreased the nonenzymatic browning of fruit purées to a larger extent than did any other chemical treatment used in his study.

The redness (a^*), and yellowness (b^*) were significantly different in Idared and Shampion samples. This variation could be due to the nature of the pigments in those two varieties. The higher a^* values of Idared purée (10.33 for control) than in Shampion (2.10) could be attributed to the presence of anthocyanins in the skin of Idared, and browning reaction usually involved high PPO enzyme activity. The colour values indicated that the addition of 0.1% ascorbic acid and microwave heating significantly decreased the redness (a^*) and yellowness (b^*) of Idared purées. This result was ascribed to the inhibition activity of oxidative enzymes present in fruits, and emphasized the importance of ascorbic acid and heat treatment to control the enzymatic browning reaction which occurred during fruit processing. Corresponding results for Shampion samples showed smaller differences between the control samples, and those prepared with ascorbic acid, and the microwave heated ones. The results supported the opinion that Shampion variety apples are a good raw material for apple purée production, characterized by the bright colour that remains stable, even without any protection.

The profiles and distribution of the polyphenol constituents of apple purées under study are presented in Tables 2 and 3. There are five major groups of polyphenolic compounds in apple, including hydroxycinnamic acids, flavan-3-ols, anthocyanidins, flavonols and dihydrochalcones. Differences were observed in the polyphenol content of Idared purées as compared with that of Shampion purées. The greatest variation occurred in the flavan-3-ols (catechins and proanthocyanidins) content of Idared and Shampion purées, i.e. in control samples (from 229 mg/100 g to 1024 mg/kg, respectively). In Shampion purées, the (−)-epicatechin and procyanidin B2 contents were higher than that of chlorogenic acid; the samples of Idared had the highest content of the chlorogenic acid. Similar results for Shampion and Idared apples were reported by Podsędek et al. (2000).

According to our measurements, the concentrations of phenolic compounds in the purées are related to their contents in raw materials but also resulted from the chemical and enzymatic oxidation reactions which occur during preparation. The use of microwave heating and vitamin C addition had a significantly different (from control and other samples ($p < 0.05$) influence on phenolic compounds, especially in Idared purée. Chlorogenic acid concentration increased 2–3 times after the microwave heating of Idared apple puree, in comparison with control samples. Microwave energy has the advantage of heating solids rapidly and uniformly, thus inactivating the enzymes more quickly; it minimizes phenolic oxidation. Chlorogenic acid is one of the most vulnerable compounds for oxidation by PPO. This result confirmed that Idared apples have a very active PPO enzyme and phenolic compounds must be protected during purée preparation. The addition of ascorbic acid and microwave heating significantly increased not only chlorogenic acid concentrations, but also polymeric procyanidin concentration from 145 mg/kg to 404 mg/kg and 620 mg/kg, respectively. Lower chlorogenic acid content, as well as PPO activity, in Shampion apples, indicated that it is a better variety than Idared for the production of light-coloured purées. Microwave heating, used for Shampion purée preparation, gave a significant ($p < 0.05$) increase in concentration of polymeric procyanidin, phloretin-2′-glucoside and quercetin glycosides. The phloretin and quercetin derivatives are abundant in apple seeds and skin, respectively. The use of entire apples and microwave heating contributed to the high content of these compounds in purées. The contents of dihydrochalcone and flavonols, in all the analyzed samples, were relatively low. They belong

Table 2
Contents of phenolic compounds (in mg/kg) in apple purées

Samples	Chlorogenic acid	p-CQA[A]	(+)-Catechin	(−)-Epicatechin	Procyanidin B2	Procyanidin B1	Procyanidin C1	Polymeric procyanidin	DP
1-Idared – control									
0 Months	87.8 ± 0.51c*	8.4 ± 0.21c	8.3 ± 0.32d	28.8 ± 0.12f	28.5 ± 0.21f	5.6 ± 0.05d	12.4 ± 0.14f	145 ± 0.99f	4.2d
3 Months	77.5 ± 0.23c	5.2 ± 0.35d	7.2 ± 0.21c	12.9 ± 0.21f	14.6 ± 0.14e	3.5 ± 0.11e	6.5 ± 0.06e	104 ± 0.67f	4.9e
6 Months	71.5 ± 0.41c	5.1 ± 0.45bc	1.9 ± 0.01d	9.7 ± 0.33f	12.3 ± 0.14e	2.7 ± 0.00b	2.9 ± 0.00d	83.5 ± 0.21e	5.7f
2-Shampion – control									
0 Months	77.1 ± 0.65d	15.1 ± 0.45a	14.0 ± 0.14b	143 ± 0.12a	172.6 ± 041a	25.7 ± .45a	84.1 ± 0.45a	584 ± 0.78d	5.6a
3 Months	65.8 ± 0.45e	11.5 ± 0.75a	13.2 ± 0.31a	89.9 ± 0.45b	116.1 ± 054b	15.3 ± 0.31b	31.4 ± 0.34c	566 ± 0.46b	5.0b
6 Months	55.6 ± 0.32e	6.5 ± 0.12b	4.9 ± 0.09c	64.4 ± 0.21c	32.9 ± 0.47c	10.6 ± 0.20a	12.5 ± 0.45b	452 ± 0.53b	9.8a
3-Idared – microwave									
0 Months	200 ± 01.21a	10.2 ± 0.11bc	13.1 ± 0.04b	56.0 ± 0.11d	83.6 ± 0.32d	20.5 ± 0.45b	27.7 ± 0.26d	620 ± 0.42c	5.6a
3 Months	193 ± 0.11a	8.4 ± 0.17bc	13.1 ± 0.17a	45.6 ± 0.32d	63.3 ± 0.45d	10.8 ± 0.14d	10.5 ± 0.21d	527 ± 1.31d	6.4a
6 Months	152 ± 0.21a	4.0 ± 0.011c	10.9 ± 0.12a	42.9 ± 0.54d	42.6 ± 0.57b	4.1 ± 0.04b	8.1 ± 0.18c	314 ± 0.54c	8.0c
4-Shampion – microwave									
0 Months	83.8 ± 1.65d	15.4 ± 0.56a	13.3 ± 0.01b	138 ± 0.45b	167 ± 0.41b	20.3 ± 0.24b	80.6 ± 0.42b	746 ± 1.47a	4.4c
3 Months	69.1 ± 0.71d	11.5 ± 0.04a	11.8 ± 0.31a	78.9 ± 0.71c	105 ± 1.11c	17.6 ± 0.42a	41.2 ± 0.47b	557 ± 0.61c	4.5d
6 Months	68.0 ± 0.02d	9.0 ± 0.00a	9.1 ± 0.11b	67.2 ± 0.45b	99.4 ± 0.54a	10.2 ± 0.31a	21.3 ± 0.12a	454 ± 0.41b	7.3d
5-Idared + vitamin C									
0 Months	179 ± 0.78b	8.5 ± 0.14c	27.3 ± 0.21a	49.3 ± 0.24e	63.1 ± 0.64e	15.3 ± 0.14c	16.8 ± 0.31e	404 ± 1.40e	3.8e
3 Months	162 ± 0.78b	6.7 ± 0.21cd	6.7 ± 0.14c	24.5 ± 0.31e	36.2 ± 0.11e	4.4 ± 0.01e	9.9 ± 0.01d	329 ± 0.34e	4.6d
6 Months	132 ± 0.12b	4.7 ± 0.11bc	1.2 ± 0.09d	21.8 ± 0.14e	6.9 ± 0.45f	3.9 ± ± 0.09b	2.7 ± 0.14d	253 ± 0.52d	8.7b
6-Shampion + vitamin C									
0 Months	63.0 ± 0.01e	11.5 ± 0.45b	10.8 ± 0.04c	133 ± 0.87c	147 ± 0.34c	16.9 ± 0.05c	72.6 ± 0.12c	675 ± 0.88b	5.1b
3 Months	59.8 ± 0.09f	9.9 ± 0.14ab	9.1 ± 0.06b	102 ± 0.25a	128 ± 0.75a	13.1 ± 0.11c	49.4 ± 0.42a	638 ± 1.29a	4.8c
6 Months	49.0 ± 0.45f	5.9 ± 0.22f	2.9 ± 0.11d	99.0 ± 0.03a	29.0 ± 0.04d	10.3 ± 0.14a	8.5 ± 0.37d	497 ± 0.75a	6.8e

[A] p-Coumaroylquinic acid.
* Values are means ± standard deviation, $n = 3$; mean values within a column of same phenolics and same time of storage marked by different letters are significantly different at $P < 0.05$.

to a class of minor apple phenolics (Sanoner et al., 1999). The processes of Idared purée preparation had a weaker effect on those compounds than on the contents of chlorogenic acid or flavonols. They are more stable and resistant to PPO oxidation.

During storage at 30 °C, significant changes were observed in the concentrations of procyanidins and cyanidin-3-galactoside of all the apple purées (Table 3). After 6 months of storage, cyanidin-3-galactoside was not detected in any samples. Anthocyanins are very sensitive to heat degradation (Garcia-Viguera et al., 1999). The procyanidins were more stable than were anthocyanins during purée storage. After 6 months of degradation at 30 °C, polymeric procyanidins in Idared ranged from 37.3% (Idared – vitamin C) to 49.5% (Idared – microwaved) and in Shampion from 22.6% (Shampion – control) to 39.1% (Shampion – microwaved), comparing to the initial values (100% at 0 month). These results indicated that use of the Shampion apple variety and the addition of vitamin C protected procyanidin polymers, whereas microwave heating increased their degradation. An increasing degree of procyanidin polymerization (DP) was observed during purée storage. Procyanidins in apple purées are better protected than are those in apple juice concentrate. Bengoechea et al. (1997) detected procyanidins in purées, but not in concentrates. During manufacturing of apple juice concentrate, they could polymerize to form insoluble forms and precipitate out, yielding lees or sediment. The procyanidins in purées are protected because of their ability to bind with cell-wall polysaccharides; they were determined in different tissue zones (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998). This feature may also affect their reactivity toward DPPH radical and can determine antioxidant capacity. Other apple phenolics, such as phenolic acids, dihydrochalcones and flavonols, were much more stable during purée storage than were flavan-3-ols, hydroxycinnamic acids and anthocyanins.

Spanos et al. (1990) reported that apple juice stored for 9 months at 25 °C, of concentrates, showed 36% degradation of hydroxycinnamic acids, 60% degradation of quercetin and phloretin glycosides, and total loss of procyanidins. After 6 months of storage at 30 °C, the apple purées had a maximal 26% degradation of chlorogenic acid and 18% degradation of phloretin-2′-glucoside.

Total antioxidant capacity, assessed by the UV–vis method, as measured by DPPH and ABTS free radical-scavenging, ranged from 0.16 to 0.81 and from 0.54 to 1.80 mg/g of purée, of trolox equivalent, respectively (Table 4). The highest level of antioxidant activity was observed in Shampion + vitamin C (0.81 and 1.80 mg/g purée of trolox equivalent for DPPH and ABTS, respectively). The antioxidant activities of Idared apple purées were less than half those of Shampion purées for all the samples, irrespective of the preparation method. The

Table 3
Contents of phenolic compounds (in mg/kg) in apple purées

Samples	Phloretin-2′-*O*-xyloglucoside	Phloretin-2′-*O*-glucoside	Quercetin-3-*O*-rutinoside	Quercetin-3-*O*-galactoside	Quercetin-3-*O*-glucoside	Quercetin-3-*O*-xyloside	Quercetin-3-*O*-arabinoside	Quercetin-3-*O*-rhamnoside	Cyanidin-3-galactoside
1-Idared – control									
0 Months	2.3 ± 0.01e[A]	59.1 ± 0.41b	0.4 ± 0.01b	12.5 ± 0.01c	2.1 ± 0.09d	5.0 ± 0.05c	11.9 ± 0.41c	4.6 ± 0.10d	8.1 ± 0.14a
3 Months	2.1 ± 0.012e	52.7 ± 0.36b	0.0 ± 0.00c	12.0 ± 0.11c	1.6 ± 0.12d	4.7 ± 0.13c	5.8 ± 0.02d	4.0 ± 0.11d	3.8 ± 0.12a
6 Months	2.0 ± 0.03f	48.4 ± 0.42c	0.0 ± 0.00c	10.4 ± 0.19c	1.3 ± 0.00e	3.3 ± 0.41d	3.6 ± 0.11d	2.7 ± 0.05e	0.0 ± 0.00e
2-Shampion – control									
0 Months	25.2 ± 0.21a	28.7 ± 0.24f	0.0 ± 0.00c	11.5 ± 0.21d	3.2 ± 0.14c	4.9 ± 0.22c	11.1 ± 0.42d	7.7 ± 0.13c	1.6 ± 0.08b
3 Months	22.5 ± 0.45a	27.9 ± 0.37f	0.0 ± 0.00c	11.5 ± 0.34d	3.1 ± 0.09c	3.5 ± 0.24d	8.4 ± 0.23c	4.8 ± 0.01c	0.0 ± 0.00e
6 Months	22.3 ± 0.31a	27.0 ± 0.24f	0.0 ± 0.00c	9.5 ± 0.14d	2.4 ± 0.16c	3.1 ± 0.12d	6.2 ± 0.17c	3.3 ± 0.14c	0.0 ± 0.00e
3-Idared – microwave									
0 Months	5.7 ± 0.01c	51.5 ± 0.46c	1.5 ± 0.01a	9.0 ± 0.12e	1.7 ± 0.08e	4.4 ± 0.08d	9.5 ± 0.23e	3.4 ± 0.06e	1.8 ± 0.04b
3 Months	4.9 ± 0.11c	51.5 ± 0.57c	0.0 ± 0.00c	9.0 ± 0.11e	1.7 ± 0.04d	3.6 ± 0.04d	4.2 ± 0.13e	3.2 ± 0.20e	0.2 ± 0.00d
6 Months	3.5 ± 0.04d	49.3 ± 0.24b	0.0 ± 0.00c	8.5 ± 0.05e	1.6 ± 0.01d	3.1 ± 0.11e	3.0 ± 0.05e	2.5 ± 0.18d	0.0 ± 0.00e
4-Shampion – microwave									
0 Months	25.0 ± 0.54a	36.1 ± 0.18e	0.0 ± 0.00c	22.2 ± 0.51a	7.2 ± 0.21a	18.1 ± 0.10a	18.1 ± 0.25a	11.4 ± 0.03a	1.3 ± 0.00b
3 Months	21.8 ± 0.12b	31.9 ± 0.20e	0.0 ± 0.00c	21.2 ± 0.21a	5.6 ± 0.43a	12.5 ± 0.34a	11.5 ± 0.41a	6.7 ± 0.14a	0.0 ± 0.00e
6 months	20.9 ± 0.24c	30.4 ± 0.45e	0.0 ± 0.00c	20.5 ± 0.34a	5.1 ± 0.33a	6.4 ± 0.20a	10.3 ± 0.22a	5.9 ± 0.22a	0.0 ± 0.00e
5-Idared + vitamin C									
0 Months	4.5 ± 0.11d	62.1 ± 0.42a	0.4 ± 0.00b	6.4 ± 0.04f	1.6 ± 0.07e	4.0 ± 0.11d	9.6 ± 0.12e	3.4 ± 0.11e	1.7 ± 0.14b
3 Months	3.4 ± 0.19d	55.8 ± 0.70a	0.0 ± 0.00c	5.5 ± 0.11f	1.2 ± 0.11e	3.1 ± 0.16e	3.8 ± 0.34f	3.3 ± 0.14e	0.3 ± 0.02d
6 Months	2.7 ± 0.07e	54.0 ± 0.15a	0.0 ± 0.00c	4.9 ± 0.17f	1.0 ± 0.04f	2.8 ± 0.08f	2.8 ± 0.08f	2.7 ± 0.06d	0.0 ± 0.00e
6-Shampion + vitamin C									
0 Months	22.7 ± 0.31b	37.5 ± 0.11d	0.0 ± 0.00c	13.9 ± 0.02b	4.1 ± 0.23b	6.1 ± 0.24b	13.8 ± 0.14b	8.7 ± 0.22b	1.2 ± 0.08b
3 Months	21.7 ± 0.29	36.1 ± 0.63d	0.0 ± 0.00c	12.7 ± 0.41b	3.6 ± 0.17b	5.5 ± 0.02b	9.6 ± 0.02b	5.8 ± 0.29b	0.0 ± 0.00e
6 Months	21.2 ± 0.37b	33.9 ± 0.24d	0.0 ± 0.00c	12.2 ± 0.31b	3.4 ± 0.16b	5.3 ± 0.16b	8.1 ± 0.10b	5.2 ± 0.04b	0.0 ± 0.00e

Mean values within a column of same phenolics and same time of storage marked by different letters are significantly different at $P < 0.05$.
[A] Values are means ± standard deviation, $n = 3$.

Table 4
Antioxidant activity and capacity measured by EPR and UV–vis spectroscopy expressed by trolox equivalent (μM trolox/100 ml)

Months	EPR measurements (μM trolox/100 ml)			UV–vis measurements (absorbance)	
	After 3 min	After 30 min	k_{obs} [s^{-1}]	DPPH[A]	ABTS[A]
1-Idared – control					
0 Months	0.116 ± 0.006	0.1815 ± 0.0038	1.45 ± 0.03	0.16 ± 0.01d[A]	0.54 ± 0.00d
3 Months	0.087 ± 0.006	0.1323 ± 0.0026	1.84 ± 0.05	0.10 ± 0.02f	0.34 ± 0.03e
6 Months	0.072 ± 0.003	0.1070 ± 0.0015	1.85 ± .0.02	0.07 ± 0.01e	0.20 ± 0.04f
2-Shampion – control					
0 Months	0.309 ± 0.012	0.7701 ± 0.0097	9.51 ± 0.13	0.40 ± 0.02b	1.27 ± 0.01c
3 Months	0.209 ± 0.017	0.5626 ± 0.0069	9.12 ± 0.11	0.30 ± 0.03c	1.18 ± 0.01b
6 Months	0.178 ± 0.008	0.4633 ± 0.0043	8.12 ± 0.09	0.12 ± 0.04bc	0.50 ± 0.01b
3-Idared – microwave					
0 Months	0.233 ± 0.015	0.6274 ± 0.0084	7.54 ± 0.08	0.20 ± 0.01c	0.54 ± 0.01d
3 Months	0.199 ± 0.013	0.5328 ± 0.0051	5.74 ± 0.07	0.19 ± 0.01d	0.48 ± 0.08c
6 Months	0.150 ± 0.007	0.3349 ± 0.0029	4.09 ± 0.06	0.10 ± 0.01d	0.38 ± 0.06e
4-Shampion – microwave					
0 Months	0.276 ± 0.013	0.6226 ± 0.0078	8.46 ± 0.07	0.41 ± 0.01b	1.50 ± 0.15b
3 Months	0.204 ± 0.011	0.5667 ± 0.0054	6.52 ± 0.06	0.36 ± 0.03b	1.19 ± 0.13b
6 Months	0.133 ± 0.009	0.4364 ± 0.0063	4.65 ± 0.07	0.13 ± 0.070b	0.47 ± 0.01c
5-Idared + vitamin C					
0 Months	0.198 ± 0.011	0.4343 ± 0.0034	8.15 ± 0.09	0.17 ± 0.01d	0.54 ± 0.04d
3 Months	0.147 ± 0.008	0.3454 ± 0.0047	5.98 ± 0.04	0.14 ± 0.02e	0.43 ± 0.02d
6 Months	0.110 ± 0.006	0.2642 ± 0.0047	5.05 ± 0.05	0.11 ± 0.07cd	0.42 ± 0.00d
6-Shampion + vitamin C					
0 Months	0.261 ± 0.017	0.6864 ± 0.0077	11.48 ± 0.17	0.81 ± 0.01a	1.80 ± 0.15a
3 Months	0.225 ± 0.009	0.6368 ± 0.0066	8.64 ± 0.09	0.50 ± 0.06a	1.44 ± 0.01a
6 Months	0.148 ± 0.005	0.4812 ± 0.0054	7.64 ± 0.08	0.16 ± 0.00a	0.57 ± 0.01a

Mean values within a column of the same free radical scavenging and same time of storage marked by different letters are significantly different at $P < 0.05$.
[A] Values are means ± standard deviation, $n = 3$.

results of antioxidant properties are in reasonable agreement with the content of phenolic compound in both varieties. There have been many studies showing correlation between polyphenolic profile and antioxidant activity (Loots, van der Westhuizen, & Jerling, 2006; Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005). Shahidi and Naczyk (2004) reported that the differences in antioxidant activities of fruits could be due to their different profiles of phenolic acids, flavonoid compounds and their derivatives. For instance, antioxidant activities of flavonoids depend on the number of hydroxyl groups in their molecules. A high concentration of procyanidins, rich in hydroxyl groups, in Shampion apple purées, contributed to a higher antioxidant activity, in comparison with Idared apple purées. The purée of Idared apple mixed with 0.1% ascorbic acid has an antioxidant activity similar to that without ascorbic acid. Rababah et al. (2005) recently reported that the addition of 0.1% ascorbic acid had no effect on the levels of antioxidants in apple purée. However, for the purée prepared from Shampion apples with and without ascorbic acid, there were statistically significant differences (significance level 0.05) between those two groups (Table 4). This could be considered as the protective effect of ascorbic acid (at a concentration of 103 mg/kg).

Principal component analysis (PCA) was performed on mean values of 18 samples and 6 variables and visualized by means of the PCA-biplot (Fig. 2). The closer the corresponding samples between the methods of analysis lay on the plot, the more similarly did the methods describe the samples. Respectively, the further the samples lay from each other, the more different they were with respect to the measured parameters. The difference between effects of the purée preparation methods explained 79.2% of the total variation in principal components. Samples of Shampion purées had higher proanthocyanidin, *p*-coumaric quinic acid, quercetin derivative contents and lightness than had Idared, which had higher chlorogenic acid and phloretin derivative contents. To achieve a higher phenolic content, apple purées should be produced with ascorbic acid addition and heated in a microwave oven. Principal component 2 explained 23.6% of the total variation, expressing the differences between the cultivars, with the some preference for Shampion which had a higher phenolic content than had Idared.

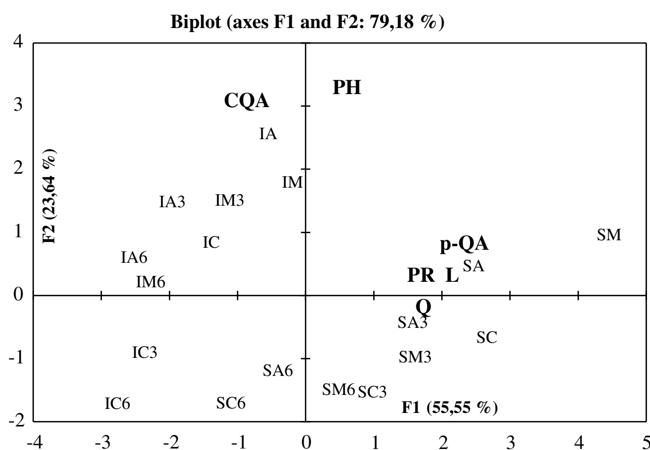

Fig. 2. Principal component analysis of apple purées prepared from two cultivars (I – Idared; S – Shampion) with ascorbic acid (A), microwave (M) and control (C) stored for 3 (3) and 6 (6) months at 30 °C. The measured variables (phenolics: **PH** = phloretin derivatives; **PR** = proanthocyanidins; **CQA** = chlorogenic acid; **p-QA** = *p*-coumaric quinic acid; **Q** = quercetin derivatives; colour parameter – **L** = lightness) are shown on the same plot.

The freshly produced purées had much higher phenolic contents than those after 3 (3) and 6 (6) months of storage 30 °C

All the purées were also examined for radical-scavenging capacity with the use of electron paramagnetic spectroscopy (EPR). The complexity of the chemical profile, and variations caused by growth period, genetic variations and processing methods, predispose to significant changes in antioxidant activity. The estimation of antioxidant capacity of purée samples was performed after 3 and 30 min after the addition of the antioxidant to the DPPH solution. As presented in Table 4, the amount of scavenged DPPH assessed by spectrophotometric measurements for apple purées was lower than that obtained using EPR spectroscopy. This may be due to the lack of background corrections in the UV–vis method. The EPR signal intensity was measured after 3 min and the reaction was allowed to proceed to the end. The first-order kinetic equation was fitted using non-linear regression analysis (carried out for $n = 30$): $I(t) = a + b^{*}\exp(-k_{obs}{}^{*}t)$, where: $I(t)$ = EPR signal intensity as a function of time; a, b = regression parameters; k_{obs} = pseudo first-order rate constant, t = time. The calculated pseudo first-order rate constants, together with total capacity (recalculated from $I(t)$ when $t \to \infty$), are given in Table 4. Different kinetics have suggested the presence of different radical-scavenging mechanisms by polyphenolic compounds (Bondet, Brand-Williams, & Berset, 1997). Polymeric products (e.g. procyanidins and larger condensed catechins) present in the reaction mixture (apple purée) may undergo various radical-scavenging reactions. Additionally, it is notable that the amount of DPPH scavenged by the same amount of apple purée is higher when assessed by EPR spectroscopy than by UV–vis measurements. The data obtained with the use of the ABTS radical are almost three times higher than those for DPPH, this is somewhat surprising, but can be linked with high cloudiness of the purées. As frequently found, radical scavenging activity was proportional to the content of polyphenols in the sample (Atoui, Mansouri, Boskou, & Kefalas, 2005). The relationships are usually linear, unless antioxidant synergism or copigmentation takes place, or measurement discrepancies appear. However, the sample for UV–vis measurements should be transparent, as in our measurements. Therefore, radical scavenging capacity was estimated by electron paramagnetic resonance (EPR) spectroscopy, which seems to be the method of choice in the case of cloudy samples of purées or fruit extracts. It is also notable that the higher

content of procyanidins was not reflected by the amount of scavenged DPPH measured by UV–vis spectrometry (Fig. 3a). Although the content of polyphenols was increased, the amount of scavenged DPPH remained unchanged until the phenolic content reached 100 mg/100 g. Using electron paramagnetic resonance (EPR) spectroscopy and estimating the percentage of scavenged DPPH after 3 min, it became evident that this method is much better for assessment of antiradical activity of plant materials. A linear relationship was obtained (Fig. 3b), whereas the data illustrated in Fig. 3a became scarce, especially at higher concentration of polyphenols.

It seemed interesting to examine the composition of apple purée in detail and to identify the most active radical-scavengers amongst its components. Using the HPLC method, we were able to identify 15 individual phenolic constituents, including 2 phenolic acids. The polyphenolic profile, however, is much more complicated because of polymerization of flavan-3-ols and glycosylation of other flavonoids. HPLC measurements also allowed a comparison of the impact of vitamin C on antioxidant activity during storage of apple purée. Different preparation methods and storage times were used, yielding a sufficient number of variables for multiple regression analysis.

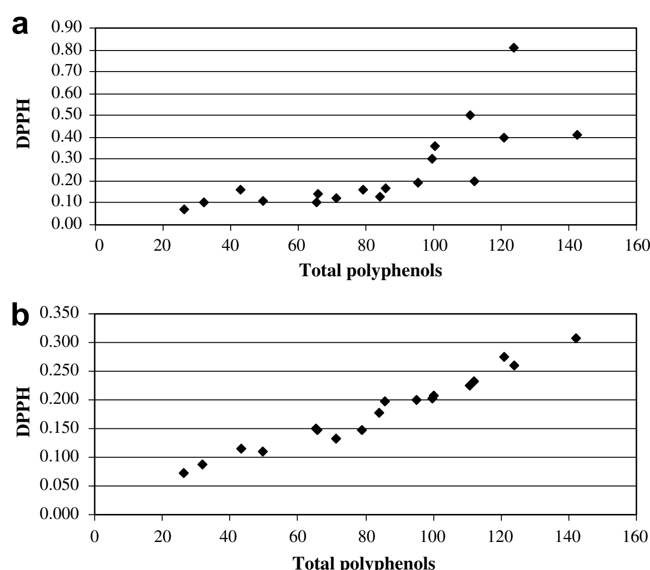

Fig. 3. Radical-scavenging activity (fraction scavenged) measured by UV–vis (a) and EPR spectroscopy (b) vs. total phenolics content (in mg/kg) in apple purée.

Using 15 predictors in a linear model, antioxidant capacity may be predicted more reliably. The linear model relating compositional data to antioxidant capacity can be written as (Soleas, Tomlinson, Diamandis, & Goldberg, 1997):

$$A = b_0 + b_1X_1 + b_2X_2 + \ldots + b_{16}X_{16}$$

where A is EPR signal intensity measured after 3 min or obtained from regression curve as $I(t)$ when $t \to \infty$ (Table 4), X are measurements of phenolic content and b are coefficients describing the contribution of the respective phenolics to A. If a coefficient for a given phenolic compound is statistically significantly different from 0, then that component is deemed to make a contribution to A.

As already mentioned, it was interesting to estimate the influence of particular compounds on the total capacity and radical-scavenging activity. According to the previously described model, the analysis was performed for all apple purées, and the results are summarized in Table 5.

Inspection of data in Table 5. shows that only a few components have a significant influence on A. Among the most active compounds are: chlorogenic acid, (−)-epicatechin and procyanidin B_2. Although polymerized procyanidins occur in the highest concentration, their impact on A was not of the highest (b reached 0.252), suggesting a scavenging mechanism different from other compounds. This was an important feature of the antioxidant activity of

Table 5
Multiple regression analysis relating individual apple purée phenolics to antioxidant activity measured after 3 min and 30 min

	After 3 min				After 30 min			
	Coefficient	SE	*t*	*P*	Coefficient	SE	*t*	*P*
Intercept	−0.013	0.026	−1.53	0.266	−0.007	0.015	−1.53	0.266
Chlorogenic acid	*0.271*	0.063	12.90	*0.006*	0.052	0.012	12.90	0.006
4′-*p*-Coumaroylquinic acid	0.022	0.027	2.49	0.130	0.004	0.005	2.49	0.130
(+)-Catechin	0.018	0.017	3.14	0.088	0.003	0.003	3.14	0.088
(−)-Epicatechin	*0.186*	0.103	5.43	*0.032*	0.036	0.020	5.43	0.032
Procyanidins B2	*0.322*	0.041	23.83	*0.002*	0.062	0.008	23.83	0.002
Procyanidins B1	0.074	0.069	3.22	0.084	0.014	0.013	3.22	0.084
Procyanidins C1	*0.105*	0.065	4.82	*0.040*	0.020	0.012	4.82	0.040
Polymeric procyanidins	*0.252*	0.045	16.90	*0.003*	*0.870*	0.009	304.46	*<0.001*
Phloretin-2′-xyloglucose	0.114	0.102	3.34	0.079	0.022	0.020	3.34	0.079
Phloridzin	0.106	0.097	3.25	0.083	0.020	0.019	3.25	0.083
Quercetin-3-*O*-galactose	0.062	0.013	14.16	0.005	0.012	0.003	14.16	0.005
Quercetin-3-*O*-glucose	−0.040	0.049	−2.48	0.131	−0.008	0.009	−2.48	0.131
Quercetin-3-O-xylose	0.017	0.014	3.47	0.074	0.003	0.003	3.47	0.074
Quercetin-3-*O*-arabinose	0.034	0.044	2.35	0.143	0.007	0.008	2.35	0.143
Quercetin-3-*O*-rhamnose	0.058	0.072	2.42	0.137	0.011	0.014	2.42	0.137

apple preparations. The radical-scavenging reaction was followed further, and total capacity was estimated from the regression curve fitted to pseudo first-order kinetics (the value of the function at infinity was treated as total capacity). The obtained values were used for prediction of A and the results are summarized in Table 5. Following this approach, the greatest influence on A is exerted mainly by polymerized procyanidins, as indicated by the value of b (up to 0.87).

The other compounds are of minor importance, and only a few of them are statistically important. Summing up, the model for assessment of radical-scavenging activity may vary between the first stage of the reaction, and later stages. When the measurement is performed after 3 min, antioxidant activity depends upon the concentrations of six phenolic compounds, namely: chlorogenic acid, (−)-epicatechin, procyanidins B2 and C1, quercetin galactoside and polymeric procyanidins. The model can be written as:

$$A = -0.013 + 0.271 * b_1 + 0.186 * b_2 + 0.322 * b_3 + 0.105 * b_4 + 0.252 * b_5 + 0.062 * b_6$$

where b_n denotes the concentration of the above-mentioned compounds. Considering the progress of the reaction after a prolonged time (the values $I(t)$, when $t \to \infty$), the influence of these compounds on antioxidant capacity appears to be different. Then the model can be better reproduced as:

$$A = -0.007 + 0.052 * b_1 + 0.036 * b_2 + 0.062 * b_3 + 0.020 * b_4 + 0.870 * b_5 + 0.012 * b_6$$

This clearly shows that the influence of non-polymeric compounds decreases and the polymerized procyanidins are mainly responsible for antioxidant capacity when the measurements are done after 10 min or later.

The results of measurements over time suggested different mechanisms of DPPH radical-scavenging. At the beginning, the reaction proceeds according to second-order kinetics and is very fast (the first points match the second-order kinetic equation, as illustrated in Fig. 4). It is probable that the effective, low molecular weight antioxidants participate in the first stage of the reaction with the DPPH radical. When the measurements after 3 min are subjected to statistical analysis, the low molecular antioxidants (chlorogenic acid, (−)-epicatechin, phloridzin) mainly contribute to antioxidant capacity. After ca. 3 min the reaction slows down and follows pseudo-first-order kinetics. Other, less active scavengers as well as the secondary reaction products, slowly decrease the amount of DPPH. The rate of depletion of DPPH is mainly determined by the amount of polymerized, high-molecular weight antioxidants.

This explanation can be confirmed by calculating the pseudo first-order rate constants presented in Table 4. The correlation of k_{obs} with the content of polymerized procyanidins in the samples shows a linear trend (Fig. 5). Thus, in order to characterize the samples containing a high content of polymerized polyphenolic compounds, measurements over time are necessary. EPR seems to be superior to UV–vis spectroscopy because it can record free radicals directly, even in cloudy, dense, or coloured products obtained from apples. Additionally, the influence of storage time and additives on antioxidant activity and phenolic profile can be easily assessed, which is extremely important during processing of apples.

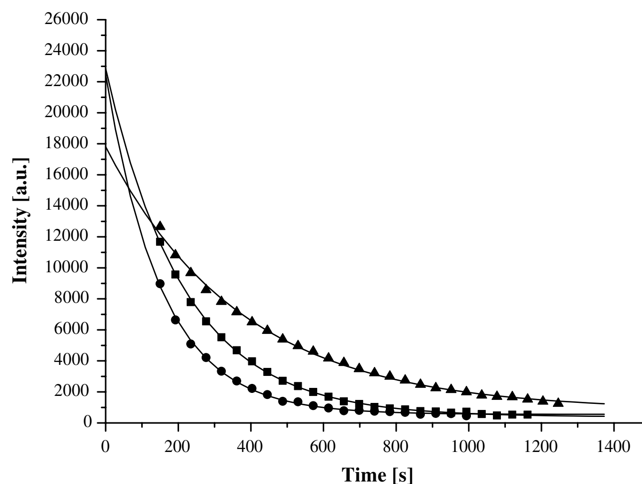

Fig. 4. A decay of EPR signal intensity after the addition of apple purée after 0 (circles), 3 (squares) and 6 (triangles) months of storage; the reactivity reflects the decreasing content of polymerized procyanidins.

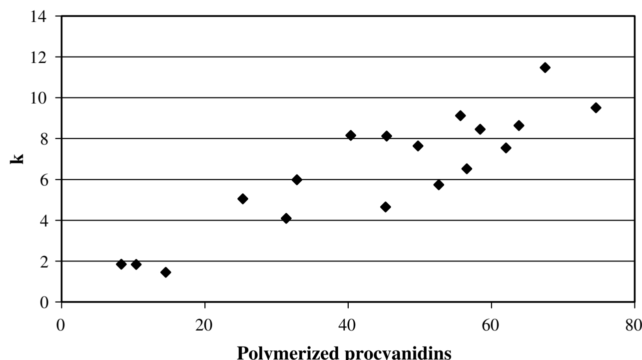

Fig. 5. Pseudo first-order rate constant k (1/s) vs. the content of polymerized procyanidins (mg/100 g).

4. Conclusions

The contents of total phenolics (especially polymerized procyanidins) were different, depending on the storage time and method of purée preparation. Thus, the radical-scavenging activity of apple purées differed significantly. This was proved by EPR spectroscopy, which appeared to be a much better method for testing purée antioxidant capacity than was UV–vis assay. In the spectrophotometric measurements, the DPPH radical-scav-

enging activity of purées was almost the same (until total polyphenol content reached 100 mg/ml), whereas in EPR assay, the values of antioxidant activity increased linearly with higher contents of total polyphenols. The HPLC technique (thiolysis method) enabled the determination of polymeric procyanidins content, which proved to be well correlated with pseudo first-order rate constant. Apple purees are a rich source of different polyphenols. Unfortunately, these compounds decompose during storage; thus measurement of their concentration, as well as assessment of antioxidant potential is of crucial importance in food processing.

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References

Aprikian, O., Busserolles, J., Manach, C., Mazur, A., Morand, C., Davicco, M., Besson, C., Rayssiguier, Y., Remesy, C., & Demigne, C. (2002). Lyophilized apple counteracts the development of hypercholesterolemia, oxidative stress, and renal dysfunction in obese Zucker rats. *Journal of Nutrition, 132*, 1969–1976.

Aprikian, O., Duclos, V., Guyot, S., Besson, C., Manach, C., Bernalier, A., Morand, C., Remesy, C., & Demigne, C. (2003). Apple pectin and a polyphenols rich apple concentrate are more effective together than separately on cercal fermentations and plasma lipids in rats. *Journal of Nutrition, 133*, 1860–1865.

Atoui, A. K., Mansouri, A., Boskou, G., & Kefalas, P. (2005). Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry, 89*, 27–36.

Bengoechea, M., Sancho, A. I., Bartolomé, B., Estrella, I., Gómez-Cordovés, C., & Hernández, M. T. (1997). Phenolic composition of industrially manufactured purées and concentrates from peach and apples fruits. *Journal of Agriculture and Food Chemistry, 45*, 4071–4075.

Bondet, V., Brand-Williams, W., & Berset, C. (1997). Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. *Lebensmittel-Wissenschaft Und-Technology, 30*, 609–615.

Cara, L., Dubois, M., Armand, N., Mekki, M., Senft, M., Portugal, H., & Lairon, D. (1993). Pectins are the components responsible for the hypercholesterolemic effect of apple fibre. *Nutrition, 12*, 66–77.

Downing, D. (Ed.). (1989). *Processed apple products*. New York: Van Nostrad Reinhold.

Eberhardt, M. V., Lee, C. Y., & Liu, R. H. (2000). Antioxidant and anticancer activities of fresh apples. *Nature, 405*, 903–904.

Garcia-Viguera, C., Zafrilla, P., Romero, F., Abellan, P., Artes, F., & Tomas-Barberan, F. A. (1999). Colour stability of strawberry jam as affected by cultivar and storage temperature. *Journal of Food Science, 64*, 243–247.

Gerard, K. A., & Roberts, J. S. (2004). Microwave heating of apple mash to improve juice yield and quality. *Zeitschrift fuer Lebensmittel-Untersuchung und Forschung, 37*, 551–557.

Guyot, S., Marnet, N., Laraba, D., Sanoner, P., & 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 Agriculture and Food Chemistry, 46*, 1698–1705.

Guyot, S., Marnet, N., Sanoner, P., & Drilleau, J. F. (2001). Direct thiolysis on crude apple materials for HPLC characterization and quantification of polyphenols in cider apple tissues and juices. *Method of Enzymology, 335*, 57–64.

Hatano, T., Edamatsu, R., Hiramatsu, M., Mori, A., Fujita, Y., Yasuhara, T., Yoshida, T., & Okuda, T. (1989). Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chemistry Pharmacology Bulletin, 37*, 2016–2021.

Ivanow, V., Carr, A. C., & Frei, B. (2001). Red wine antioxidants bind to human lipoproteins and protect them from metal-dependent and -independent oxidation. *Journal of Agriculture and Food Chemistry, 49*, 4442–4449.

Lea, A. G. H. (1995). Enzymes in the production of beverages and fruit juices. In G. A. Tucker & L. F. J. Woods (Eds.). *Enzymes in foods processing* (246). London: Blackie Academic & Professional.

Lee, C. Y., & Whitaker, J. R. (1995). *Enzymatic browning and its prevention*. Washington, DC: American Chemical Society.

Leite da Silva Porto, P. A., Nave Laranjinha, J. A., & Pereira de Freitas, V. A. (2003). Antioxidant protection of low density lipoprotein by procyanidins: Structure/activity relationships. *Biochemical Pharmacology, 66*, 947–954.

Loots, D. T., van der Westhuizen, F. H., & Jerling, J. (2006). Polyphenol composition and antioxidant activity of Kei-apple (*Dovyalis caffra*) juice. *Journal of Agriculture and Food Chemistry, 54*, 1271–1276.

Lotito, S. B., Actis-Goretta, L., Renart, M. L., Caligiuri, M., Rein, D., Schmitz, H. H., Steinberg, F. M., Keen, C. L., & Fraga, C. G. (2000). Influence of oligomer chain length on the antioxidant activity of procyanidins. *Biochemistry and Biophysical Research Communications, 276*, 945–951.

Nicolas, J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J., & Aubert, S. Y. (1995). Enzymatic browning reactions in apple and apple products. *Critical Review of Food Science Nutrition, 34*, 109–157.

Oszmiański, J., & Bourzeix, M. (1995). Preparation of catechin and procyanidin standards from hawthorn (*Crategus azorus* L.) and pine (*Pinus mesogeensis* Fieschi) barks. *Polish Journal Food Nutrition and Science, 4/45*(2), 91–98.

Oszmiański, J., Wolniak, M., Wojdyło, A., & Wawer, I. (2007). Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. *Journal of Science and Food Agricultural, 87*(4), 573–579.

Parpinello, G. P., Chinnici, F., Versari, A., & Riponi, C. (2002). Preliminary study on glucose oxidase–catalase enzyme system to control the browning of apple and pear purées. *Zeitschrift fuer Lebensmittel-Untersuchung und Forschung, 35*, 239–243.

Podsędek, A., Wilska-Jeszka, J., Anders, B., & Markowski, J. (2000). Compositional characterisation of same apple varieties. *European Food Research & Technology, 210*, 268–272.

Rababah, T. M., Ereifej, K. I., & Howard, L. (2005). Effect of ascorbic acid and dehydration on concentrations of total phenolics, antioxidant capacity, anthocyanins, and colour in fruits. *Journal of Agriculture and Food Chemistry, 53*, 4444–4447.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biology & Medicine, 26*(9/10), 1231–1237.

Renard, C., Baron, A., Guyot, S., & Drilleau, J. (2001). Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences. *International Journal of Biology Macromolecules, 29*, 115–125.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology & Medicine, 20*, 933–956.

Sable-Amplis, R., Sicart, R., & Bluthe, E. (1983). Decreased cholesterol ester levels in tissues of hamsters fed with apple fibre enriched diet. *Nutrition Reports International, 27*, 881–889.

Sanoner, P., Guyot, S., Marnet, N., Molle, D., & Drilleau, J. F. (1999). Polyphenol profiles of French cider apple varieties (*Malus domestica* sp.). *Journal of Agriculture and Food Chemistry, 47*, 4847–4853.

Shahidi, F., & Naczyk, M. (2004). *Phenolics in food and nutraceuticals*. Boca Raton, FL: CRC Press.

Soleas, G. J., Tomlinson, G., Diamandis, E. P., & Goldberg, D. M. (1997). Relative contributions of polyphenolic constituents to the antioxidant status of wines: Development of a predictive model. *Journal of Agriculture and Food Chemistry, 45*, 3995–4003.

Spanos, G. A., & Wrolstad, R. E. (1992). Phenolics of apple, pear, and white grape juices and their changes with processing and storage. *Journal of Agriculture and Food Chemistry, 40*, 1478–1487.

Spanos, G. A., Wrolstad, R. E., & Heatherbell, D. A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agriculture and Food Chemistry, 38*, 1572–1579.

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

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agriculture and Food Chemistry, 43*, 27–32.