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Effect of Activated Charcoal on Some Phenolic Compounds of Apple Juice

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The effect of activated charcoal on the content of several phenolic compounds in apple juice has been studied. In this study, 0, 0.5, 1.0, 2.0 and 3.0 g/L amounts of activated charcoal were added into apple juice. Apple juice samples were then shaken gently for 0, 5, 10, 20 and 30 min, respectively. The quantitative analysis of phenolic compounds using high-performance liquid chromatography with diode-array detection was carried out. Considerable reduction in the content of phenolic compounds (gallic acid, chlorogenic acid, caffeic acid, *p*-cumaric acid, quercetin, phloridzin, (+) -catechin, catechol, (-) -epicatechin and protocatechuic acid) of apple juice was found. The highest decrement in the content of phenolic compounds was obtained at 3.0 g/L activated charcoal mixed for 5 min. The length of mixing period did not seem to have any more effect on the content of phenolic compounds of apple juice.

Key Words: Apple juice, Activated charcoal, Phenolic, HPLC.

INTRODUCTION

Phenolic compounds are found in plants and thus are part of the human diet[1]. Dietary phenolic compounds and especially the flavonoids, consist mainly of anthocyanidins, flavonols and catechins[2,1]. The main phenolic compounds of apples are esters of caffeic and *p*-coumaric acid with quinic acid, flavanol monomers, di- and oligomers, quercetin glycosides and dihydrochalcones[3-8]. Phenolic compounds in apples are of undoubted importance because of their contribution to the colour, taste and flavour characteristics of apples and its derivative products[9]. Their use as indicators of the physiological stages during fruit development and their participation in technological processes leading to low quality products and in possible alterations (browning, formation of hazes and sediments, *etc*.)[10]. Recently, polyphenols have been the subject of increasing interest because of their biological properties, namely antiinflammatory, antihistaminic and antitumor activities and as free radical scavengers and protection against cardiovascular diseases[11-13].

HPLC techniques have proved to be the most appropriate ones, allowing polyphenol analysis with sufficient precision, sensitivity and within a reasonable time[10]. Polyphenolic composition from apples has been studied using HPLC reversed-phase chromatography employing gradient elution methods with phosphoric acid-methanol[3,14,15,5,16,1] and acetic acid-acetonitrile[17,4,8] mixtures as mobile phase and conventional C-18 columns (25-3030.46 cm I.D.) have been reported in most of the cases[3-5,8,10,14,15,18].

The use of the activated charcoal for colour and patulin control in the production of apple juice and apple juice concentrate is a novel procedure in the world. In generally, activated charcoal varying from 0.5 to 2 g/L is used in apple juice plants[19-21]. Gökmen *et al.*,[22] reported a significant decrease in apple juice phenolics in conventional clarification using gelatin, bentonite and activated charcoal. However, the effects of different doses of activated charcoal with various mixing periods on the content of phenolic compound of apple juice are not studied by Gökmen *et al.*[22]. The objective of this study was to determine the phenolic compounds of apple juice when activated charcoal in different levels and periods were applied.

EXPERIMENTAL

In this research, apple juice and activated charcoal (Granucol FA) were used as the materials. The apples used for the production of apple juice were obtained from a well-established local factory (Çal town in Denizli/Turkey). The activated charcoal was provided from Erbslöh Geisenheim Inc., Germany and it has soluble property when directly stirred into the solution. It was found that the activated charcoal used in this research had a pH of 5.02, 8.8 % moisture, 4.12 % ash (dry matter), 1.98 % water soluble matter, a 0.9 ± 0.2 molasses factor, a 60 % methylene blue adsorption and a 1600 m^2/g total surface area.

HPLC-grade methanol and formic acid were obtained from Merck (Darmstadt, Germany). Water used in all the experiments was doubly distilled and deionized. The standards

gallic acid, chlorogenic acid, caffeic acid, phloridzin, (+)-catechin, catechol, (-)-epicatechin, protocatechuic acid were procured from Sigma (St. Louis, MO, USA); *p*-cumaric acid and quercetin were obtained from Roth (Karlsruhe, Germany). All the samples (solutions and extracts) were filtered through 0.45 mm membranes (Millipore) and degassed by an ultrasonic bath before use. For preparing calibration curve, five different concentration levels of each standard were used. Thus, a calibration curve was prepared for each phenolic compound. Correlation coefficients of gallic acid, chlorogenic acid, caffeic acid, *p*-cumaric acid, quercetin, phloridzin, (+)-catechin, catechol, (-)-epicatechin and protocatechuic acid based on the concentration (mg/L) *versus* peak area (mAU) were found to be higher than 0.99.

Production of apple juice: The apples were cut into quarters with stainless steel knives, smashed (Beko, model BKK 1146, Istanbul-Turkey) and pressed by using a hydraulic press (Bucher-Guyer AG, Niederweningen, Switzerland) to obtain cloudy (unclarified) apple juice. The cloudy apple juice was heated in a tubular heat exchanger (Armfield, Model FT74, Chicago-USA) at 80 ºC for 3-5 min and cooled down to 45-50 ºC in a container in circulating cooling water. Then, 1 mL/L of pectolytic enzyme (pectinex 100-L, Nova Nordisk, Istanbul, Turkey) was added for the hot enzymatic fermentation and the temperature was kept in the stated range during treatment (2 h). Following the pectolytic treatment, 500 mg/L of gelatine (Type A, 75-100 bloom, Sigma-aldrich Chemie GmbH, Deisenhofen, Germany) and 2500 mg/L bentonite (Sigma-aldrich Chemie GmbH, Deisenhofen-Germany) were added. After 1.5-2 h, the apple juice was filtered through a Whatman filter paper (grade 40, Sigma-aldrich Chemie GmbH, Deisenhofen-Germany) with a 8 mm particle retention under vacuum. The filtered juice was then pasteurized in a plate heat exchanger (Gemak Ltd. Sti, Ankara-Turkey) at 90 ºC for1 min.

Activated charcoal treatment: For each treatment, 1000 mL apple juice was used. The activated charcoal was added into a glass beaker at 0, 0.5, 1.0, 2.0 and 3.0 g/L concentrations and the experiments were performed in batch with stirring. In each case, the activated charcoal was removed by filtering it on a filter paper after stirring each sample for 5, 10, 20 and 30 min. The experiments were carried out with two replicates and the analysis was made on the samples which were treated by activated charcoal during different periods.

For the HPLC analysis, a Shimadzu model HPLC (Shimadzu corporation, Kyoto, Japan) system consisting of a column oven (Shimadzu, Model CTO-10 ASVP), a photodiode array detector (Shimadzu, Model SPD-M10 AVP-UV-VIS), a degasser (Shimadzu, Model DGU 14A), a liquid chromatography pump (Shimadzu, Model LC-10AT-VP) and a Software Program (Shimadzu) was used. The sample (20 mL) was injected with a syringe (Hamilton Co., Reno, NV, USA) into the HPLC. The column used was a 5 mm C_{18} YMC-pack ODS-AM (250 × 4.6 mm I.D.) (SGE GmbH, Darmstadt, Germany) and a guard C_{18} YMC-Guard Pack ODS-AM (10 × 4.0 I.D.) (SGE GmbH, Darmstadt, Germany).

Extraction procedure: The original pH of the juice samples was not modified. All the samples were diluted to 11.2 brix before extraction. Apple juice samples were centrifuged for 10 min at 5000 rpm (Sigma, Bioblock Scientific 2-16). Then, supernatant was filtered using FP 30/45 CA-S filters (Schleicher and Schuell, Germany) with 0.45 mm (7 bar max) pore size[23] and injected directly into the HPLC.

HPLC conditions: Detection was performed at 280 nm and the absorption spectra of compounds were recorded between 210 and 350 nm. The elution solvents used were A (formic acid: Water, 5:95 v/v) and B (100 % methanol). The samples were eluted according to the following gradient: 17-22 % B as initial condition; 22-30 % B for 10-12 min; 30-37 % B for 12-20 min, 37-45 % B for 20-25 min and finally 40-57 % B for 25-40 min[24]. The chromatographic data on the peaks were integrated up to 40 min. The flow-rate was 1 mL/min. The column was operated at room temperature. The sample injection volume was 20 mL. Identification of compounds was achieved by comparing their retention time values and UV spectra with those of standards stored in a data bank. Concentrations of the phenolic compounds were calculated from integrated areas of the sample and the corresponding standards.

Test of recovery: The recovery efficiency was determined by adding measured amounts of pure standards to the samples prior to extraction of apple juice. Apple juice samples containing known amounts of gallic acid, chlorogenic acid, caffeic acid, *p*-cumaric acid, quercetin, phloridzin, (+)-catechin, catechol, (-)-epicatechin and protocatechuic acid were spiked with the different levels (5, 10, 25, 50, 75 mg/L) of gallic acid, chlorogenic acid, caffeic acid, *p*-cumaric acid, quercetin, phloridzin, (+)-catechin, catechol, (-)-epicatechin and protocatechuic acid to determine the recovery of the extraction procedure.

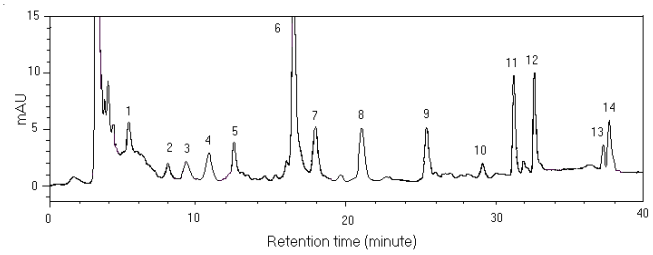
Further determinations: The water soluble (brix) was determined by using digital refractometer (RFM, Model 340, Istanbul, Turkey). The pH (potentiometric) was measured with the pH meter (WTW GmbH and Co., Model 537, Weilheim, Germany)[25]. The moisture was determined by drying until getting a fixed weight and the ash amounth was determined by burning at 500 ± 25 ºC[25]. All results were expressed as average of duplicate samples. Total surface area (m^2/g), molasses factor and methylene blue adsorption (%) of activated charcoal was provided from the manufacturing company.

Statistical analysis: Each experiment was done in duplicates with two replicates. Statistical analysis of the data was performed using SAS® software (SAS, 1985)[26]. When analysis of variance (ANOVA) revealed a significant effect ($p < 0.05$), data means were compared with the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Fig. 1 illustrates the separation of phenolic compounds of control sample by HPLC. As shown in Fig. 1, a good separation can be achieved in 40 min. As can be seen from the chromatogram, gallic acid, chlorogenic acid, caffeic acid, *p*-cumaric acid, quercetin, phloridzin, (+)-catechin, catechol, (-) -epicatechin and protocatechuic acid are separated well. Identification of compounds was achieved by comparing their retention time values and UV spectra with those of standards stored in a data bank. The elutions of four unknown peaks not included in the standards were noticed. But the identification

of these unknown peaks was not achieved without standards. The average percentage recoveries of gallic acid, chlorogenic acid, caffeic acid, *p*-cumaric acid, quercetin, phloridzin, (+)-catechin, catechol, (-s)-epicatechin and protocatechuic acid in apple juice were found to be 95.66, 97.23, 102.46, 95.21, 94.98, 96.78, 103.47, 95.83, 96.30 and 96.12 %, respectively, for added levels of five different concentrations. The contents of phenolic compounds in apple juice samples were corrected for the average per cent recoveries.



Peaks: 1) gallic acid 2) protocatechuic acid 3) catechol 4) (+)-catechin 5) unknown peak 6) chlorogenic acid 7) cafeic acid 8) (-)-epicatechin 9) p-cumaric acid 10) unknown peak 11) unknown peak 12) phloridzin 13) quercetin 14) unknown peak

Fig. 1. Separation of phenolic compounds of apple juice (control) by HPLC

To the best of our knowledge, there are no published research studies about the effect of activated charcoal on the phenolic compounds of apple juice. The effects of different doses of activated charcoal with various mixing periods on the content of phenolic compound of apple juice are shown in Table-1. The levels of phenolic compounds of control sample were decreased by the treatment of activated charcoal. In general, it was observed that increasing the amount of activated charcoal decreases the level of phenolic compounds in apple juice. However, 5 min of treatment of the apple juice with activated charcoal was found to be sufficient with respect to other treatments. Treatments with activated charcoal for a longer time period have only a little effect on the reduction of phenolic compounds of apple juice. Statistical analysis of the data showed that there were significant differences ($p < 0.05$) in the phenolic compounds of the apple juice samples between the dosages of activated charcoal. However, no significant differences were determined in the phenolic compounds of the apple juice between the mixing periods. The pH value of apple juice ranged from 3.94 to 3.88 and brix from 11.35 to 11.44 with the treatment of activated charcoal varying from 0.0 to 3.0 g/L.

Variations of gallic acid, phrotocatechin; (-)-epicatechin, (+)-catechin, catechol; quercetin, floridzin; chlorogenic acid, caffeic acid, *p*-cumaric acid in apple juice samples treated with different doses and mixing periods of activated charcoal are presented in Figs. 2-5, respectively. As shown in Table-1 and Figs. 2-5, the predominant phenolic constituents in the control apple juice were chlorogenic acid and (-)-epicatechin, followed by quercetin. On the other hand, the minor compound found in control apple juice was catechol followed by gallic acid. Results indicated that there was a linear relationship between the concentration of activated charcoal and gallic acid, phrotocatechin; (-)-epicatechin, (+)-catechin, catechol, quercetin, phloridzin; chlorogenic acid, caffeic acid, *p*-cumaric acid

TABLE-1
EFFECT OF DIFFERENT DOSES OF ACTIVATED CHARCOAL ON PHENOLIC COMPOUNDS (mg/L) OF APPLE JUICE SAMPLES

Phenolic compound	Activated charcoal dose (g/L)				
	Control	0.5	1.0	2.0	3.0
Gallic acid [x]	13.4 a	12.8ab	11.8 b	9.6 c	7.9 d
Protocatechin [x]	21.6 a	20.0 ab	18.1 b	15.3 c	13.7 d
(-)-Epicatechin[x]	70.8 a	66.7 b	58.5 c	50.4 d	43.9 e
(+)-Catechin[x]	20.1 a	18.5 ab	16.7 c	15.1 d	14.3 e
Catechol [x]	9.3 a	9.0 a	7.2 b	6.2 c	5.3 d
Quercetin [x]	42.6 a	37.4 b	28.7 c	22.7 d	14.7 e
Phloridzin [x]	22.8 a	18.5 b	14.8 c	11.4 d	9.9 e
Chlorogenic acid[x]	88.3 a	82.3 b	74.5 c	62.4 d	54.7 e
Caffeic acid[x]	36.6 a	32.9 b	28.2 c	24.4 d	20.4 e
p-cumaric acid[x]	19.8 a	17.9 b	15.5 c	13.8 d	10.7 e

[x]; Results are the mean of 5, 10, 20, 30 min mixing periods
*,**; Different letters in the same line are significantly at $p < 0.05$, $p < 0.01$, respectively

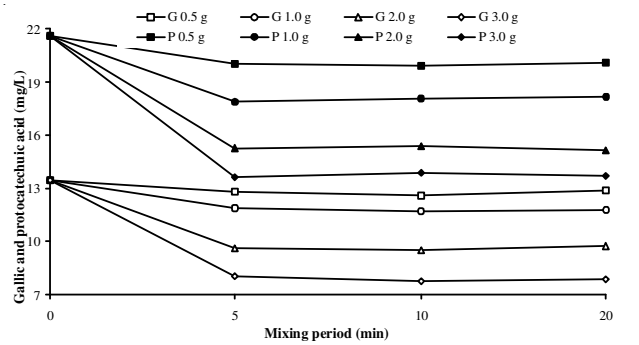


Fig. 2. Variations of the gallic acid (G) and protocatechiuc acid (P) content of apple juice samples treated with activated charcoal

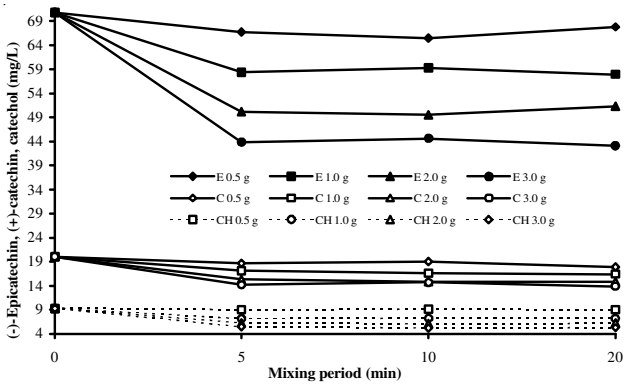


Fig. 3. Variations of the (-)-epicatechin, (+)-catechin and catechol content of apple juice samples treated with activated charcoal

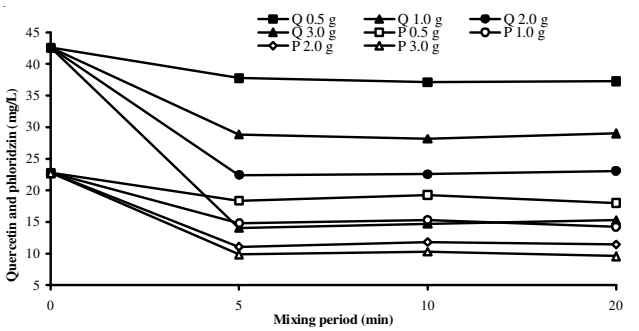


Fig. 4. Variations of the quercetin and phloridzin content of apple juice samples treated with activated charcoal

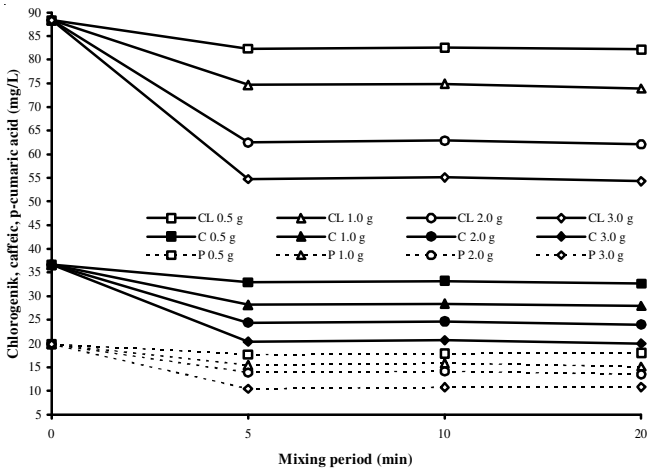


Fig. 5. Variations of the chlorogenic, caffeic and *p*-cumaric acid content of apple juice samples treated with activated charcoal

losses of the apple juice. The highest decrement was obtained in quercetin. A 65.49 % of the quercetin was adsorbed with the treatment of 3 g/L activated charcoal. In contrast, the lowest decrement was determined in (+)-catechin. A 28.86 % decrement was determined in catechin with the treatment of 3 g/L activated charcoal when compared with the control. The loss (%) in gallic acid, protocatechin, (-)-epicatechin, catechol, phloridzin, chlorogenic acid, caffeic acid, *p*-cumaric acid contents of juice samples treated with 3.0 g/L activated charcoal were found as 41.04, 36.57, 39.64, 43.01, 56.58, 38.05, 44.26 and 45.96 %, respectively.

Conclusion

1) The method used in this study [elution solvents A (formic acid: water, 5:95 v/v) and B (100 % methanol] enabled the quantitative determination of the phenolic compounds present in apple juice treated with activated charcoal.

2) Use of activated charcoal for colour and patulin control in the production of apple juice is a novel procedure. But, activated charcoal has decreasing effect on the phenolic compound content of apple juice. So, if it is not necessary (such as patulin and colour control), activated charcoal should not be used for the production of apple juice.

3) The concentration of phenolic compounds in apple juice samples decrease with the activated charcoal. There was a linear relationship between the concentration of activated charcoal and decrement of phenolic compounds of apple juice.

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