A RESEARCH NOTE

EFFECT OF ACTIVATED CHARCOAL ON WATER-SOLUBLE VITAMIN CONTENT OF APPLE JUICE

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ABSTRACT

The effect of activated charcoals (0, 0.5, 1.0, 2.0 and 3.0 g/L of powdered and granular activated charcoals) on the content of several water-soluble vitamins in apple juice was studied. Apple juice samples with activated carbon added were mixed for 0 (control), 5, 10, 20, and 30 min, respectively. The content of water-soluble vitamins was analyzed by HPLC. Considerable reduction in ascorbic acid (Vit C), niacin, pyridoxine (Vitamin B_6), thiamine (Vitamin B_1) and biotin concentrations was found while there was a dramatic improvement in the color and clearness of apple juice. The highest decrement in water-soluble vitamins was obtained at 3.0 g/L powdered activated charcoal. Statistical analysis of the data showed highly significant differences ($P < 0.05$, $P < 0.01$) in the water soluble vitamins, color and clearness of the apple juice samples between the dosages of activated charcoals but no significant differences induced by the mixing periods.

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INTRODUCTION

Vitamins can be classified in two main groups: water-soluble and fat-soluble vitamins. Among the B group of water-soluble vitamins, both thiamin (B_1) and pyridoxine (B_6) are important vitamins. They play different specific and vital functions in metabolism, and their lack produces specific diseases (Moreno and Salvado 2000). Vitamins are relatively unstable, affected by factors such as heat, light, air, other food components and food processing conditions (Machlin 1991; Ottaway 1993). Because of the critical role of vitamins in nutrition and their relative instability, qualitative and quantitative analyses are needed in food manufacturing. HPLC is the preferred technique for vitamin separation because of its high selectivity (de Leenheer *et al.* 1985).

Use of activated charcoal for color and patulin control in the production of apple juice and apple juice concentrate isn't a novel procedure in the world. However, there is no published report investigating changes in the concentration of water-soluble vitamins of apple juice from activated charcoal treatment.

The objective of this study was to determine the water-soluble vitamin, color and clearness of apple juice when powdered and granular activated charcoals in different levels and stages of application were investigated and to help the apple juice manufacturing industry select an appropriate procedure.

MATERIALS AND METHODS

Materials

Apple juice and activated charcoals [Powdered (Carbopal Gn-A ultra), Granular (Granucol FA)] were used as the materials. The apples (Golden delicious) used for the production of apple juice were obtained from a well-established local factory (Denizli, Turkey). The granular activated charcoal was provided by Erbslöh Geisenheim GmbH & Co. KG, Geisenheim, Germany and the powdered activated charcoal by LURGI Aktivkohle GmbH, Frankfurt, Germany. The granular activated charcoal is soluble when directly stirred into the solution. Some properties of powdered and granular activated charcoals are given in Table 1.

Reagents

Acetonitrile (HPLC grade) and K_2HPO_4 (extra pure) were obtained from Merck (Darmstadt, Germany). Water used in all the experiments was doubly distilled and deionized. The vitamin standards (ascorbic acid, niacin, pyridoxine, thiamine and biotin) were obtained from Sigma (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Stock and standard solutions of water-soluble vitamins

were prepared in water. For preparing calibration curve, five different concentration levels of each standard were used. Correlation coefficients of ascorbic acid, niacin, pyridoxine, thiamin and biotin based on the concentration (μg/mL) versus peak area (mAU) were found to be 0.99, 0.98, 0.99, 0.97 and 0.98, respectively.

TABLE 1.
PROPERTIES OF ACTIVATED CHARCOALS

Specifications	Activated Charcoal	
	Powdered Activated Charcoal	Granucol Fa
Moisture (%)	<10	5
pH value	2-5	6
Ash (%, dry matter)	<5	-
Molasses factor	0.8 ± 0.2	0.3
Methylene blue adsorption (MBA)	10	60
Total surface area (m^2/g)	1350	1600
Water-soluble matter (25C, %)	-	-

Production of Apple Juice

The apples were cut into quarters with stainless steel knives, crushed (Beko, model BKK 1146, İstanbul, 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, IL) at 80C for 3-5 min and cooled down to 45-50C in a container in circulating cooling water. Then, 1 mL L^{-1} of pectolytic enzyme (Pectinex 100 - L, Nova Nordisk, İstanbul, Turkey) was added and the temperature was kept in the stated range during treatment (2 h). Following the pectolytic treatment, 500 mg L^{-1} of gelatin (Type A, 75-100 bloom, Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) and 2500 mg L^{-1} bentonite (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) were added. After resting for about 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 μm particle retention under vacuum. The filtered juice was then pasteurized in a plate heat exchanger (Gemak Ltd. Şti, Ankara, Turkey) at 90C for 1 min.

For each treatment, 1,000 mL of the produced apple juice (12.0 brix-regulated) was used. The activated charcoals were added into a glass beaker at 0, 0.5, 1.0, 2.0 and 3.0 g/L concentrations and followed by stirring. In each case, the activated charcoal was removed by filtering through filter paper (İnterlab, Cat. No 47.05.001, İstanbul, Turkey) after stirring each sample for 5, 10, 20, and 30 min. All the treatments (dosage levels and mixing periods) were replicated.

Sample Preparation

The original pH of the juice samples was not modified. The juice samples were centrifuged for 10 min at 14×10^3 g (Sigma, Bioblock Scientific 2-16). Prior to HPLC analysis, all samples were filtered using FP 30/45 CA-S filters (Schleicher & Schuell, Darmstadt, Germany) with 0.45 μm (7 bar max) pore size.

Methods

The determination of water-soluble vitamins was carried out by using a Shimadzu VP series high pressure liquid chromatography apparatus (Shimadzu Corp., Kyoto, Japan), as suggested by Sigma-Aldrich Co. (SUPELCO 2000, Bellfonte, PA). The mobile phase employed was KH_2PO_4-acetonitrile (99/1) with a flow rate of 1.0 mL/min. For the analysis, a Discovery C18 150X4.6 mm column (Cat. No: 504955), a UV-VIS diode array detector (Shimadzu, model SPD-M10 Avp) set at 220 nm, a LC-10AT-VP Shimadzu HPLC pump and a Software program (Shimadzu) were used. A typical chromatogram of the apple juice on a discovery C-18 (150X4.6 mm I.D.) column using KH_2PO_4-acetonitrile (99/1) as the mobile phase with a flow rate of 1.0 mL/min is illustrated in Fig. 1.

Measurement of color and clearness of 12 brix-regulated apple juice compared with distilled water was carried out spectrophotometrically (Shimadzu, Model UV-1201V, Kyoto, Japan). To measure the color, the spectrophotometer was adjusted at 440 nm, whereas to measure clearness, the spectrophotometer was set at 620 nm (Kadakal and Nas 2002). The soluble solid (brix) was determined by using a digital refractometer (RFM Model 340, İstanbul, Turkey) (AOAC 1980). The pH was measured with pH meter (WTW GmbH & Co., Model 537, Weilheim, Germany) (AOAC 1980). All samples were diluted to 11.2 °Bx at the time of analysis.

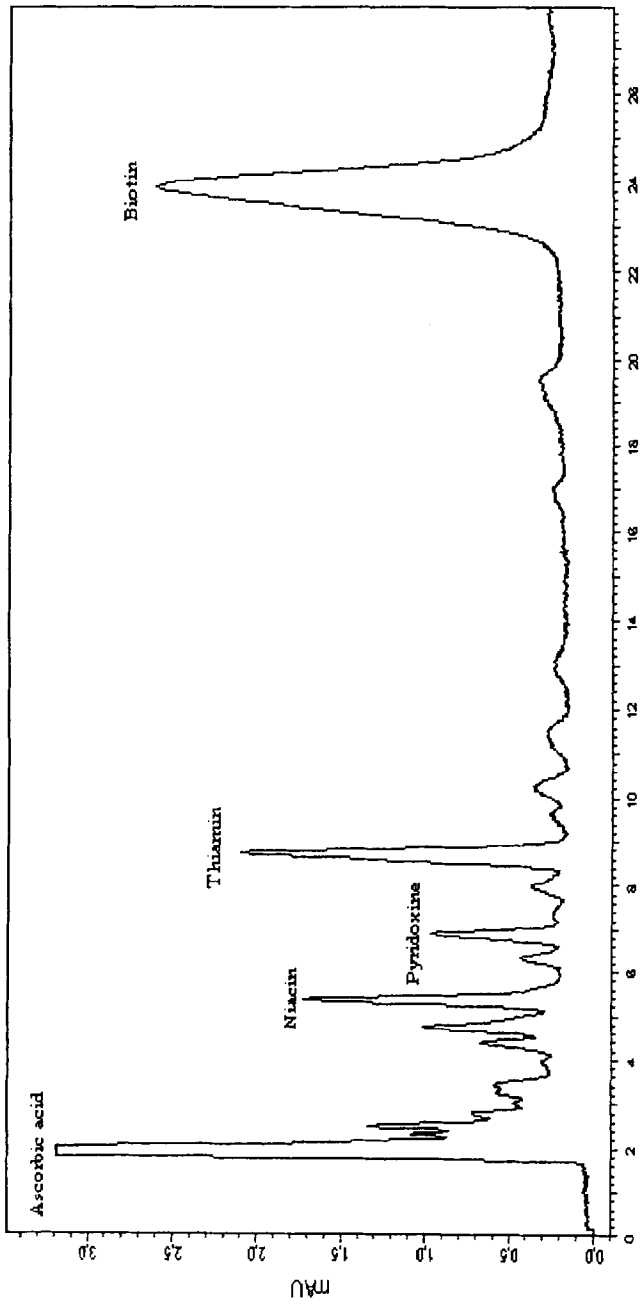

FIG. 1. SEPARATION OF WATER-SOLUBLE VITAMINS OF APPLE JUICE BY HPLC

Recovery of Water-soluble Vitamins

Apple juice samples containing known amounts of ascorbic acid, niacin, pyridoxine, thiamine and biotin were spiked with the different levels (25, 50, 75, 100, 200 μg/L) of the vitamins to determine recovery of each vitamin. The average percentage recoveries of ascorbic acid, niacin, pyridoxine, thiamine and biotin in apple juice were found to be 96.86%, 95.3%, 102.1%, 101.9%, and 95.8%, respectively, for levels added at five different concentrations. The levels of ascorbic acid, niacin, pyridoxine, thiamine and biotin in apple juice samples were corrected for the average percent recoveries.

Statistical Analysis

Statistical analysis of the data was performed using SAS (SAS Institute, SAS 1985, Cary, NC). When analysis of variance (ANOVA) revealed a significant effect ($P < 0.05$, $P < 0.01$), data means were compared with the least significant difference (LSD) test.

RESULTS AND DISCUSSION

To the best of our knowledge, there is no published research on the effect of activated charcoal on the water-soluble vitamin content of apple juice. Statistical analysis of the data showed that there were significant differences ($P < 0.05$) in the water-soluble vitamins of the apple juice samples between the dosages of activated charcoals. However, no significant differences were determined between the mixing periods. Data for the 5 min mixing periods were used for the evaluation of the results because, the least mixing period is most useful in view of time possession for the plants. The pH value of apple juice ranged from 3.85 to 3.82 and brix from 11.20 to 11.28. The effects of different doses of activated charcoals with 5 min mixing periods are shown in Table 2.

The water-soluble vitamin content of a control sample was decreased by the treatment of activated charcoal. Increasing amounts of activated charcoal decreased the water-soluble vitamin level in apple juice. However, it was observed that 5 min of treatment of the apple juice with activated charcoal was sufficient with respect to other treatments. Powdered activated charcoal was more effective in reducing the water-soluble vitamins than the granular activated charcoal. The highest losses of ascorbic acid, niacin, pyridoxine, thiamine and biotin in apple juice were found to be 16.5%, 31.5%, 48%, 53.5%, and 44.2%, respectively, with the treatment of 3 g/L powdered activated charcoal when compared with the control sample. The lowest losses of ascorbic acid, niacin, pyridoxine, thiamine and biotin in apple juice were found to be 3.1%, 4%, 8.8%, 9.3%, and 4%, respectively, with the treatment of 0.5 g [illegible]lar

TABLE 2.
THE EFFECT OF DIFFERENT DOSES OF POWDERED AND GRANULAR ACTIVATED CHARCOALS ON ASCORBIC ACID, NIACIN, PYRIDOXINE, THIAMINE AND BIOTIN CONTENT (μg/100 g) OF APPLE JUICE SAMPLES

Activated charcoal dose (g/L)	Activated charcoal									
	Powdered activated charcoal					Granucol Fa				
	Ascorbic acid [x]	Niacin[x]	Pyridoxine[x]	Thiamine[x]	Biotin[x]	Ascorbic acid [x]	Niacin[x]	Pyridoxine [x]	Thiamine[x]	Biotin [x]
Control	796 a*	130 a*	54 a*	430 a*	520 a*	796 a*	130 a*	54 a**	430 a*	520 a*
0.5	761 b	121 bc	45.3 b	370 b	460 b	771 b	124.7 b	49.2 bc	390 b	500 b
1.0	733 c	114,6 c	40.4 c	320 c	410 c	748 c	118 c	45.6 c	350 c	450 c
2.0	701 d	101,5 d	34 d	260 d	350 d	719 d	112.9 d	39.8 d	310 d	400 d
3.0	665 e	89 e	28.1 e	200 e	290 e	691 e	99.4 e	33.4 e	270 e	330 e

[x] Results are the mean of 5 min mixing periods with two replicates
*, **, Different letters in the same column are significantly different at $P < 0.05$, $P < 0.01$, respectively.

activated charcoal when compared with the control sample. The losses of ascorbic acid, niacin, pyridoxine, thiamine and biotin were 13.2%, 23.5%, 38.1%, 37.2%, and 36.5%, respectively, with the treatment of 3.0 g/L granular activated charcoal. In general, activated charcoal varying from 0.5 to 2.0 g/L is used in apple juice plants (Artık *et al.* 1992; Kadakal and Nas 2002).

The effects of different doses of activated charcoal with 5 min mixing periods on the color and clearness value of apple juice are shown in Table 3. Statistical analysis of the data showed that there were significant differences ($P < 0.05$) in the color and clearness of the apple juice samples between the dosages of activated charcoal. However, no significant differences were determined in the color and clearness of the apple juice between the mixing periods.

TABLE 3.
THE EFFECT OF DIFFERENT DOSES OF POWDERED AND GRANULAR ACTIVATED CHARCOALS ON COLOR AND CLEARNESS OF APPLE JUICE SAMPLES

Activated charcoal dose (g/L)	Activated charcoal			
	Powdered activated charcoal*		Granucoal Fa*	
	Colour[xz] [440 nm]	Clearness[xz] [620 nm]	Colour[xz] [440 nm]	Clearness[xz] [620 nm]
Control	63.3 e*	97,6 e**	63.3 e*	97,6 e**
0.5	70.0 d	98.0 d	68.5 d	97.9 d
1.0	74.60 c	98.3 c	72.4 c	98.0 c
2.0	83.7 b	99.6 b	81.6 b	98.5 b
3.0	89.4 a	99.8 a	86.8 a	99.2 a

[x] Transmittance

*, **, Different letters in the same column are significantly different at $P < 0.05$, $P < 0.01$, respectively.

[z] Results are the mean of 5 min mixing periods with two replicates

Transmittance value of the control sample at 440 nm was 63.3%. The transmittance values of the juice samples increased with the treatment of activated charcoals. Linear increment on the transmittance value of the juice samples was determined as the dosages of activated charcoals were increased. The best improvement in color of the juice samples were observed with the treatment by powdered activated charcoal of 5 min at the level of 3 g/L.

CONCLUSION

(1) Use of activated charcoal for color and patulin control in the production of apple juice isn't a novel procedure in the world. Powdered activated charcoal and granular activated charcoals have decreasing effect on the water-soluble vitamin content of apple juice. If it is not necessary for color and patulin control in the production of apple juice, activated charcoal should not be used.

(2) The concentration of water-soluble vitamins in apple juice samples decreased with the increasing amounts of activated charcoal. Powdered activated charcoal was more effective than granular activated charcoal.

(3) There was a linear relationship between the concentration of activated charcoal and ascorbic acid, niacin, pyridoxine, thiamine and biotin losses of the apple juice but no relationship with the mixing period.

(4) There was a linear relationship between the concentration of activated charcoal and improvement of color and clearness of apple juice.

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