Effects of Blackcurrant and Apple Mash Blending on the Phenolics Contents, Antioxidant Capacity, and Colour of Juices

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Abstract


The objective of this research was to evaluate the effect of blackcurrant mash blended with apple pulp during juice production and storage on its phenolic composition, antioxidant activity, l-ascorbic acid, and colour. Five variants of samples were prepared: apple juices from two cultivars: the Shampion and Idared cultivars without and with 20% of blackcurrant pulp and blackcurrant juice which were stored at 4°C and 30°C for 6 months. The apple juices prepared from the Idared and Shampion cultivars had a very low l-ascorbic acid contents (1.32 mg/l and 6.26 mg/l, respectively) whereas blackcurrant juice showed the highest amount of l-ascorbic acid, i.e. 704.3 mg/l. The addition of 20% of blackcurrant pulp before apple crushing resulted in a great difference between l-ascorbic acid contents in juices. The addition of blackcurrant fruits before apple crushing had a statistically significantly different (P < 0.05) influence on phenolic compounds, especially in Idared blended pulp. As compared with the control samples, flavan-3-ol concentration increased 4 times in juices made from 80% of Idared apples blended with 20% of blackcurrant fruits. Apple pulp blended with blackcurrant was richer in hydroxycinnamic acids (especially caffeic, p-coumaric, and neo-chlorogenic acids) than juices made only from apples. The results ranged from 83.05 to 3297.6µM T/100 ml for DPPH (1,1-diphenyl-2-picrylhydrazyl radical), from 20.64 to 490.93µM T/100 ml for ABTS (2,2'azinobis-(3-ethylbenzthiazoline-6-sulphonic acid)), and from 1.52 to 37.35µM T/ml for FRAP (Ferric reducing antioxidant power assay) for apple juice made from the Idared cultivar and for blackcurrant juice, respectively. The highest level of the antioxidant capacity (P < 0.05) observed in the blackcurrant sample was due to the effect of the high anthocyanin and ascorbic acid contents. The apple juice colour showed a moderate degradation with time as indicated by the slight reduction of L* values in the samples stored at 4°C for 6 months, and a much higher decrease of L* values in the samples stored at 30°C. The lightness of the apple blended with blackcurrant increased during storage as a result of the coloured anthocyanin degradation. The temperature during the sample storage (30°C) had a significant influence, resulting in a higher degradation of all phenolics compounds analysed, colour and antioxidant activity.

Keywords: blackcurrant; apple; juice; phenolic compounds; l-ascorbic acid; antioxidant activity; colour; storage

A high intake of fruits and their products is generally acknowledged to promote good health and lower the risk of diseases, such as coronary heart disease and cancer, which implicate the oxidative stress as part of their pathogenesis or progression.

Oxygen free radicals and lipid peroxidation may be involved in pathological conditions, such as arteriosclerosis, cancer, and chronic inflammation (LEONTOWICZ et al. 2003; LOTITO & FREI 2004).

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The high content of antioxidants in fruits has been proved to play a significant role in the disease prevention. Apples and apple products are widely consumed in USA and Europe. Raw apples have been found to possess a very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower the content of cholesterol. Phenolics are important, biologically active constituents of apples. This fruit contains phytochemicals, including procyanidins, quercetin, catechin, chlorogenic acid, and all of which are strong antioxidants (Eberhardt et al. 2000).

The processing of apples has been found to affect phytochemicals content. In the conventional apple juice production, the juice obtained is poor in phenolics and has 3–10% of the antioxidant activity of the original fruit (Van der Sluis et al. 2002). This results from the oxidative conditions in the mash treatment, used in the common practice for clear apple juice production (Schols et al. 1991; Will et al. 2002). The apple juice obtained from Jonagold apples by pulping and straight pressing had 10% of the antioxidant activity of fresh apples, while the juice obtained after pulp enzyming had only 3% of the antioxidant activity. After pulp enzyming, the juice contained 31% less phloridzin, 44% less chlorogenic acid, and 58% less catechin.

Apple mash undergoes the oxidative reaction very easily which results in the oxidation of phenolic compounds into o-quinones, which subsequently polymerise into complex dark coloured pigments (Nicolas et al. 1995).

To satisfy consumers’ growing demand for the products containing bioactive components, certain protection of phenolics in apple juices is of vital importance.

To reduce apple pulp browning, chemical antioxidant was used (Lee & Whitaker 1995). Considerable research was conducted into the natural antioxidant substances, such as honey (Oszmiański et al. 1990), pineapple juice (Lzoano-de-Gonzalez et al. 1993), and rhubarb juice (Son et al. 2000).

Rhubarb juice was also used to protect apple juice against browning and its phenolic compounds against oxidation. The addition of 2–3% of rhubarb juice to apples brings about the same effect (protection against oxidation) as the application of 0.5% ascorbic acid (Oszmiański et al. 1995b).

Blackcurrant, being a very rich source of ascorbic acid and phenolics, was used for the improvement on the biological activity of apple juice compounds. Spayd et al. (1984) blended apple juices with 5%, 10%, and 20% anthocyanin contained in fruit juices, and Nani et al. (1993) blended apple juice with 10–27.5% berry juice. Blackcurrant (Ribes nigrum L.) is the most commercially important bush fruit in Europe with an annual production of ~500 000–600 000 t/year. The overwhelming majority of this production is used for processed products, especially jams and juices (Hummer & Barney 2002). Blackcurrant is rich in phenolic compounds, notably anthocyanins. The dark red coloration of the blackcurrant berries and of the products derived from them is thus a result of a very high level of anthocyanins present mainly in the skin. The total content of anthocyanins is at least 2000 mg/kg of the fresh weight of skin (Koeppen & Herrmann 1977). Flavonoid glycosides and hydroxycinnamic acids are also found in blackcurrant. According to Bermudez-Soto and Tomas-Barberan (2004), the most abundant in the blackcurrant flavonol composition were myricetin glycosides, followed by quercetin glycosides. Phenolics-rich blackcurrant is also rich in ascorbic acid, which is a very good phenolics oxidation protector.

Therefore, the objective of this research was to evaluate the effect of blackcurrant pulp blended with apple pulp during the juice production and storage on its phenolic composition, antioxidant activity, and colour. Two apple cultivars and 14 phenolic compounds plus the polymer procyanidins and the degree of polymerisation (DP) were investigated.

**MATERIALS AND METHODS**

**Chemicals.** DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS (2,2’-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid), potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethoxychroman-2-carboxylic acid), (−)-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-xilosidase, β-galactosidase and β-hesperidinase came from Sigma Chemical Co. (Steinheim, Germany). Acetonitrile was the product of Merck (Darmstadt, Germany).

**Plant material.** The material used in this study comprised two apple cultivars: Shampion and
Idared, harvested at the stage of commercial maturity during the 2005 season in the experimental orchard of the Agricultural University, near Wrocław, Poland. Blackcurrant cultivar Titania was harvested near Lubin from a private plantation. The berries were picked at the commercially ripe stage, washed, frozen, and then stored in polyethylene bags at –20°C (up to 1 month) until juice processing.

**Preparation of apple and blackcurrant blended juices on a laboratory scale.** Five combinations of samples were prepared: 100% apple juices from Shampion and Idared, mixtures of apple pulp from each cultivar with 20% blackcurrant pulp, and 100% of blackcurrant juice. In the two first variants the apples (1 kg of each cultivar) were washed with water to remove the surface dirt, and blended with apples using a Thermomix (Vorwerk, Germany) ground to 20°C and next blended with apples (0.8 kg), and ground for 20 seconds. The first frozen blackcurrant fruits (0.2 kg) were crushed in laboratory mill, and ground to 20°C, and next, blended with apples (0.8 kg), and ground for 20 seconds. The blackcurrant fruits (1 kg) were crushed in the laboratory mill, thawed, and heated to 20°C. All five prepared mashses were incubated for 1 h at 20°C with 0.4 ml of pectolytic enzyme preparation (Pectinex Color), and then pressed using a laboratory hydraulic press (Type Zodiac, Poland) for 10 min with 85% yield. The resulting juices were heated in a microwave oven to 90°C for 5 min, while hot transferred into 0.2 l glass jars at 90°C, immediately sealed with plastisol lined metal lids, and inverted for 5 min to sterilise the lids. The jars were then brought back to normal position for cooling. Three replicates were made of the juices preparations. After the processing and storage at 4°C and 30°C for 6 months, the juices were subjected to analyses.

**HPLC analysis of polyphenols.** Before analysis, the juices were centrifuged at 15 000 rpm (20.878 g). The determination of flavan-3-ols, hydroxycinnamates, dihydrochalcones, and flavonol glycosides was carried out on a Merck-Hitachi L-7455 liquid chromatograph with a diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan) and autosampler L-7200. The separation was performed on a Synergi Fusion RP-80A 150 mm × 4.6 mm (4 µm) Phenomenex (Torrance, USA) column. Oven temperature was set to 20°C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 36 min 25% B, followed by the column washing and reconditioning. 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. Photo Diode Array (PAD) spectra were measured over the wavelength range 200–600 nm in steps of 2 nm. The retention times and spectra were compared with those of pure standards between 200–600 nm.

Additionally, enzymatic hydrolysis of flavonol glycosides was performed. The apple juice was diluted with the citrate buffer solution at pH 5 and specific enzymes were added: β-glucosidase, β-xilosidase, 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 incubation at 38°C with a specific enzyme. The amounts of phenolics in the samples were determined by HPLC. The calibration curves were made with (--)-epicatechin, (+)-catechin, chlorogenic acid, phloridzin, isoquercitin, and procyanidin B2, C1, B1 as standards, using the method of OSZMIAŃSKI and BOURZEIX (1995a).

**Procyanidins analysis by thiolysis.** Direct thiolysis of freeze-dried juice was performed as described by GUYOT et al. (2001). Portions (0.5 ml) of juices were precisely measured in 1.5 ml Eppendorf vials and freeze-dried, then acidic methanol (3.3% (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 minutes. Next, the vials were cooled in ice water and centrifuged immediately at 4°C and 14 000 rpm (20 000 g) for 10 minutes. The samples were stored at 4°C until RP-HPLC analysis. All incubations were done in triplicates. The thiolysis products were separated on a Merck Purospher RP 18 end-capped column 250 mm × 4 mm, 5 µm (Merck, Darmstadt, Germany). The liquid chromatograph used was the Waters (Milford, USA) system equipped with DAD and Scanning Fluorescence detectors. Solvent A (aqueous acetic acid, 2.5% (v/v)) and solvent B (acetonitrile) were used as the following gradient: initial 3% B, 0–5 min, 9% B linear; 5–15 min, 16% B linear; and 15–45 min, 50% B linear, followed by the column washing and reconditioning. Flow rate of 1 ml/min
and oven temperature of 30°C were used. The compounds for which reference standards were available (synthesised or isolated previously) were identified on the chromatograms according to their retention times and UV-vis spectra. Fluorescence was recorded at the excitation wavelength of 278 nm and emission wavelength of 360 nm. The calibration curves were established using flavan-3-ol and benzylthioether standards prepared in our laboratory. The average degree of polymerisation (DP) was determined by calculating the molar ratio of all the flavan-3-ol units (thioether adducts + terminal units) to (−)–epicatechin and (+)–catechin.

**DPPH radical scavenging spectrophotometric assay.** The DPPH radical scavenging activity of the juices was determined according to the method of Yen and Chen (1995). The centrifuged juice (1 ml) was diluted with methanol. An aliquot (1 ml) of the diluted juice 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. The reference cuvette contained all 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). Apple juices are coloured and/or cloudy, thus for the spectrophotometric UV-vis measurements the background corrections for the absorbance are necessary.

**Ferric reducing/antioxidant power (FRAP) assay.** The total antioxidant potential of the samples was determined using a ferric reducing ability of plasma FRAP assay by Benzie et al. (1996) as a measure of the antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe³⁺) to the ferrous ion (Fe²⁺); the latter forms a blue complex (Fe²⁺/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300µM, pH 3.6), a solution of 10µM TPTZ in 40µM HCl, and 20µM FeCl₃ at 10:1:1 (v/v/v) ratio. The reagent (300 µl) and sample solutions (10 µl) were added to each sample and mixed thoroughly. The absorbance was read at 593 nm after 10 minutes. The standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. The results were corrected for dilution and expressed in µM Trolox per 100 ml. All the determinations were performed in triplicates.

**Free radical scavenging ability by the use of a stable ABTS radical cation.** The free radical scavenging activity was determined by ABTS radical cation decolourisation assay described previously by Re et al. (1999). ABTS was dissolved in water to a 7µM concentration. ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution with 2.45µM potassium persulfate (final concentration) and kept in dark at room temperature for 12–16 h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion, the samples containing the ABTS⁺ solution were diluted with redistilled water to an absorbance of 0.700(± 0.02) at 734 nm and equilibrated at 30°C. The reagent blank reading was taken (Aₒ). After the addition of 3.0 ml of diluted ABTS⁺ solution (Aₚ), 0.700 ± 0.02) to 30 µl of polyphenolic extracts, the absorbance reading was taken exactly 6 min after the initial mixing (Aₚ). The results were corrected for the dilution and expressed in µM Trolox per 100 ml. All determinations were performed in triplicates.

**Juice colour assessment.** The juice colour was measured with Color Quest XE (HunterLab). The juices were placed in a glass cuvette (1 cm path) and the colour was recorded using CIE L* a* b* 10°/D₆₅ and CIE L* a* b* 10°/D₆₅ colour spaces. L* indicates lightness and its value ranges from 0 (an ideal black object) to 100 (an ideal white object).

**L-ascorbic acid analysis.** The juices were diluted with 0.1M phosophoric acids and centrifuged at 14 000 rpm (20 000 g) for 10 minutes. The estimation of L-ascorbic acid was carried out on the Waters liquid chromatograph with a Tunable Absorbance Detector (Waters 486) and quaternary pump with Waters 600 Controller apparatus (Waters Associates, Milford, USA). A 20 µl sample was injected into a Chromolith Performance RP-18e column (100–4.6 mm) (Merck, Darmstadt, Germany). The elution was carried out using 0.1M phosphoric acid, the flow rate was 1 ml/min. The absorbance was monitored at 254 nm. L-Ascorbic acid was identified by comparison with the standard. The calibration curve was prepared by plotting different concentrations of the standard versus the area measurements in HPLC.

**Statistical analysis.** The results were given as mean ± standard deviation of three independent determinations. All statistical analyses were
performed with Statistica Version 7.0. One-way analysis of variance (ANOVA) by Duncan’s test was used to compare the means. The differences were considered significant at $P < 0.05$.

**RESULTS AND DISCUSSION**

Figure 1 shows $L$-ascorbic acid contents of apple juice, blackcurrant juice, and apple-blackcurrant juice. The apple juices prepared from the Idared and Shampion cultivars had very low amounts of this compound, with average values of 1.3 mg/l and 6.2 mg/l, respectively. The addition of 20% of blackcurrant pulp before apple pressing had a detrimental effect on $L$-ascorbic acid content in blended samples. The juice obtained from the Shampion cultivar and blackcurrant pulp mixture had a much higher ascorbic acid content (144.2 mg/l) than that prepared from Idared cultivar (5.7 mg/l). This may have resulted from the higher susceptibility of ascorbic acid to oxidation in Idared apples than in those of the Shampion cultivar. The rate of oxidation, observed after the apples were ground, depended on different factors. However, the most important of these were polyphenoloxidase (PPO) activity and polyphenol composition and content. First, PPO oxidises $o$-diphenols to $o$-quinones, which results in a series of coupled oxide-reduction reactions with other compounds e.g. ascorbic acid. This compound is easily oxidised in apple pulp by the $o$-quinons formed by PPO enzymatic oxidation of apple phenols, mainly such as catechins and hydroxycinnamic acids (NICOLAS et al. 1994). The polyphenoloxidase activity in the Idared cultivar (1560 U/g) was 5.8 times higher than in the Shampion cultivar (270 U/g) (PODSEDEK et al. 2000).

Blackcurrant juice showed the highest amount of $L$-ascorbic acid, i.e. 704.3 mg/l (Figure 1). PINELO et al. (2006) found 603 mg/l of ascorbic acid in raw blackcurrant juice. Blackcurrants are known to contain high amounts of $L$-ascorbic acid and to have a low pH of 2.6–2.8 which influences its stabilisation in juices.

The losses of $L$-ascorbic in juices after six-month storage at 30°C were found to be: 100% in apple, 30–50% in apple juice with blackcurrant, and 41% in blackcurrant juices, whereas after storage at 4°C the respective losses were 58%, 7–19%, and 26%. The highest $L$-ascorbic acid stability was found in the juices containing blackcurrant and in those stored at a lower temperature.

Tables 1–4 show the phenolic compounds amounts and profiles in apples, apples blended with blackcurrant, and blackcurrant juices before and after 6-month storage at 4°C and 30°C. The flavonols, quercetin derivatives (quercetin-3-O-glactose, quercetin-3-O-glucose, quercetin-3-O-xylose, quercetin-3-O-arabinoase and quercetin-3-O-rhamnose), as shown in Table 1, were present in considerably lower quantities in

![Figure 1](image-url)
Table 1. Contents of flavonols (in mg/l) in apple, apple-blackcurrant, and blackcurrant juices

<table>
<thead>
<tr>
<th>Kind of sample</th>
<th>Time and temperature of storage</th>
<th>Quercetin-3-O-rutinoside</th>
<th>Quercetin-3-O-galactoside</th>
<th>Quercetin-3-O-glucoside</th>
<th>Quercetin-3-O-arabinoside</th>
<th>Quercetin-3-O-xyloside</th>
<th>Quercetin-3-O-rhamonoside</th>
<th>Derivatives of myricitrin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampion</td>
<td>0 months</td>
<td>nd</td>
<td>0.80 ± 0.00</td>
<td>0.48 ± 0.00</td>
<td>0.43 ± 0.02</td>
<td>0.43 ± 0.14</td>
<td>1.32 ± 0.24</td>
<td>nd</td>
<td>3.46</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>nd</td>
<td>0.65 ± 0.00</td>
<td>0.32 ± 0.04</td>
<td>0.43 ± 0.12</td>
<td>0.08 ± 0.00</td>
<td>1.22 ± 0.45</td>
<td>nd</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>nd</td>
<td>0.54 ± 0.11</td>
<td>0.30 ± 0.11</td>
<td>0.16 ± 0.03</td>
<td>0.03 ± 0.54</td>
<td>1.14 ± 0.24</td>
<td>nd</td>
<td>2.17</td>
</tr>
<tr>
<td>Idared</td>
<td>0 months</td>
<td>0.14 ± 0.00</td>
<td>2.08 ± 0.24</td>
<td>0.63 ± 0.05</td>
<td>0.84 ± 0.14</td>
<td>0.09 ± 0.01</td>
<td>1.15 ± 0.34</td>
<td>nd</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>0.07 ± 0.01</td>
<td>1.51 ± 0.14</td>
<td>0.34 ± 0.08</td>
<td>0.92 ± 0.24</td>
<td>0.05 ± 0.01</td>
<td>0.55 ± 0.45</td>
<td>nd</td>
<td>3.44</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.06 ± 0.00</td>
<td>1.29 ± 0.34</td>
<td>0.31 ± 0.90</td>
<td>0.33 ± 0.13</td>
<td>0.08 ± 0.00</td>
<td>0.37 ± 0.01</td>
<td>nd</td>
<td>2.44</td>
</tr>
<tr>
<td>Shampion + blackcurrant</td>
<td>0 months</td>
<td>2.57 ± 0.14</td>
<td>2.72 ± 0.87</td>
<td>3.78 ± 1.05</td>
<td>1.53 ± 0.54</td>
<td>0.76 ± 0.12</td>
<td>2.50 ± 0.56</td>
<td>16.50 ± 2.42</td>
<td>30.36</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>0.22 ± 0.12</td>
<td>2.08 ± 1.04</td>
<td>2.86 ± 1.01</td>
<td>1.17 ± 0.23</td>
<td>0.64 ± 0.11</td>
<td>2.37 ± 0.75</td>
<td>13.64 ± 3.45</td>
<td>22.98</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.10 ± 0.07</td>
<td>1.70 ± 1.11</td>
<td>1.63 ± 0.56</td>
<td>1.16 ± 0.42</td>
<td>0.54 ± 0.09</td>
<td>1.25 ± 0.64</td>
<td>12.18 ± 2.69</td>
<td>18.72</td>
</tr>
<tr>
<td>Idared + blackcurrant</td>
<td>0 months</td>
<td>2.74 ± 1.03</td>
<td>1.69 ± 1.15</td>
<td>2.73 ± 0.45</td>
<td>1.75 ± 0.23</td>
<td>0.75 ± 0.04</td>
<td>1.41 ± 0.78</td>
<td>17.12 ± 1.42</td>
<td>28.19</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>0.10 ± 0.00</td>
<td>1.04 ± 1.24</td>
<td>1.82 ± 0.62</td>
<td>0.48 ± 0.11</td>
<td>0.46 ± 0.08</td>
<td>0.76 ± 0.05</td>
<td>13.50 ± 1.03</td>
<td>18.16</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.08 ± 0.00</td>
<td>1.24 ± 0.34</td>
<td>1.57 ± 0.57</td>
<td>0.99 ± 0.42</td>
<td>0.61 ± 0.45</td>
<td>1.00 ± 0.08</td>
<td>12.34 ± 0.99</td>
<td>17.83</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>0 months</td>
<td>14.15 ± 1.56</td>
<td>nd</td>
<td>12.43 ± 0.23</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>86.60 ± 2.64</td>
<td>113.18</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>13.35 ± 1.34</td>
<td>nd</td>
<td>12.39 ± 1.45</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>82.81 ± 0.85</td>
<td>108.55</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>11.35 ± 2.14</td>
<td>nd</td>
<td>10.38 ± 1.86</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>74.98 ± 0.54</td>
<td>96.71</td>
</tr>
</tbody>
</table>

nd – not detected; values are means ± standard deviation, n = 3; the letters are significantly different at P < 0.05
## Table 2. Contents of catechins and proanthocyanidins (in mg/l) in apple, apple-blackcurrant, and blackcurrant juices

<table>
<thead>
<tr>
<th>Kind of sample</th>
<th>Time and temperature of storage</th>
<th>(+)-Catechin</th>
<th>(-)-Epicatechin</th>
<th>Procyanidins B2</th>
<th>Procyanidins B1</th>
<th>Procyanidins C1</th>
<th>Oligomeric proanthocyanidins</th>
<th>Degree of polymerisation of proanthocyanidins</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 month</td>
<td>10.66 ± 2.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.17 ± 1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.98 ± 2.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.33 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.41 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210.89 ± 4.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.95&lt;sup&gt;k&lt;/sup&gt;</td>
<td>309.39</td>
</tr>
<tr>
<td>Shampion</td>
<td>6 months 4°C</td>
<td>5.25 ± 2.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.68 ± 1.34&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.10 ± 1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.73 ± 0.56&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.73 ± 0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>119.38 ± 1.54&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2.95&lt;sup&gt;k&lt;/sup&gt;</td>
<td>163.82</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>3.18 ± 1.11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.52 ± 1.04&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.04 ± 0.45&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.28 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.41 ± 0.34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>114.02 ± 1.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.23&lt;sup&gt;f&lt;/sup&gt;</td>
<td>133.68</td>
</tr>
<tr>
<td></td>
<td>0 months</td>
<td>1.32 ± 0.353&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.11 ± 0.56&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.97 ± 1.25&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.94 ± 0.23&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.34 ± 0.42&lt;sup&gt;f&lt;/sup&gt;</td>
<td>38.71 ± 0.68&lt;sup&gt;m&lt;/sup&gt;</td>
<td>3.77&lt;sup&gt;i&lt;/sup&gt;</td>
<td>61.39</td>
</tr>
<tr>
<td>Idared</td>
<td>6 months 4°C</td>
<td>0.22 ± 0.89&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.75 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09 ± 0.56&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.56 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.67 ± 0.11&lt;sup&gt;h&lt;/sup&gt;</td>
<td>20.22 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.01&lt;sup&gt;k&lt;/sup&gt;</td>
<td>26.52</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.04 ± 0.56&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.50 ± 0.04&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.35 ± 0.01&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.05 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19 ± 0.12&lt;sup&gt;i&lt;/sup&gt;</td>
<td>8.46 ± 0.16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.43&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15.02</td>
</tr>
<tr>
<td></td>
<td>0 months</td>
<td>13.92 ± 1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.98 ± 0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.19 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.6 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.03 ± 0.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>275.83 ± 0.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.91&lt;sup&gt;d&lt;/sup&gt;</td>
<td>388.46</td>
</tr>
<tr>
<td>Shampion + blackcurrant</td>
<td>6 months 4°C</td>
<td>11.15 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.72 ± 2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.74 ± 0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.71 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.66 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>212.53 ± 2.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.59&lt;sup&gt;e&lt;/sup&gt;</td>
<td>309.10</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>3.31 ± 2.65&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.62 ± 0.19&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.77 ± 0.14&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.04 ± 0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.45 ± 0.11&lt;sup&gt;i&lt;/sup&gt;</td>
<td>100.83 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;j&lt;/sup&gt;</td>
<td>111.62</td>
</tr>
<tr>
<td>Idared + blackcurrant</td>
<td>0 months</td>
<td>5.18 ± 1.25&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.56 ± 1.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.04 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.06 ± 0.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.35 ± 0.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>192.45 ± 1.024&lt;sup&lt;f&lt;/sup&gt;</td>
<td>4.05&lt;sup&gt;h&lt;/sup&gt;</td>
<td>248.69</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>0.28 ± 0.01&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.16 ± 0.12&lt;sup&gt;k&lt;/sup&gt;</td>
<td>2.48 ± 1.00&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.16 ± 0.43&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.60 ± 0.12&lt;sup&gt;h&lt;/sup&gt;</td>
<td>137.09 ± 2.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.92&lt;sup&gt;h&lt;/sup&gt;</td>
<td>146.69</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.20 ± 0.00&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.13 ± 0.14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.36 ± 1.05&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.28 ± 0.01&lt;sup&gt;j&lt;/sup&gt;</td>
<td>89.59 ± 3.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.21&lt;sup&gt;g&lt;/sup&gt;</td>
<td>97.90</td>
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<td>0 months</td>
<td>9.27 ± 1.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>574.01 ± 3.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.86&lt;sup&gt;h&lt;/sup&gt;</td>
<td>592.14</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>6 months 4°C</td>
<td>7.79 ± 1.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>348.8 ± 4.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>363.04</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.00 ± 0.00&lt;sup&gt;h&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>161.47 ± 2.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174.82</td>
</tr>
</tbody>
</table>

nd – not detected; values are means ± standard deviation, n = 3; the means are significantly different at P < 0.05.
apple juices (3.46–4.93 mg/l). Quercetin-3-rutinose was found only in trace amounts in Idared apple juice (0.14 mg/l). This result is in agreement with Price et al. (1999) findings, that a majority of the flavonol glycosides are retained in pomace, which is conceivable, since these compounds are almost exclusively located in the skins. Kahle et al. (2005) has found small amount of quercetin derivatives (from 0.4–4 mg/l) also in juices made from dessert apples.

Blackcurrant juice and juice from apples blended with blackcurrant had much higher amounts of flavonols, 113.18 mg/l and 28.19–30.36 mg/l, respectively. Myricetin glycosides were the most abundant, followed by quercetin glycosides, but only in blackcurrant and apple-blackcurrant juices. These results were also confirmed by other authors who previously studied blackcurrant flavonol composition (Bermudez-Soto & Tomas-Barberan 2004).

The following derivatives of myricetin and quercetin were found and identified in blackcurrant fruits: glucose, rutinoside (rhamnosylglucose), and malonylgucose conjugates of these flavonols (Macheix et al. 1990; Maatta et al. 2003) as well as recently found flavonols such as: kaempferol glucoside, kaempferol rutinoside and isorhamnetin rutinoside (Aanttonen et al. 2006). The juices made from apples blended with blackcurrant pulp had 20% higher flavonol content (28.2–30.4 mg/l) of which 20% was obtained from blackcurrant and 80% from the apple juice (25.4–26.5 mg/l). These results indicated some protection of apple flavonol by blackcurrant fruits. A higher stability of flavonols was found in juices stored for 6 months at the lower temperature (4°C).

The profiles and distributions of the flavan-3-ol constituents of the apple and blackcurrant juices under study are presented in Table 2. There are two catechins, two dimers, a trimer, and oligomeric proanthocyanidins, moreover the degree of its polymerisation is presented. A great difference occurred between flavan-3-ols (catechins and proanthocyanidins) contents in Idared and Shampion juices, i.e. 61.39 mg/l and 309.39 mg/l, respectively. In Shampion juice the (+)-catechin, (−)-epicatechin, and proanthocyanidins amounts were much higher than those in Idared juices. The concentrations of phenolic compounds in our juices were related to their contents in raw materials but they also resulted from the enzymatic oxidation reactions which occur during preparation. Catechins are compounds very sensitive to oxidation by PPO. This result confirmed that Idared apples have a very active PPO enzyme, thus phenolic compounds must be protected during the juice preparation (Podsędek et al. 2000; Oszmiański & Wojdyło 2006).

The addition of blackcurrant mash before apple crushing had different statistically significant (P < 0.05) influence on phenolic compounds, especially in Idared blended juice. Flavan-3-ol concentration increased 4 times in juices made of 80% of Idared apples blended with 20% of blackcurrant fruits, compared to control samples. It is probable that L-ascorbic acid of blackcurrant fruits has a protective effect on the PPO enzyme oxidation of Idared apple phenolics. The addition of blackcurrant ascorbic acid increased significantly not only catechin concentrations but also procyanidin concentrations, from 4.43 mg/l to 14.74 mg/l and from 56.96 mg/l to 229.90 mg/l, respectively (Table 2). The juices made from Idared apples blended with blackcurrant pulp had a high flavan-3-ol content (248.69 mg/l), 20% of which came from blackcurrant and 80% from the apple juice (167.54 mg/l). These results confirmed that L-ascorbic acid used in the preparation of Idared and blackcurrant blended juice (Figure 1) protected the apple flavan-3-ols.

A smaller effect was found with Shampion and blackcurrant blended juice. This might be the result of a lower phenolics sensitivity to oxidation as well as PPO activity in Shampion apples.

A similar effect of blackcurrant protection of Idared apple phenolics was observed in relation to chlorogenic acid (Table 3). The sample of juice prepared from blackcurrant fruits blended with Idared apples gave more than 50% (P < 0.05) increase in the concentration of chlorogenic acid as compared to control sample. Such results were observed despite the fact that this compound is non-existent in blackcurrant fruits (Pinelo et al. 2006). Chlorogenic acid as well as catechins in apples are compounds which are the most sensitive to oxidation by PPO (Nicolas et al. 1995). This result confirmed that polyphenoloxidase from apples of the Idared cultivar possesses a high activity, and that phenolic compounds have to be protected during the juice preparation.

The apple juices blended with blackcurrant are richer in hydroxycinnamic acids than those made from apples only. Blackcurrant juices contained higher concentrations of caffeic and p-coumaric acids derivatives than the apple juices. Among hydroxycinnamic acids, neochlorogenic acid content
Table 3. Contents of phenolic acids and dihydrochalcones (in mg/l) in apple, apple-blackcurrant and blackcurrant juices

<table>
<thead>
<tr>
<th>Kind of sample</th>
<th>Time and temperature of storage</th>
<th>Chlorogenic acid</th>
<th>Caffeoyl-glucoside</th>
<th>Neochlorogenic acid</th>
<th>p-Cumaroyl-quinic acid</th>
<th>Phloretin-2'-O-xylloglucoside</th>
<th>Phloretin-2'-O-glucoside</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampion</td>
<td>0 month</td>
<td>32.69 ± 1.56e</td>
<td>1.96 ± 0.11k</td>
<td>nd</td>
<td>6.32 ± 1.03d</td>
<td>7.44 ± 0.24c</td>
<td>5.35 ± 0.54g</td>
<td>53.76</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>32.57 ± 1.23e</td>
<td>1.93 ± 0.01k</td>
<td>nd</td>
<td>6.28 ± 1.04d</td>
<td>6.78 ± 0.78d</td>
<td>5.04 ± 0.85b</td>
<td>52.60</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>28.48 ± 2.01h</td>
<td>1.13 ± 0.15l</td>
<td>nd</td>
<td>4.87 ± 0.56f</td>
<td>6.58 ± 0.64e</td>
<td>4.85 ± 0.18i</td>
<td>45.91</td>
</tr>
<tr>
<td>Idared</td>
<td>0 months</td>
<td>69.08 ± 1.47c</td>
<td>7.27 ± 0.64e</td>
<td>nd</td>
<td>3.98 ± 0.45g</td>
<td>3.14 ± 0.45b</td>
<td>16.03 ± 0.16a</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>69.01 ± 3.01c</td>
<td>5.09 ± 0.25i</td>
<td>nd</td>
<td>3.30 ± 1.11i</td>
<td>1.95 ± 0.62k</td>
<td>13.17 ± 0.34b</td>
<td>92.52</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>62.67 ± 3.11d</td>
<td>3.65 ± 0.56j</td>
<td>nd</td>
<td>3.03 ± 0.89h</td>
<td>1.84 ± 0.31k</td>
<td>12.56 ± 0.25c</td>
<td>83.75</td>
</tr>
<tr>
<td>Shampion + blackcurrant</td>
<td>0 months</td>
<td>31.11 ± 1.56f</td>
<td>7.00 ± 0.64f</td>
<td>4.63 ± 0.64f</td>
<td>6.31 ± 0.56d</td>
<td>8.93 ± 0.11e</td>
<td>11.36 ± 0.98f</td>
<td>69.34</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>30.04 ± 1.49s</td>
<td>6.48 ± 0.25s</td>
<td>4.56 ± 0.52f</td>
<td>6.25 ± 0.42d</td>
<td>8.15 ± 0.19b</td>
<td>8.09 ± 0.24d</td>
<td>63.57</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>25.92 ± 3.64l</td>
<td>5.60 ± 0.45h</td>
<td>2.73 ± 0.34f</td>
<td>5.99 ± 0.34g</td>
<td>6.16 ± 0.34f</td>
<td>6.15 ± 0.45g</td>
<td>52.55</td>
</tr>
<tr>
<td>Idared + blackcurrant</td>
<td>0 months</td>
<td>104.50 ± 1.05a</td>
<td>9.97 ± 0.34c</td>
<td>5.15 ± 0.86d</td>
<td>3.02 ± 0.21l</td>
<td>3.62 ± 0.34e</td>
<td>16.02 ± 1.08a</td>
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<tr>
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<td>6 months 4°C</td>
<td>104.22 ± 0.99a</td>
<td>9.96 ± 1.05c</td>
<td>5.11 ± 0.75d</td>
<td>2.82 ± 0.11k</td>
<td>2.37 ± 0.45j</td>
<td>12.59 ± 0.34c</td>
<td>137.07</td>
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<tr>
<td></td>
<td>6 months 30°C</td>
<td>85.09 ± 0.89b</td>
<td>7.30 ± 2.04e</td>
<td>2.81 ± 0.42f</td>
<td>2.73 ± 0.19k</td>
<td>2.09 ± 0.11ij</td>
<td>11.15 ± 0.19d</td>
<td>111.17</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>0 months</td>
<td>nd</td>
<td>13.16 ± 1.64a</td>
<td>23.79 ± 0.56a</td>
<td>14.03 ± 1.05a</td>
<td>nd</td>
<td>nd</td>
<td>50.98</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>nd</td>
<td>12.03 ± 1.45b</td>
<td>22.24 ± 0.88b</td>
<td>13.29 ± 1.42b</td>
<td>nd</td>
<td>nd</td>
<td>48.54</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>nd</td>
<td>8.37 ± 2.34d</td>
<td>15.82 ± 0.42c</td>
<td>12.92 ± 1.56c</td>
<td>nd</td>
<td>nd</td>
<td>37.11</td>
</tr>
</tbody>
</table>

nd – not detected; values are means ± standard deviation, n = 3; the means are significantly different at P < 0.05
Table 4. Contents of anthocyanins (in mg/l) in apple, apple-blackcurrant, and blackcurrant juice

<table>
<thead>
<tr>
<th>Kind of sample</th>
<th>Time and temperature of storage</th>
<th>Cyanidin-3-O-glucoside</th>
<th>Cyanidin-3-O-rutinoside</th>
<th>Delphinidin-3-O-glucoside</th>
<th>Delphinidin-3-O-rutinoside</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampion</td>
<td>0 month</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td>Idared</td>
<td>0 months</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.00</td>
</tr>
<tr>
<td>Shampion + blackcurrant</td>
<td>0 months</td>
<td>15.75 ± 2.34d</td>
<td>131.25 ± 2.34e</td>
<td>114.42 ± 3.07d</td>
<td>305.37 ± 0.89d</td>
<td>566.79</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>8.61 ± 1.56e</td>
<td>88.84 ± 0.35f</td>
<td>75.21 ± 1.45e</td>
<td>205.72 ± 2.15e</td>
<td>378.38</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.16 ± 0.03b</td>
<td>1.80 ± 0.18i</td>
<td>0.41 ± 0.00b</td>
<td>1.88 ± 1.08b</td>
<td>4.25</td>
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<tr>
<td>Idared + blackcurrant</td>
<td>0 months</td>
<td>16.35 ± 1.56e</td>
<td>139.11 ± 0.36d</td>
<td>115.48 ± 2.69e</td>
<td>318.04 ± 3.45e</td>
<td>588.98</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>5.75 ± 1.05f</td>
<td>48.10 ± 3.42g</td>
<td>22.51 ± 1.64f</td>
<td>162.35 ± 2.45f</td>
<td>238.71</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>0.08 ± 0.00hi</td>
<td>1.17 ± 0.15b</td>
<td>0.24 ± 0.04l</td>
<td>1.05 ± 0.24i</td>
<td>2.54</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>0 months</td>
<td>88.98 ± 0.75e</td>
<td>716.3 ± 2.68a</td>
<td>685.28 ± 5.06e</td>
<td>1684.21 ± 4.56e</td>
<td>3174.77</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>34.15 ± 0.64b</td>
<td>571.9 ± 3.05b</td>
<td>384.28 ± 0.34b</td>
<td>903.07 ± 1.67b</td>
<td>1893.42</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>2.03 ± 0.34c</td>
<td>49.3 ± 0.16c</td>
<td>13.22 ± 2.65g</td>
<td>44.42 ± 2.45g</td>
<td>108.97</td>
</tr>
</tbody>
</table>

nd – not detected; values are means ± standard deviation, n = 3; ** the means are significantly different at P < 0.05

(not identified in apples) was the highest in the blackcurrant juice. This result is in agreement with the literature data presented recently by Pinelo et al. (2006). Hydroxycinnamic acids, with chlorogenic acid as the dominating constituent, ranged from 57 mg/l to 68 mg/l in apple juices as presented by Kahle et al. (2005). In these studies, the apple juice made from the Idared cultivar contained 69.08 mg/l of chlorogenic acid, and the total of hydroxycinnamic acid was equal to 80.31 mg/l; and for the Shampion cultivar 32.70 mg/l and 41.00 mg/l, respectively. Phenolic acids and dihydrochalcones were much more stable during the juices storage in comparison with flavan-3-ols (Tables 2 and 3). Spanos et al. (1990) reported that 9-month storage at 25°C of apple juice concentrates showed 36% degradation of hydroxycinnamics, 60% degradation of phloretin glycosides, and total loss of procyanidins. The temperature at the sample storage, especially 30°C, had a significant influence on the high degradation of all phenolics analysed.

Anthocyanins contents in blackcurrant and blackcurrant blended with apple juices are shown in Table 4. The four major anthocyanins in blackcurrant, delphinidin-3-O-glucoside, delphinidin-3-O-rutinoside, cyanidin-3-O-glucoside, and cyanidin-3-O-rutinoside, were estimated by HPLC method. Recently, Slimestad and Solheim (2002) reported that these four compounds made up > 97% of the total fifteen anthocyanins of blackcurrant berries (Ribes nigrum L.). Delphinidin-3-O-rutinoside was identified as the main anthocyanin (1684.20 mg/l), and cyanidin-3-O-glucoside as the minor compound (88.98 mg/l) in blackcurrant juice. The total anthocyanin content in blackcurrant juice was 3174.77 mg/l. This was higher than given in blackcurrant juices by Buchert et al. (2005) (2000 mg/l) and Pinelo et al. (2006) (2380 mg/l) and in agreement with Landbo and Meyer (2004) (1340–3220 mg/l) results.

In these studies, the total anthocyanin content was 588.98 mg/l and 566.79 mg/l in the apple juices made from the Idared and Shampion cultivars and blended with blackcurrant. These anthocyanin amounts represented about 18% of anthocyanins of blackcurrant juice. This high content of anthocyanin was obtained from 20% of blackcurrant blended with the apple mash which was used for the juice extraction. The anthocyanin enzymatic
Table 5. Antioxidant activity of apple, apple-blackcurrant and blackcurrant juice as determined by the DPPH, ABTS and FRAP assays before and after 6 months of storage in different conditions (4°C and 30°C)

<table>
<thead>
<tr>
<th>Kind of sample</th>
<th>Time and temperature of storage</th>
<th>DPPH (µM T/100 ml)</th>
<th>ABTS (µM T/100 ml)</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampion</td>
<td>0 month</td>
<td>94.21 ± 0.05d</td>
<td>28.50 ± 0.75j</td>
<td>2.13 ± 0.99i</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>86.24 ± 0.09d</td>
<td>23.64 ± 1.23l</td>
<td>1.42 ± 0.24l</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>89.03 ± 0.78b</td>
<td>24.82 ± 0.75k</td>
<td>1.69 ± 0.65j</td>
</tr>
<tr>
<td>Idared</td>
<td>0 months</td>
<td>83.05 ± 1.02m</td>
<td>20.67 ± 1.65o</td>
<td>1.52 ± 1.65o</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>78.03 ± 0.54o</td>
<td>16.15 ± 1.56o</td>
<td>1.19 ± 0.75o</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>78.23 ± 0.87m</td>
<td>16.58 ± 1.98o</td>
<td>1.27 ± 0.05m</td>
</tr>
<tr>
<td>Shampion +</td>
<td>0 months</td>
<td>748.44 ± 1.04d</td>
<td>133.68 ± 2.09d</td>
<td>8.99 ± 0.56d</td>
</tr>
<tr>
<td>blackcurrant</td>
<td>6 months 4°C</td>
<td>619.38 ± 0.96b</td>
<td>97.41 ± 1.85g</td>
<td>6.08 ± 0.45b</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>703.05 ± 0.85c</td>
<td>105.18 ± 1.35f</td>
<td>7.75 ± 0.63c</td>
</tr>
<tr>
<td>Idared +</td>
<td>0 months</td>
<td>636.89 ± 0.34d</td>
<td>125.39 ± 1.67e</td>
<td>9.01 ± 0.24d</td>
</tr>
<tr>
<td>blackcurrant</td>
<td>6 months 4°C</td>
<td>597.47 ± 0.64f</td>
<td>81.87 ± 1.45d</td>
<td>6.48 ± 0.19b</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>631.33 ± 1.05g</td>
<td>93.01 ± 2.06e</td>
<td>7.24 ± 0.53f</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>0 months</td>
<td>3297.6 ± 2.75a</td>
<td>490.93 ± 3.06a</td>
<td>37.35 ± 0.34a</td>
</tr>
<tr>
<td></td>
<td>6 months 4°C</td>
<td>2844.06 ± 1.68c</td>
<td>330.31 ± 1.54c</td>
<td>29.96 ± 0.67c</td>
</tr>
<tr>
<td></td>
<td>6 months 30°C</td>
<td>3043.26 ± 1.67b</td>
<td>349.74 ± 1.11b</td>
<td>35.03 ± 0.42b</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation, n = 3; the means are significantly different at P < 0.05.

oxidation by PPO and apple phenolics in this mash was stopped by blackcurrant L-ascorbic acid.

Quinones play an important role in enzymatic degradation of anthocyanins. First, enzymes oxidise other phenolic compounds in the media to their corresponding quinones, which then react with anthocyanins, the result being anthocyanin degradation and the formation of brown condensation products. This was observed in many studies with berry products (Yokotsuka & Singleton 1997; Kader et al. 1999; Skrede et al. 2000; Kader et al. 2002).

In our studies, the losses of anthocyanins were found to be: 96% and 40% in blackcurrant juice after six months of storage at 4°C and 30°C, respectively. Similarly, the anthocyanin losses in the apple-blackcurrant juice were found to be: 33% and 99% at 4°C and 30°C, respectively, for the Shampion cultivar, and 60% and 99.5% at 4°C and 30°C, respectively, for the Idared cultivar.

The higher stability of anthocyanins found in blended juices made from Shampion apples and stored at 4°C was probably due to copigmentation with the high amount of flavan-3-ols in the Shampion cultivar. In blackcurrant juice, the increased anthocyanin concentration promotes also a higher colour stability (Giusti & Wrolstad 2003).

Iversen (1999) reported that after 6-month storage of blackcurrant juice at 20°C, about 50% remained of the monomeric anthocyanins original content in blackcurrant nectar. The stabilities of delphinidins and cyanidins during storage were very similar. Spayd et al. (1984) determined the colour stability of apple and pear juices blended with juices which contained anthocyanin, and reported that the losses of anthocyanins in juices after three months of storage at 25°C were 20% in black raspberry blend, 26% in Bing cherry blend, 31% in Concord grape blend, and 42% in red raspberry blend. The anthocyanin losses in the blends of apple juice after 4 months of storage at room temperature were found to be: 46–69% with blackcurrant juice and 50–56% with redcurrant juice (Nani et al. 1993).

The degradation rate of anthocyanins significantly increases during storage as the temperature rises. The temperature rise at the pH values equal 2–4 induces the loss of the glycosyl moieties of the anthocyanins by hydrolysis of the glycosidic
bond. This leads to a further loss of anthocyanin colour, since the aglycones are much less stable than their glycosidic forms. It is postulated that the formation of a chalcone is the first step in thermal degradation of anthocyanins (Adams et al. 1973). Eventually, thermal degradation leads to brown products, especially in the presence of oxygen. Thermal degradation of anthocyanins follows the first order kinetics (Ahmed et al. 2004).

The antioxidant activity of the apple, blackcurrant, and blended juices tested was very strongly correlated with the contents of phenolic compounds. The antioxidant capacity of the prepared juices measured with the use of DPPH, ABTS free radical scavenging, and FRAP assays is shown in Table 5. The results ranged from 83.05 to 3297.6µM T/100 ml for DPPH, from 20.64 to 490.93µM T/100 ml for ABTS, and 1.52 to 37.35µM T/ml for FRAP for apple juice made from the Idared cultivar and for blackcurrant juice, respectively. The highest level of antioxidant capacity ($P < 0.05$) observed in the blackcurrant sample is the effect of a high anthocyanin content (Rice-Evans et al. 1997). However, nonphenolic components present in blackcurrant, such as ascorbic acid, might contribute to the radical scavenging activity of the juice (Kapasakalidis et al. 2006). The apple juices blended with blackcurrant had a much higher antioxidant activity than those made only from apples. In the comparison of the control apple samples blended with blackcurrant and apple samples, the following results were obtained: 7.9 and 7.6 (for DPPH); and 6.1 and 4.7 (for ABTS free radical scavenging activity); and 5.9 and 4.2 (for FRAP assays) times higher for the Idared and Shampion cultivars apples, respectively.

A higher decrease of juices antioxidant capacity on 6 months storage was observed at 30°C than at 4°C. The degradation products formed during juices storage at the higher temperature may have influenced the juice antioxidant activity.

The lightness ($L^*$) values of juices prepared from apples and blackcurrant are shown in Figure 2. The results showed that Shampion apple juice had higher $L^*$ values than that from Idared. Blackcurrant had a significant decreasing effect on $L^*$ value, which caused a darker colour. The lowest $L^*$ value (1.26) was found for the blackcurrant juice with dark colour. This is related to the presence of red pigments (anthocyanins) in blackcurrant. The apple juice colour showed a moderate degradation with time as indicated by the slight reduction of $L^*$ values in the samples stored at 4°C for 6 months, and a much higher decrease of $L^*$ values in those stored at 30°C. The lightness of blended apple and blackcurrant juices increased during storage as a result of coloured anthocyanin degradation.

**CONCLUSION**

The results showed that the positive effect of apple pulp with blackcurrant mash blended during the juice production and storage resided in the

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**Figure 2.** The lightness $L^*$ in apple, apple-blackcurrant, and blackcurrant juices before and after 6 months of storage in different conditions (R – 4°C and H – 30°C). The values are mean values within a column and different temperature of storage marked by different letters is significantly different at $P < 0.05$.
enrichment of these products with new phenolic
compounds, especially with proanthocyanidins,
anthocyanidins, hydroxyxycinnamic acid (especially
caffeic, p-coumaric, and neochlorogenic acids), and
vitamin C. The addition of blackcurrant mash to
apple juice, especially juice made from the Idared
cultivar, had a beneficial influence on the conserva-
tion of phenolic compounds before oxidation
in this juice during the preparation and storage
time. The production of mixed apple-blackcurrant
juices had a good effect on the antioxidant
activity; therefore, these juices can be used as a
good source of antioxidants in our diet and may
be relevant in the prevention of diseases in which
free radicals are implicated.

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