DOI:10.1021/jf900806u

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effect of Enzymatic Mash Treatment and Storage on Phenolic Composition, Antioxidant Activity, and Turbidity of Cloudy Apple Juice

Jan Oszmiański,* Aneta Wojdyło, and Joanna Kolniak

Department of Fruit and Vegetable Processing, Wroclaw University of Environmental and Life Science, 25 Norwida Street, 50-375 Wroclaw, Poland

The effects of different commercial enzymatic mash treatments on yield, turbidity, color, and polyphenolic and sediment of procyanidins content of cloudy apple juice were studied. Addition of pectolytic enzymes to mash treatment had positive effect on the production of cloud apple juices by improving polyphenolic contents, especially procyanidins and juice yields (68.3% in control samples to 77% after Pectinex Yield Mash). As summary of the effect of enzymatic mash treatment, polyphenol contents in cloudy apple juices significantly increased after Pectinex Yield Mash, Pectinex Smash XXL, and Pectinex XXL maceration were applied but no effect was observed after Pectinex Ultra-SPL I Panzym XXL use, compared to the control samples. The content of polymeric procyanidins represented 50−70% of total polyphenols, but in the present study, polymeric procyanidins were significantly lower in juices than in fruits and also affected by enzymatic treatment (Pectinex AFP L-4 and Panzym Yield Mash) compared to the control samples. The enzymatic treatment decreased procyanidin content in most sediment with the exception of Pectinex Smash XXL and Pectinex AFP L-4. Generally in samples that were treated by pectinase, radical scavenging activity of cloudy apple juices was increased compared to the untreated reference samples. The highest radical scavenging activity was associated with Pectinex Yield Mash, Pectinex Smash XXL, and Pectinex XXL enzyme and the lowest activity with Pectinex Ultra SP-L and Pectinex APFL-4. However, in the case of enzymatic mash treatment cloudy apple juices showed instability of turbidity and low viscosity. These results must be ascribed to the much higher hydrolysis of pectin by enzymatic preparation which is responsible for viscosity. During 6 months of storage at 4 °C small changes in analyzed parameters of apple juices were observed.

KEYWORDS: Cloudy apple juice; phenolic compounds; procyanidins; mash maceration; enzymes; antioxidant activity; cloud stability

INTRODUCTION

In epidemiological research, the intake of fruits and vegetables has been widely acknowledged to be inversely related to cancer incidence and cardiovascular diseases (*1*, *2*). Regular consumption of fruits and vegetables is associated with a reduced risk of these diseases (*3*). About one-third of all cancer deaths could be avoided through appropriate dietary modification by increasing the consumption of fruits, vegetables, and whole grains (*4*). Therefore, there has been growing interest to identify the bioactive components of plant foods. In several studies dealing with the cancer-preventive aspect of fruit or juice constituents, the main focus has been on the polyphenolic constituents, which are present at particularly high concentrations in apple (*5*) and apple juice (*6*) than other compounds. Apple juice was an important component of fruit intake in Europe. Recent animal experiments have shown a protective activity of cloudy apple juice with respect to carcinogenesis. Barth et al. (*7*) compared the effects of clear and cloudy apple juice in a rat model for colon carcinogenesis using dimethylhydrazine (DMH) as a carcinogen. After intervention for 8 weeks, cloudy apple juice was more potent in inhibiting carcinogen-induced epithelial cell proliferation and DNA damage than clear apple juice. Also, cloudy apple juice reduced the number of aberrant crypt foci by 28% as a preneoplastic marker for colon carcinogenesis, whereas clear apple juice was ineffective. Production of cloudy juices from stored apples is of increasing technological importance, especially with regard to growing consumer demand for direct ("not produced from concentrate") fruit juices, including the "freshly pressed" type (*8*).

Apples present a wide diversity of polyphenols that were classified into several major classes. The flavan-3-ols include monomeric (catechins) and polymeric (procyanidins) forms, mainly constituted by (−)-epicatechin units. Among the hydroxycinnamic acids, 5-caffeoylquinic acid and 4-*p*-coumaroylquinic acid are present in the highest concentrations. The major types of dihydrochalcones are phloretin glucoside and xyloglucoside.

*To whom correspondence should be addressed. Phone/fax: (+48) 71-3205 477. E-mail: oszm@wnoz.up.wroc.pl.

 Published on Web 07/02/2009

Phenolic compounds of apples are not equally distributed within the fruit (*9*). Flavonols and anthocyanins are essentially present in apple peels, main catechins, and procyanidins and are mostly located in the skin, but lower levels are also present in the flesh and core, including the seeds. Chlorogenic acid, the predominant hydroxycinnamate, is mainly present in the core, whereas lower levels are found in the flesh and skin. Dihydrochalcones are mainly located in the seed and core, and lower levels are present in skin and flesh. The concentrations of apple phenols are dependent on the variety, maturity stage, and environmental growth (*10*). However, the process of clear apple juice production has greater influence on phenolic composition than the variation of cultivars and other factors. Clear apple juice technology is primarily aimed at polyphenol reduction by juice fining, (*11*, *12*). At present, enzyme treatment of crushed apple mash with pectolytic enzymes is followed by mash extraction. Apple mash maceration with the use of pectolytic enzyme is widely used in the clear juice technology to improve the juice yield and to facilitate the pressing operation, clarification, and filterability of juice (*13*).

The effect of enzymation on polyphenolic content of apple juice has been studied in common production practice of clear apple juice concentrate (*14*−*16*). During liquefaction, cell walls of fruits or vegetables are degraded to release the cell contents. Recently, apple pomace liquefication with pectolytic enzymes has been shown to increase the polyphenolic content, especially dihydrochalcones and quercetin glycosides (*17*).

The reason for the use of mash enzymes is the development of mashes with lower viscosities and lower water binding capacities, thus resulting in an easier juice extraction and a better working capacity of presses or decanters. In the case of apple processing, higher juice yield is the main goal of the fruit juice industry. However, in cloudy apple juice technology the amount of colloid material in juice was strongly influenced by the method of processing and enzyme preparation used. Cloudy apple juices are complex systems containing fine pulp particles dispersed in a serum constituted by macromolecules (pectins, proteins, etc.) colloidally dissolved in a true solution of low-molecular weight components (sugars, organic acids, etc.) (*18*). Light scattering of these particles, which represent cell wall fragments surrounded by negatively charged adsorbed pectin, is perceived as juice turbidity (*19*).Cloud stability is one of the visual quality attributes that is decisive for consumer acceptance of cloudy apple juices (*20*).

Recently, the effects of enzymatic mash treatments on yield, turbidity, color, and polyphenolic content of cloudy apple juice were studied. The results obtained demonstrate the possibility of production of cloudstable apple juice with high polyphenolic content due to mash maceration (*21*). However, the objective of this study was only to investigate the effect of mash maceration on nonpolymeric polyphenol content of cloudy apple juice. On the basis of recent data, it is most suggestible that the polyphenols derived from apple cell wall belong to the group of polymeric procyanidins, since the affinity constants of polyphenols with apple pectin were the highest for high molecular weight procyanidins. The apple cell wall polysaccharides develop secondary structures forming hydrophobic pockets to complex polyphenols during cloudy juice processing (*22*). Monomeric apple polyphenols such as hydroxycinnamic acids and (−)-epicatechin did not bind to apple cell wall polysaccharides. Procyanidins with high degrees of polymerization could be particularly affected because of their capacity to be selectively adsorbed from cell-wall material (*23*). Since there are strong interactions between polymeric procyanidins and polysaccarides, polyphenolic constituents in the cloud were examined with regard to their quality and quantity. The amount of bound procyanidins can decrease when the polysaccharides decrease after an enzymatic apple mush treatment. Procyanidins mainly bound to pectins compared to other cell wall compounds. Their adsorption to cell walls reduced the depolymerization of pectins induced by pectin lyase (*24*).

This paper focuses on the production of cloudy apple juices with the usual method of enzymatic nonoxidative mash maceration. New generations of commercial pectolytic enzyme preparations were tested. The effects of mash maceration on yield, turbidity, cloud stability, the composition of phenolics, antioxidant activity, and color properties were studied to evaluate the potential application of enzyme preparations in cloudy apple juice production. Main emphasis was given to the high molecular weight procyanidins.

MATERIALS AND METHODS

Chemicals. DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine), (−)-epicatechin, (+)-catechin, chlorogenic acid, acetic acid, phloroglucinol, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, chlorogenic acid, phloretin 2′-*O*-glucoside, quercetin-3-*O*-glucoside, procyanidins B1, and procyanidins B2 were purchased from Extrasynthese (Lyon, France). Acetonitrile and ascorbic acid were from Merck (Darmstadt, Germany). The following commercial pectolytic enzymes Pectinex Yield Mash, Pectinex Smash XXL, Pectinex XXL, Pectinex Ultra-SPL, and Pectinex AFP L-4 were kindly supplied by Novoenzym (Bagsvaerd, Denmark), and Panzym XXL, Panzym Yield Mash were kindly supplied by Begerow GmbH & Co. (Darmstadt, Germany).

Plant Material. The material used in this study was Shampion variety collected of apples (*Malus domestica* cv. Shampion) at commercial maturity during the 2007 season in the experimental orchard of Agricultural University, near Wroclaw, Poland.

Preparation of Apple Juices on a Laboratory Scale. Apples (15 kg) were ground using a Thermomix (Wuppertal, Vorwerk, Germany) laboratory mill during 20 s with ascorbic acid (0.5 g/kg). Mash was divided into seven lots (400 g) and treated with pectolytic enzyme preparation: Pectinex Yield Mash, Pectinex Smash XXL, Pectinex XXL, Pectinex Ultra-SPL, Pectinex AFP L-4, Panzym XXL, and Panzym Yield Mash and control without enzyme. Enzymes were used at the average dosage recommended by the manufacturers, resulting in a range of 10−50 mL/100 kg mash. Enzymatic mash maceration was carried out under periodic stirring at 20 °C for 1 h. After incubation mash was pressed in a laboratory hydraulic press (SRSE, Warsaw, Poland). After pressing the juices were heated in Thermomix to 90 °C for 4 min, hot fillet at 0.13 L glass jars, immediately inverted for 10 min to sterilize the lids, and cooled to 20 °C. Three replicates of apple juice preparation were carried out. Directly after processing and 6 months of storage in a cold room (4 °C) the juices were subjected to analyses.

Preparation of Fresh Pulp to Polyphenol Analysis. Apple pulp was obtain by grounding using a Thermomix (Wuppertal, Vorwerk, Germany) laboratory mill during 20 s with ascorbic acid (0.5 g/kg). Directly after grounded apple pulp was frozen and freeze-dried (24 h; Christ Alpha 1-4 LSC, Germany). The homogeneous powder was obtained by crushing the dried tissues with the use of closed laboratory mill to avoid hydration and analyzed.

HPLC Analysis of Polyphenols. Apple juice (5 mL) was from 10 min of centrifugation 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 chromatography 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. Separation was performed on a on a Cadenza CD C18 (75 mm × 4.6 mm, 5 μm) column (Imtakt, Japan). Oven temperature was set to 20 °C. The mobile phase was composed of solvent A (2.5% acetic acid, v/v) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 25% B in 36 min, 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 and dihydrochalcones at 280 nm, hydroxycinnamates at 320 nm, and flavonol glycosides at 360 nm. Diode array detector (DAD) spectra were measured over the wavelength range 200−600 nm in steps of 2 nm. Retention times and spectra were compared with those of pure standards within 200−600 nm. The calibration curves were made from (−)-epicatechin, (+)-catechin, chlorogenic acid, phloretin-2′*O*-glucose, quercetin-3-*O*-glucoside, and procyanidin B2, C1, B1 as standards.

Identification of Polyphenols by the Liquid Chromatography−Mass Spectrometry (LC−MS) Method. Identification of polyphenol apple extracts was conducted using a LC−MS system consisting of a Waters 2690 gradient high-performance liquid chromatography (HPLC) separation module, an autoinjector, a 996 ultraviolet−visible (UV−vis) absorbance detector (Waters Corp., Milford, MA), and a quadrupole ion tunnel mass spectrometer (Quattro Ultima, Micromass Ltd., Manchester, U.K.) equipped with a ZQ-spray electrospray ionization (ESI) source. Separations were carried out using a Symmetry C18 (Waters Corp., Milford, MA) 5 μm column (150 mm × 4.6 mm i.d.) at 20 °C. The mobile phase was composed of solvent A (10% formic acid, v/v) and solvent B (100% of acetonitrile). The program began with isocratic elution with 95% A (0−1 min), and then a linear gradient was used until 41 min, lowering A to 0%; from 42 to 51 min, a decrease to 0% A. The flow rate was set at 0.5 mL/min. Analysis was carried out using full scan, data-dependent MS scanning from *m*/*z* 100 to 1000. The capillary temperature was 300 °C; the sheath gas and auxiliary gas were 50 and 5 units, respectively; and the source voltage was 3 kV for negative ionization and 0.1 kV for positive ionization.

Analysis of Proanthocyanidins by Phloroglucinolysis. Direct phloroglucinolysis of freeze-dried apple juices was performed as described by Kennedy et al. (*25*). Portions (0.5 mL) of juices were precisely measured into 2 mL Eppendorf vials and freeze-dried; then 0.8 mL of the methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) was added. After addition of 0.4 mL of methanolic HCl (0.3 M), vials were closed and incubated for 30 min at 50 °C with vortexing by thermoshaker (TS-100, BIOSAN). The reaction was stopped by placing the vials in an ice bath, withdrawing 0.5 mL of the reaction medium, and diluting with 0.5 mL of sodium acetate buffer 0.2 M. Next the vials were cooled in ice−water and centrifuged immediately at 20000*g* for 10 min at 4 °C. Samples were stored at 4 °C before reverse phase HPLC (RP-HPLC) analysis. All incubations were done in triplicate. Phloroglucinolysis products were separated in a on a Cadenza CD C18 (75 mm × 4.6 mm, 3 μm) column (Imtakt, Japan). Liquid chromatography was done with a Waters (Milford, MA) system equipped with a diode array and scanning fluorescence detectors (Waters 474) and autosampler (Waters 717plus). Solvent A (25 mL of aqueous acetic acid and 975 mL of water) and solvent B (acetonitrile) were used in the following gradient: initial, 5% B; 0−15 min, 10% B linear; 15−25 min, 60% B linear; followed by washing and reconditioning of the column. The flow rate was 1 mL/min, and the oven temperature was 15 °C with injection of the filtrate (20 μL) on the HPLC system. Compounds for which reference standards were available were identified on chromatograms according to their retention times and UV−visible spectra. The fluorescence detection was recorded at excitation wavelength of 278 nm and emission wavelength of 360 nm. The calibration curves that were based on peak area were established using (+)-catechin, (−)-epicatechin, and (+)-catechins and (−)-epicatechin−phloroglucinol adducts standards. The average degree of polymerization was measured by calculating the molar ratio of all the flavan-3-ol units (phloroglucinol adducts + terminal units) to (−)-epicatechin and (+)-catechin which correspond to terminal units. Quantification (mg/L of juices) of the (+)-catechin, (−)-epicatechin, (+)-catechins and (−)-epicatechin−phloroglucinol adducts was achieved by using the calibration curves of the corresponding standards (Extrasynthese; Lyon, France).

Ferric Reducing/Antioxidant Power (FRAP) Assay. The total antioxidant potential of a sample was determined using a ferric reducing ability of plasma FRAP assay by Benzie et al. (*26*) as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (300 μL) and sample solutions (10 μL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. 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 of juices. All determinations were performed in triplicate using Shimadzu UV-2401 PC spectrophotometer (Tokyo, Japan).

Free Radical Scavenging Ability by the Use of a Stable DPPH Radical. The DPPH radical scavenging activity was determined using the method proposed by Yen et al. (*26*). An amount of 100 μM DPPH was dissolved in pure ethanol (96%). The radical stock solution was prepared fresh daily. The DPPH solution (1 mL) was added to 1 mL of polyphenol extracts with 3 mL of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were corrected for dilution and expressed in μM Trolox per 100 mL of juices. All determinations were performed in triplicate using a Shimadzu UV-2401 PC spectrophotometer (Tokyo, Japan).

Free Radical Scavenging Ability by the Use of a Stable ABTS Radical Cation. The free radical scavenging activity was determined by ABTS radical cation decolorization assay described by Re et al. (*27*). ABTS was dissolved in water to a 7 μM concentration. ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution with 2.45 μM potassium persulfate (final concentration) and kept in the dark at room temperature for 12−16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. 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. After addition of 3.0 mL of diluted $ABTS^{\bullet+}$ solution ($A_{734\ nm}$ = 0.700 ± 0.02) to 30 μL of polyphenolic extracts, the absorbance reading was taken exactly 6 min after initial mixing. The results were corrected for dilution and expressed in μM Trolox per 100 of juices. All determinations were performed in triplicate using a Shimadzu UV-2401 PC spectrophotometer (Tokyo, Japan).

Color Measurement. Color properties (*L**, *a**, *b**) of juices were determined with Color Quest XE Hunter Lab colorimeter. The samples were filled in a 1 cm cell, and *L**, *a**, *b**, and WL (dominant wavelength) values were determined using Illuminant D_{65} and a 10° observer angle.

Turbidity Measurement. The turbidity of juices was measured with a turbidimeter Turbiquant 3000T (Merck, Germany) using 2.5 cm round cuvettes. Turbidity was expressed in nephelometric turbidity units (NTU). The resistance to clarification (cloud stability) was deduced from the relative turbidity (% *T*):

$$T(\%) = (T_c/T_o) \times 100$$

where T_o and T_c are the juice turbidities before and after centrifugation at 4200*g* for 15 min at 20 °C, respectively (*28*).

Viscosities Measurement. The viscosities of the cloudy apple juices were measured with a rotation viscometer (DV-II+PRO viscometer, Brookfield, England) MC1. Both measurements were carried out at 20 °C.

Isolation of Colloids. Juice polysaccharides were isolated by alcoholic precipitation. Then milliliter of juices was mixed with (50 mL) ethanol (96%). The mixture was thoroughly stirred for 5 min and stored overnight in the cold. After centrifugation (4200*g* for 10 min at 4 °C), the supernatant was discarded, and the residue obtained after centrifugation was freeze-dried and finally weighed. Direct phloroglucinolysis of freeze-dried apple juice precipitate was performed as described above.

Statistical Analysis. Results were given as the mean ± standard deviation of three independent determinations. All statistical analyses were performed with Statistica, version 7.0 (StatSoft, Poland). One-way analysis of variance (ANOVA) by Duncan's test was used to compare the means. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

The enzymatic apple mash treatment resulted in a higher cloudy juice yield in all samples than the control without enzyme

Table 1. Effect of Enzymatic Pulp Treatment on the Juice Yield, Content of Chemical Property, and Physical Parameters Shampion Apple Juices before (0 Month) and after Storage Time (6 Months)[a]

						sediment and colloid concentration [g/L]		viscosity [mPas]		lightness L^*	
	juice yield [%]	turbidity [NTU], 0m	stability of turbidity [%], 0m	turbidity [NTU], 6m	stability of turbidity [%], 6m	0m	6m	0 m	6m	0m	6m
control	68.3 ± 1.2	3055 ± 23	66 ± 5	2877 ± 21	74 ± 6	18.7 ± 0.5	20.5 ± 0.9	40.9 ± 1.3	34.0 ± 2.3	49.1 ± 0.2a	50.5 ± 2.1a
Pectinex Yield Mash	77.0 ± 1.5	2707 ± 34	9 ± 3	2520 ± 16	10 ± 1	2.9 ± 0.2	3.3 ± 0.3	3.7 ± 0.4	1.6 ± 0.7	45.5 ± 0.5c	47.1 ± 0.3b
Pectinex Smash XXL	75.4 ± 1.4	2950 ± 12	14 ± 2	2714 ± 11	16 ± 1	6.5 ± 0.4	8.3 ± 0.5	6.3 ± 0.7	1.8 ± 0.4	47.0 ± 0.8b	48.7 ± 2.3b
Pectinex XXL	73.7 ± 2.9	2786 ± 25	16 ± 1	2589 ± 25	18 ± 2	9.8 ± 0.1	12.9 ± 0.2	5.5 ± 0.4	1.9 ± 0.6	46.5 ± 0.6b	47.2 ± 0.9b
Pectinex Ultra SP-L	72.6 ± 1.6	2818 ± 28	37 ± 5	2655 ± 19	43 ± 4	6.1 ± 0.5	10.5 ± 0.4	7.2 ± 1.0	2.6 ± 0.6	47.6 ± 1.1b	48.9 ± 0.5b
Pectinex APFL-4	76.2 ± 2.4	2689 ± 10	18 ± 2	2513 ± 26	19 ± 2	8.3 ± 0.3	9.0 ± 0.2	5.2 ± 1.1	2.1 ± 0.2	46.2 ± 1.5b	47.7 ± 1.1b
Panzym XXL	76.5 ± 1.7	2505 ± 27	12 ± 3	2354 ± 32	12 ± 1	10.7 ± 0.7	12.5 ± 0.5	4.5 ± 1.3	1.9 ± 0.3	45.1 ± 1.1c	46.5 ± 0.5bc
Panzym Yield Mash	76.2 ± 2.4	2133 ± 21	6 ± 1	1965 ± 11	6 ± 1	3.1 ± 0.0	6.6 ± 0.2	3.9 ± 0.5	1.6 ± 0.0	42.4 ± 1.8d	44.0 ± 1.1d

[a] 0m: 0 month. 6m: 6 months.

(**Table 1**). The juice yield varied from 68.3% in control samples to 77% after Pectinex Yield Mash enzyme preparation treatment. The smallest effect was obtained after enzymatic mash maceration with Pectinex Ultra-SPL (72,6%). Generally 6−13% increase of the yield of juices was observed. These results were smaller than those of Mihalev et al. (*21*) who obtained cloudy juice yield of 77.5% without enzymation and 82.9% after treatment with Rohavin CXL (which is a special pectinase for winemaking) with addition of ascorbic acid. It may have resulted not only from the use of the type of enzyme in our experiment but also from the apple variety and treatment and pressing conditions.

With respect to turbidity, the following quality requirements for cloudy apple juices have been established: turbidity more than 250 NTU and stability turbidity more than 50% (*29*). As shown in **Table 1**, all juices matched the reference, with higher than 250 NTU value for the turbidity. However, when enzymatic mash treatment was not applied, cloudy apple juice met the optimal requirement of turbidity stability of less than 50%. These results must be ascribed to the much higher hydrolysis of pectin by enzymatic preparation which is responsible for viscosity. As shown in **Table 1**, viscosity of apple juices significantly decreased from 40.9 mPas without enzymation to 3.66−7.2 mPas with the enzymatic mash treatment, respectively. It is known that the reasons for the use of mash enzymes is development of mash with lower viscosities, thus resulting in an easier juice extraction and better working capacity of presses or decanters. These effect are positive in clear apple juice production, but in cloudy juices the use of enzymatic mash maceration caused instability of turbidity and sediment decantation.

The sediment and colloid concentrations expressed as grams dw per liter of different juices are shown in **Table 1**. The highest value of 18.65 g/L was found in control juices. This sediment obtained by ethanol precipitation contained colloids and insoluble juice substances. Respectively, smaller values ranging from 2.89 to 10.65 g of sediment per liter were found in juices with mash enzymatic maceration. The highest value was discovered for Panzym XXL and the lowest for Pectinex Yield Mash, respectively. Mehrlander et al. (*30*) found colloid content ranging from 9.7 to 19.6 g/L recovered from the apple juices by ethanol precipitation obtained by enzymatic pomace liquefaction. In the case of cloudy apple juices, turbidity and color are decisive quality attributes.

The effect of enzymatic treatment of apple mash on color parameters representing the lightness L^* is summarized in **Table 1**. There is not much difference in the comparison of nontreated control juices with value 49.1 and with enzymation mash 42.4−47.6, respectively. Cloudy apple juice parameter L^* for Golden Delicious was similar (41.06 ± 4.11) in the study described by Ozoglu et al. (*31*). Control juices had higher turbidity which influenced the color measured by light scattering of cloud particles. Mihalev et al. (*21*) showed that the color properties of cloudy apple juices changed with the oxidation but not after enzymatic treatment with enzymatic preparations was applied. We used the addition of ascorbic acid as antioxidant for nonenzymatic oxidation enzymatic mash treatment because Shampion cultivar has extremely low PPO activity and a low rate of enzymatic browning (*32*).

During 6 months of storage at 4 °C small changes in analyzed parameters of apple juices were observed (**Table 1**). The effect of enzymatic pulp treatment on the content of polyphenolic compounds of juices is shown in **Table 2**. Qualitative analysis obtained by LC−MS methods and quantitative analysis obtained by HPLC (quantified using DAD and fluorescence detection) were presented in our previous paper (*33*). However, some differences in comparison to previous results were found, e.g., absence of anthocyanin content and presence of 4-caffeoylquinic acid in fresh apple and juices. Mihalev et al. (*21*) also showed the presence of 4-caffeoylquinic acid in apple juices.

A total of 17 kinds of polyphenolic compounds found in apple tissues were identified and are presented in **Table 2**: three hydroxycinnamates (*p*-coumaroylquinic acid, chlorogenic acid (5-*O*-caffeoylquinic acid), and cryptochlorogenic acid (4-*O*-caffeoylquinic acid)), five flavan-3-ols ((+)-catechin, (−)-epicatechin, and procyanidin B1, B2, and C1), two dihydrochalcones (phloretin-2′-*O*-xyloglucoside and phloretin-2′-*O*-glucoside), and six flavonols (quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-orutinoside, quercetin-3-*O*-arabinoside, and quercetin-3-*O*-xyloside).

Contents of polyphenolic compounds of fresh apples and juices are shown in **Table 2**. The amounts of polyphenolic classes such as hydroxycinnamates, catechins, and procyanidin dimers (B1, B2) were found to be comparable or higher in juices than in raw material. These effects could be ascribed to good solubility of these compounds in juices during mash maceration and good stability of oxidation by ascorbic acid added during apple crushing. Two of seven enzymatic preparations (Pectinex Yield Mash and Pectinex Smash XXL) gave a statistically significant increase of chlorogenic acid contents in juices compared to the fresh apple pulp which produced juices. Treatment of apple mash with Pectinex Yield Mash, Pectinex Smash XXL, and Pectinex XXL showed also a statistically important increase in the (−)-epicatechin and procyanidin B1 and B2 contents, whereas flavonol glycoside, dihydrochalcone, and polymeric procyanidin contents were considerably lower in juices than in apples. The latter result is in line with the findings that most of these compounds are

Table 2. Effect of Enzymatic Pulp Treatment on the Content of Phenolic Compounds (mg/L) in Shampion Apple Juices before Storage Time

		apple juices							
phenolic compd	fresh pulp [mg/kg]	control	Pectinex Yield Mash	Pectinex Smash XXL	Pectinex XXL	Pectinex Ultra-SPL	Pectinex AFP L-4	Panzym XXL	Panzym Yield Mash
chlorogenic acid	49.1 ± 2.5e	56.1 ± 2.4b	58.1 ± 4.6a	58.3 ± 2.7a	56.4 ± 4.4b	55.0 ± 2.6c	54.5 ± 3.4d	55.2 ± 3.5c	55.0 ± 2.9c
4-caffeoylquinic acid	3.5 ± 0.4c	5.0 ± 1.1a	4.7 ± 0.5b	4.9 ± 1.1ab	5.1 ± 1.1a	5.2 ± 1.3a	5.0 ± 1.3a	5.2 ± 2.1a	5.2 ± 1.3a
p-coumaroyloquinic acid	5.1 ± 1.0c	6.1 ± 1.7a	6.1 ± 0.6a	6.1 ± 2.7a	6.2 ± 1.3a	6.0 ± 1.4ab	6.0 ± 1.5ab	6.0 ± 1.3ab	5.9 ± 1.3b
(−)-epicatechin	61.4 ± 3.6b	62.9 ± 5.9b	63.8 ± 3.1a	64.3 ± 4.5a	64.0 ± 3.1a	61.6 ± 3.8b	60.7 ± 3.2c	62.2 ± 3.9b	61.0 ± 4.5b
procyanidin B2	78.7 ± 4.7d	86.3 ± 5.2b	88.5 ± 6.9b	90.8 ± 6.6a	89.7 ± 6.2a	87.4 ± 5.8b	85.3 ± 5.1c	87.0 ± 5.0b	86.1 ± 7.9b
procyanidin C1	32.6 ± 1.3a	28.3 ± 1.2c	29.8 ± 1.3a	30.4 ± 2.2b	29.5 ± 1.5b	28.7 ± 4.1c	26.9 ± 2.4d	28.6 ± 1.1c	28.0 ± 2.1c
(+)-catechin	13.9 ± 1.2b	16.4 ± 1.7ab	16.7 ± 1.6ab	17.2 ± 1.5a	17.1 ± 1.7a	17.0 ± 1.4a	16.2 ± 1.0b	16.5 ± 1.4ab	16.2 ± 1.7b
procyanidin B1	11.8 ± 01.7c	13.9 ± 1.4ab	14.5 ± 1.4a	14.7 ± 3.2a	14.1 ± 1.5a	13.7 ± 1.1b	13.5 ± 1.3b	13.9 ± 2.6ab	13.6 ± 2.9b
phloretin 2′-*O*-xyloglucoside	9.0 ± 1.6a	4.9 ± 0.2b	5.1 ± 0.3b	4.9 ± 1.1	4.8 ± 1.3b	4.8 ± 0.6b	4.7 ± 1.1b	4.7 ± 1.1b	4.8 ± 0.6b
phloretin 2′-*O*-glucoside	8.1 ± 0.4a	5.5 ± 0.7b	5.5 ± 0.7b	5.4 ± 0.9c	5.5 ± 1.3b	5.2 ± 0.9c	5.6 ± 1.5b	5.2 ± 1.3c	5.8 ± 0.9b
quercetin-3-*O*-rutinoside	0.6 ± 0.1a	0.2 ± 0.0c	0.3 ± 0.0b	0.3 ± 0.0b	0.3 ± 0.1b	0.3 ± 0.1b	0.3 ± 0.0b	0.3 ± 0.1b	0.3 ± 0.0b
quercetin-3-*O*-galactoside	12.8 ± 1.6a	3.0 ± 0.4b	2.8 ± 0.2b	2.7 ± 0.6b	2.8 ± 0.6b	2.7 ± 0.4b	2.8 ± 0.2b	2.8 ± 1.0b	2.5 ± 0.0bc
quercetin-3-*O*-glucoside	1.8 ± 0.2a	0.6 ± 0.1b	0.7 ± 0.1b	0.6 ± 0.1b	0.6 ± 0.2b	0.6 ± 0.1b	0.7 ± 0.3b	0.7 ± 0.2b	0.5 ± 0.0bc
quercetin-3-*O*-arabinoside	3.1 ± 0.6a	0.7 ± 0.0bc	0.6 ± 0.1c	0.6 ± 0.1c	0.8 ± 0.1b	0.9 ± 0.2b	0.6 ± 0.1c	0.8 ± 0.1b	0.9 ± 0.2b
quercetin-3-*O*-ksyloside	6.2 ± 1.0a	1.6 ± 0.2b	1.6 ± 0.4b	1.5 ± 0.2b	1.4 ± 0.7bc	1.5 ± 0.2b	1.4 ± 0.4bc	1.5 ± 0.4b	1.6 ± 0.4b
quercetin-3-*O*-rhamnoside	4.3 ± 1.1a	1.5 ± 0.3c	1.5 ± 0.5c	1.6 ± 0.3bc	1.7 ± 0.3b	1.6 ± 0.1bc	1.6 ± 0.2bc	1.7 ± 0.4b	1.4 ± 0.1c
polymeric procyanidins	770.8 ± 23a	422.2 ± 23d	489.5 ± 34c	522.4 ± 31b	477.9 ± 24c	431.1 ± 29d	381.2 ± 32e	465.5 ± 34c	360.3 ± 27ef
total of polyphenols	1072.7a	715.3d	789.7c	826.6b	777.9c	722.9d	666.9e	757.8 cd	649.0e

retained in the pomace, which is conceivable because these compounds had the capacity to bind more strongly with the solid parts of apples (*22*, *23*). Flavonol glucosides are almost exclusively located in skins and dihydrohalcone in seeds (*34*). Dihydrochalcones were found in juices in appreciable amounts, whereas flavonol glycoside contents were considerably lower (*21*). The enzymatic mash treatment had no statistically significantly influence on most of these compounds compared to the control samples.

Polymeric procyanidins analyzed by phloroglucinolysis were the main polyphenolic compounds of fresh apples and juices (**Table 2**). The content of polymeric procyanidins represented 50−70% of total polyphenols. Consequently, consistent with the results of Guyot et. al. (*35*), procyanidins constituted the main polyphenol class in apples, accounting for more than 50% of total polyphenols depending on the variety. In the present study, as shown in **Table 2**, polymeric procyanidins were significantly lower in juices than in fruits and also affected by enzymatic treatment. Most of enzymatic preparations increased polymeric procyanidin contents in juices with the exception of Pectinex AFP L-4 and Panzym Yield Mash compared to the control samples. Polymeric procyanidins were also found in sediment precipitated from juices by ethanol (**Figure 1**). The enzymatic treatment decreased procyanidin content in most sediment with the exception of Pectinex Smash XXL and Pectinex AFP L-4. Probably this situation was dependent on the influence of enzyme on apple mash pulp. Contained enzymes in preparations could hydrolyze pectins in a specific way and influence their bonds from procyanidins.

The recent results showed that apple cell walls had the capacity to bind apple procyanidins and that this retention depends upon compositional and structural parameters, such as stereochemistry, conformational flexibility, molecular weight, and procyanidin concentrations (*22*, *23*). They also showed that during apple pressing procyanidins are transferred from fruit vacuoles to juice (*36*). Procyanidins mainly bind to pectin compared to other cell wall compounds and can form bridges between readily soluble pectin and insoluble protopectin (*24*). In the present study, our results revealed that the degree of polymerization of procyanidins (DP) found in sediment was 2−3 times higher than in juices (**Figure 2**). The (−)-epicatechin−phloroglucinol extension unit (1) in sediment samples was much higher than the

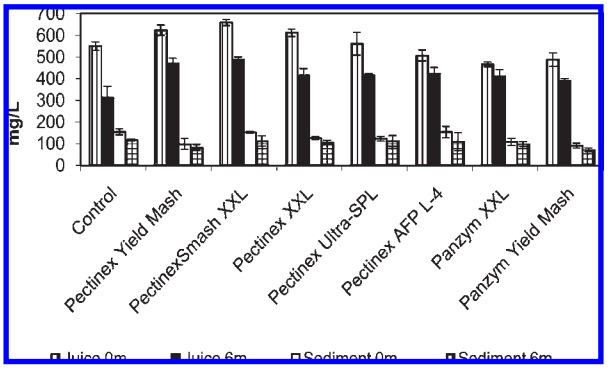

Figure 1. Effect of enzymatic pulp treatment on the concentration of procyanidin (mg/L) in Shampion apple juices and sediments before (0m) and after 6 months (6m) of storage at 4 °C.

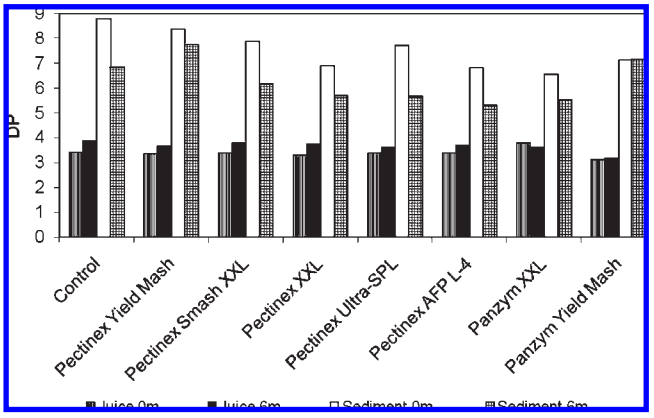

Figure 2. Effect of enzymatic pulp treatment on the degree polymerization of procyanidins (DP) in Shampion apple juices and sediments before (0m) and after 6 months (6m) of storage at 4 °C.

terminal unit of (−)-epcatechin (3) and (+)-catechin (2) compared to juices (**Figure 3**). This result is in agreement with the findings reported by other work who detected that the amounts of procyanidins bound to polysaccharides increased with the initial concentration and with DP (*23*). Higher polymers were bound

selectively to procyanidin mixtures, and apple procyanidins were selectively retained by apple cell walls, the more so as their degree of polymerization increased (*36*).

In summary, the effect of enzymatic mash treatment and polyphenol content in cloudy apple juices significantly increased after Pectinex Yield Mash, Pectinex Smash XXL, and Pectinex XXL maceration were applied; no effect was observed after Pectinex Ultra-SPL I Panzym XXL use, whereas polyphenols in apple juices decreased after Pectinex AFPL-4 and Panzym Yield Mash were used, compared to the control samples.

During storage at 4 °C, significant changes were observed in the concentrations of procyanidins in all cloudy apple juices (**Table 3**). After 6 months of storage, control sample showed 50% degradation of polymeric procyanidins. Juices obtained after enzymatic maceration had smaller degradation of procyanidins which ranged from 15% for Pectinex AFP L-4 to 27% Pectinex Smash XXL compared to the initial values (100% at 0 month), respectively. The procyanidin propensity to form complexes with other compounds (proteins, carbohydrates,) is believed to be responsible for many of their interactions with biological systems (*37*, *38*) which could change the stability of these complexes. The enzymatic maceration of apple mash could change polysaccharides composition in juices, which probably affects the ability to stabilize procyanidins.

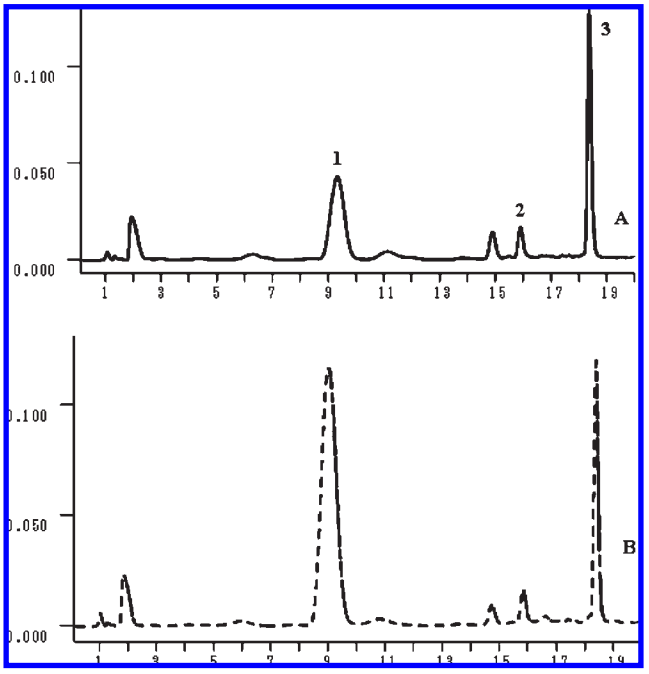

Figure 3. Comparison of chromatograms (HPLC-FD) after phloroglucinolysis of the apple juice (**A**) and the sediment precipitated by ethanol from juices (**B**, dotted line) 1- phloroglucinol−(−)-epicatechin, 2 (+)-catechin, 3 (−)-epicatechin.

Other determinate phenolics were more stable than procyanidins in stored juices. During the storage time at 4 °C no significant changes were observed in concentrations of quercetin glycosides, catechins, hydroxycinnamates, and dihydrochacones. Low temperature apple juice storage reduced the hydrolysis process of phenolic glycoside to unstable aglycone. In our previous results in strawberry juices stored at 30 °C the flavonoid glycosides were much more degraded than at 4 °C (*39*). Spanos et al. (*12*) reported that apple juice stored for 9 months at 25 °C showed 36% degradation of hydroxycinnamic acids, 60% degradation of quercetin and phloretin glycosides, and total loss of procyanidins.

Van der Sluis et. al (*40*) indicated that after 1 month of storage of apple juice in a refrigerator or even at room temperature, lower concentration of present polyphenolic antioxidants was not observed. Phloridzin and chlorogenic acid showed minor degradation rates. Both compounds showed 20−30% decrease in levels over 100 h of incubation at 80 °C. According to this simulation, both chlorogenic acid and quercetin glycosides declined roughly by 40%, a small amount of quercetin aglycon was formed, and phloridzin remained quite stable. In cartonlaminated packed commercial apple juices a decline of phenolic acids (5−21%) and in flavonoid content (14−18%, quercetin glycosides and phloridzin together) was reported after 11 months of storage at room temperature (*41*).

Three methods were used to test the antioxidant capacities of apple juices treatment by different enzymes. On the basis of the data presented in **Table 4**, the enzymatic treatments had considerable influence on capacities of antioxidant activities. In some samples of apple juices radical scavenging activity increased about 4−19% after enzymatic treatments. The scavenging DPPH

Table 3. Effect of Enzymatic Pulp Treatment on the Content of Phenolic Compounds (mg/L) in Shampion Apple Juices after 6 Months of Storage at 4 °C

	apple juices							
phenolic compd	control	Pectinex Yield Mash	Pectinex Smash XXL	Pectinex XXL	Pectinex Ultra-SPL	Pectinex AFP L-4	Panzym XXL	Panzym Yield Mash
chlorogenic acid	51.3 ± 1.9c	54.2 ± 2.4a	52.6 ± 2.9b	50.5 ± 4.7c	49.9 ± 4.1c	50.2 ± 3.4c	50.4 ± 2.3c	50.3 ± 4.3c
4-caffeoylquinic acid	4.5 ± 0.5b	4.2 ± 0.6b	4.4 ± 0.2b	4.5 ± 0.7b	4.7 ± 0.4a	4.5 ± 1.2b	4.7 ± 1.2a	4.7 ± 1.9a
p-coumaroyloquinic acid	6.2 ± 0.6a	6.1 ± 0.8a	6.1 ± 0.4a	6.0 ± 1.1a	5.9 ± 0.8ab	5.9 ± 2.0ab	6.0 ± 1.4a	5.9 ± 1.4ab
(−)-epicatechin	56.7 ± 4.1a	56.7 ± 3.6a	56.8 ± 3.7a	55.1 ± 3.9b	54.5 ± 2.7b	55.5 ± 4.1b	56.3 ± 5.8a	55.2 ± 2.2b
procyanidin B2	71.4 ± 9.0a	71.2 ± 7.3a	71.0 ± 0.4a	69.2 ± 4.4ab	67.8 ± 4.2c	69.7 ± 5.3ab	70.2 ± 3.9a	69.3 ± 3.4ab
procyanidin C1	22.0 ± 2.6ab	23.2 ± 2.6a	22.8 ± 1.8ab	21.7 ± 2.9ab	20.8 ± 1.2b	21.4 ± 1.8ab	21.9 ± 2.2ab	21.7 ± 1.6ab
(+)-catechin	10.9 ± 1.1b	14.5 ± 1.7a	14.4 ± 3.0a	10.7 ± 1.6b	10.7 ± 2.3b	10.8 ± 2.0b	10.9 ± 1.8b	10.8 ± 1.1b
procyanidin B1	9.6 ± 3.0b	11.7 ± 1.5a	11.5 ± 1.8a	9.4 ± 2.0b	9.1 ± 1.4b	9.0 ± 1.2b	7.9 ± 2.0c	9.4 ± 1.7b
phloretin 2′-*O*-xyloglucoside	4.5 ± 0.4b	5.0 ± 1.3a	4.7 ± 0.4ab	4.5 ± 0.5b	4.3 ± 0.4b	4.4 ± 2.1b	4.3 ± 1.1b	4.2 ± 0.8b
phloretin 2′-*O*-glucoside	5.2 ± 0.5a	5.1 ± 0.7ab	5.1 ± 0.6ab	5.1 ± 0.9ab	4.8 ± 0.4c	5.2 ± 1.1a	5.0 ± 1.6b	5.5 ± 0.3a
quercetin-3-*O*-rutinoside	0.1 ± 0.0b	0.1 ± 0.0b	0.0 ± 0.0c	0.1 ± 0.0b	0.1 ± 0.0b	0.2 ± 0.0a	0.1 ± 0.0b	0.1 ± 0.0b
quercetin-3-*O*-galactoside	2.7 ± 0.5a	2.4 ± 0.3b	2.4 ± 0.4b	2.5 ± 0.3b	2.3 ± 1.3bc	2.4 ± 0.0b	2.6 ± 0.1ab	2.4 ± 0.2b
quercetin-3-*O*-glucoside	0.5 ± 0.0a	0.6 ± 0.1a	0.6 ± 0.3a	0.5 ± 0.1a	0.6 ± 0.1a	0.5 ± 0.0a	0.6 ± 0.1a	0.5 ± 0.1a
quercetin-3-*O*-arabinoside	0.6 ± 0.1a	0.6 ± 0.2a	0.5 ± 0.1a	0.6 ± 0.0a	0.6 ± 1.1a	0.5 ± 0.0a	0.5 ± 0.0a	0.5 ± 0.1a
quercetin-3-*O*-ksyloside	1.4 ± 0.4a	1.3 ± 0.2a	1.3 ± 0.3a	1.2 ± 0.2ab	1.4 ± 0.3a	1.2 ± 0.1ab	1.3 ± 0.3a	1.2 ± 0.0ab
quercetin-3-*O*-rhamnoside	1.5 ± 0.3a	1.4 ± 0.3ab	1.4 ± 0.3ab	1.5 ± 0.4a	1.4 ± 0.1ab	1.4 ± 0.2ab	1.5 ± 0.7a	1.4 ± 0.5ab
polymeric procyanidins	209.3 ± 22e	364 ± 12b	380.6 ± 25a	314 ± 33c	317.3 ± 24c	321.9 ± 39c	308.9 ± 14 cd	288.8 ± 29d
total of polyphenols	458.4d	622.1a	636.2a	557.0b	556.1b	564.7b	553.1b	531.8bc

Table 4. Effect of Enzymatic Mush Maceration on Antioxidant Activity in Shampion Apple Juices before and after Storage Time[a]

	DPPH [μM/mL]		ABTS [μM/mL]		FRAP [μM/mL]	
sample	0m	6m	0m	6m	0m	6m
control	828.5 ± 5.8	803.2 ± 7.9	94.8 ± 2.0	49.0 ± 2.1a	807.7 ± 24.9	733.6 ± 3.3
Pectinex Yield Mash	778.7 ± 1.4	740.4 ± 34.0	98.2 ± 15.9	50.0 ± 2.5a	876.1 ± 25.9	739.3 ± 19.7
Pectinex Smash XXL	830.5 ± 9.2	749.4 ± 19.9	94.8 ± 2.5	48.1 ± 2.6b	930.2 ± 2.9	764.9 ± 18.3
Pectinex XXL	806.6 ± 0.0	623.9 ± 15.0	89.1 ± 0.0	49.0 ± 1.8a	955.9 ± 11.9	633.8 ± 18.5
Pectinex Ultra SP-L	752.8 ± 2.8	797.2 ± 9.1	87.0 ± 2.7	44.7 ± 3.7d	782.0 ± 21.5	679.4 ± 5.7
Pectinex APFL-4	741.8 ± 13.4	755.4 ± 15.3	91.5 ± 2.0	46.2 ± 1.5 cd	742.1 ± 4.4	665.1 ± 5.9
Panzym XXL	779.7 ± 2.1	692.6 ± 30.1	89.4 ± 3.3	47.1 ± 3.5c	844.7 ± 10.3	519.8 ± 14.1
Panzym Yield Mash	724.9 ± 8.5	701.6 ± 9.0	99.5 ± 1.1	44.7 ± 2.3d	864.7 ± 26.5	747.8 ± 24.4

[a] 0m: 0 month. 6m: 6 months.

ranged from 724.9 to 830.5 mM Trolox/mL, the ABTS method ranged from 87.0 to 98.2 mM Trolox/mL, and the ability to reduce Fe^{3+} to Fe^{2+} measured by FRAP assay ranged from 742.1 to 955.9 mM Trolox/mL. However, it was observed that the control juices extracted without enzyme contained relatively more antioxidant activity than samples treated with enzymes. Bagger-Jorgensen et al. (*42*) suggested that different antioxidant effects were presumably due to the difference in phenolic composition of samples resulting from a better extraction of the same phenol compounds than others by enzymes (**Table 1**).

Generally in samples that were treated by pectinase radical scavenging activity of cloudy apple juices was increased compared to the untreated reference samples (**Table 4**). However, the highest radical scavenging activity was associated with Pectinex Yield Mash, Pectinex Smash XXL, and Pectinex XXL enzyme and the lowest activity with Pectinex Ultra SP-L and Pectinex APFL-4. These three enzymes also gave the highest amounts of polyphenol contents, especially of polymeric procyanidins which have high antioxidant activity (*43*, *44*). The juice prepared after mash maceration with Pectinex Ultra SP-L had the lowest ABTS scavenging activity (87.0 mM Trolox/mL), but Pectinex APFL-4 had the smallest DPPH (741.8 mM Trolox/mL) radical and FRAP assay (742.1 mM Trolox/mL). The antioxidant and antimicrobial potency of juices (i.e., billberry juice) clearly increased as a consequence of increased amounts of phenolic compounds (*45*). Radical scavenging activity of apple products can be increased with the use of enzymes, which may be positively reflected on human health. Products that increase antioxidant status may have many applications in the future as components of functional foods. After storage time, the antioxidant activity of all investigated apple juices significantly decreased, especially for ABTS capacities. A good correlation ($R^2 > 0.78$) was found between in vitro antioxidant activity ABTS and FRAP and the total phenolic content and poor correlation between DPPH assay and total phenolic compounds ($R^2 > 0.48$) for the juices produced by mash enzymating. On the other hand, DPPH assay did not show any good correlation between antioxidant activity and content of procyanidins, the main phenolic group in apple and investigated apple juices. Nevertheless, the rest of the antioxidant method showed a good correlation between procyanidins and ABTS and FRAP in the juices ($R^2 > 0.74$ and 0.75, respectively). Fernandez-Pachon et al. (*46*) showed that 50% of the total radical scavenging activity (TEAC, DPPH assays) was attributable to polymeric phenolic compounds. The remaining activity was mainly attributed to anthocyanins and flavan-3-ols, followed by phenolic acids and flavonols.

The results of the present study demonstrate that enzymatic mash treatment had a positive effect on the production of cloud apple juices by improving polyphenolic contents and juice yields. However, in the case of enzymatic mash treatment cloudy apple juices showed instability of turbidity and low viscosity. The enzyme composition and dose used for apple mash maceration had caused too high hydrolysis of pectins.

ACKNOWLEDGMENT

Donation of enzyme samples by Begerow GmbH & Co. (Germany) and Novoenzym (Denmark) is gratefully acknowledged.

LITERATURE CITED

(1) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1–29.
(2) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer prevention: a review. *J. Am. Diet. Assoc.* **1996**, *96*, 1027–39.
(3) Willett, W. C. Balancing life-style and genomics research for disease prevention. *Science* **2002**, *296*, 695–698.
(4) Kelloff, G. J.; Corwell, J. A.; Steele, V. E.; Lubet, R. A.; Malone, W. A.; Boone, C. W.; Kopelovich, L.; Hawk, E. T.; Lieberman, R.; Lawrence, J. A.; Ali, I.; Sigman, C. C. Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *J. Nutr.* **2000**, *130* (Suppl. 2S), 467S–471S.
(5) Alonso-Salces, R. M.; Herrero, C.; Barranco, A.; Berrueta, L. A.; Gallo, B.; Vicente, F. Technological classification of basque cider apple cultivars according to their polyphenolic profiles by pattern recognition analysis. *J. Agric. Food Chem.* **2004**, *52* (26), 8006–8016.
(6) Kahle, K.; Kraus, M.; Richling, E. Polyphenol profiles of apple juices. *Mol. Nutr. Food Res.* **2005**, *49*, 797–806.
(7) Barth, S. W.; Fahndrich, C.; Bub, A.; Dietrich, H. Cloudy apple juice decreases DNA damage, hyperproliferation and aberrant crypt foci development in the distal colon of DMH-initiated rats. *Carcinogenesis* **2005**, *26*, 1414–1421.
(8) Oszmianski, J.; Wolniak, M.; Wojdylo, A.; Wawer, I. Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. *J. Sci. Food Agric.* **2007**, *87*, 573–579.
(9) Escarpa, A.; González, M. C. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *J. Chromatogr., A* **1998**, *823*, 331–337.
(10) Sanoner, P.; Guyot, S.; Marnet, N.; Molle, D.; Drilleau, J. F. Polyphenol profiles of French cider apple varieties (*Malus domestica* sp.). *J. Agric. Food Chem.* **1999**, *47*, 4847–4853.
(11) Van der Sluis, A. A.; Dekker, M.; Skrede, G.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *J. Agric. Food Chem.* **2002**, *50*, 7211–7219.
(12) Spanos, G. A.; Wrolstad, R. E.; Heatherbell, D. E. Influence of processing and storage on the phenolic composition of apple juice. *J. Agric. Food Chem.* **1990**, *38*, 1572–1579.
(13) Pilnik W.; Voragen A. G. J. Pectic Enzymes in Fruit and Vegetable Juice Manufacture. In *Enzymes in Food Processing*; Nagodawithana, T., Reed, G.; , Eds.; Academic Press, Inc.: London, 1993; pp 363−399.
(14) Will, F.; Schulz, K.; Ludwig, M.; Otto, K.; Dietrich, H. The influence of enzymatic treatment of mash on the analytical composition of apple juice. *Int. J. Food Sci. Technol.* **2002**, *37*, 653–660.

(15) Schols, H. A.; In't Veld, P. H.; van Deelen, W.; Voragen, A. G. J. The effect of the manufacturing method on the characteristics of apple juice. *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 142–148.
(16) Dietrich, H.; Rechner, A.; Patz, C.-D.; Bitsch, R.; Böhm, V.; Bitsch, I.; Netzel, M. Einfluss der Verbeitung auf die phenolischen antioxidanzien von apfelsäften. *Dtsch. Lebensm.-Rundsch.* **2003**, *99*, 1–11.
(17) Will, F.; Bauckhage, K.; Dietrich, H. Apple pomace liquefaction with pectinases and cellulases: analytical data of the corresponding juices. *Eur. Food Res. Technol.* **2000**, *211*, 291–297.
(18) Wucherpfennig, K.; Dietrich, H.; Kanzler, K.; Will, F. Origin, structure and molecular weight of colloids present in fruit juices and fruit wines and their significance for clarification and filtration processes. *Confructa-Studien* **1987**, *31*, 80–96.
(19) Gierschner, K.; Valet, R.; Endress, H. Neuere erkenntnisse in theorie und praxis des klären bzw. Schönens von fruchtsäften. *Fluess. Obst.* **1982**, *49*, 574–582.
(20) Beveridge, T. Opalescent and cloudy fruit juices: formation and particle stability. *Crit. Rev. Food Sci. Nutr.* **2002**, *42*, 317–337.
(21) Mihalev, K.; Schieber, A.; Mollov, P.; Carle, R. Effect of mash maceration on the polyphenolic content and visual quality attributes of cloudy apple juice. *J. Agric. Food Chem.* **2004**, *52*, 7306–7310.
(22) Le Bourvellec, C.; Bouchet, B.; Renard, C. M. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochim. Biophys. Acta* **2005**, *1725*, 10–18.
(23) Renard, C. M. G. C.; Baron, A.; Guyot, S.; Drilleau, J. F. Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *Int. J. Biol. Macromol.* **2001**, *29*, 115–125.
(24) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Interactions between apple (Malus x domestica Borkh.) polyphenols and cell walls modulate the extractability of polysaccharides. *Carbohydr. Polym.* **2009**, *75*, 251–261.
(25) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740–1746.
(26) Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27–32.
(27) Re, R.; Pellegrini, N.; Proteggente, A; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26* (9/10), 1231–1237.
(28) Zimmer, E.; Pecoroni, S.; Dietrich, H.; Gierschner, K. Verfahrenstechnische und chemische grundlagen der herstellung von trübem apfelsaft unter besonderer berücksichtigung von kontinuierlichen verfahren. I. Beiträge zur analytik "naturtrüber" apfelsäfte unter einbeziehung physikalischer parameter. *Ind. Obst. Gemueseverwert.* **1994**, *79*, 405–412.
(29) Dietrich, H.; Gierschner, K.; Pecoroni, S.; Zimmer, E.; Will, F. Neue erkenntnisse zu dem phänomen der trübungsstabilitäts erste ergebnisse aus einem laufenden forschungsprogramm. *Fluess. Obst.* **1996**, *63*, 7–10.
(30) Mehrlander, K.; Dietrich, H.; Sembries, S.; Dongowski, G.; Will, F. Structural characterization of oligosaccharides and polysaccharides from apple juices produced by enzymatic pomace liquefaction. *J. Agric. Food Chem.* **2002**, *50*, 1230–1236.
(31) Ozoglu, H.; Baymdirh, A. Inhibition of enzymic browning in cloudy apple juice with selected antibrowning agents. *Food Control* **2002**, *13*, 213–221.
(32) Podsędek, A.; Wilska-Jeszka, J.; Anders, B.; Markowski, J. Compositional characterization of some apple varieties. *Eur. Food Res. Technol.* **2000**, *210*, 268–272.
(33) Wojdyło, A.; OszmiaŃski, J.; Laskowski, P. Polyphenolic compounds and antioxidant activity of new and old apple varieties. *J. Agric. Food Chem.* **2008**, *56*, 6520–6530.
(34) Price, K. R.; Prosser, T.; Richetin, A. M. F.; Rhodes, M. J. C. A comparison of the flavonol content and composition in dessert, cooking and cider-making apples: distribution within the fruit and effect of juicing. *Food Chem.* **1999**, *66*, 489–494.
(35) Guyot, S.; Marnet, N.; Sanoner, P.; Drilleau, J. F. Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. *J. Agric. Food Chem.* **2003**, *51*, 6240–6247.
(36) Le Bourvellec, C.; Le Quéré, J. M.; Sanoner, P.; Guyot, S.; Drilleau, J. F. Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. *J. Agric. Food Chem.* **2004**, *52*, 122–130.
(37) De Bruyne, T.; Pieters, L.; Deelstra, H.; Vlietnic, A. Condensed vegetable tannins: biodiversity in structure and biological activities. *Biochem. Syst. Ecol.* **1999**, *27*, 445–459.
(38) Dixon, R. A.; Xie, D.-Y.; Sharma, S. B. Proanthocyanidinssa final frontier in flavonoid research. *New Phytol.* **2005**, *165*, 9–28.
(39) Oszmiański, J.; Wojdyło, A. Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. *Eur. Food Res. Technol.* **2009**, *228*, 623–639.
(40) Van der Sluis, A. A.; Dekker, M.; Skrede, G.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple juice. 3. Stability during storage. *J. Agric. Food Chem.* **2005**, *53*, 1073–1080.
(41) Gliszczynska-Świgło, A.; Tyrakowska, B. Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. *J. Food Sci.* **2003**, *68*, 1844–1849.
(42) Bagger-Jorgensen, R.; Meyer, A. Effects of different enzymatic prepress maceration treatments on the release of phenols into blackcurrant juice. *Eur. Food Res. Technol.* **2004**, *219*, 620–629.
(43) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903–904.
(44) Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Rice-Evans, C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.* **1995**, *322*, 339–346.
(45) Puupponen-Pimiä, R.; Nohynek, L.; Ammann, S.; Oksman-Caldentey, K.; Buchert, J. Enzyme-assisted processing increases antimicrobial and antioxidant activity of bilberry. *J. Agric. Food Chem.* **2008**, *56*, 681–688.
(46) Fernandez-Pachon, M. S.; Villano, D.; Garcia-Parrilla, M. C.; Troncoso, A. M. Antioxidant activity of wines and relation with their polyphenolic composition. *Anal. Chim. Acta* **2004**, *513*, 113–118.

Received March 10, 2009. Revised manuscript received May 24, 2009. Accepted June 17, 2009. This work was financially supported by Grant N N312 199835 from the Ministry of Science and Higher Education of Poland.