Effect of activated charcoal on patulin, fumaric acid and some other properties of apple juice

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In this study, 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g/l amounts of activated charcoal (AC) were added into apple juice with a patulin content of 62.3 ppb obtained from a well-established manufacturing company. Apple juice samples were then mixed for 0, 5, 10, 20, and 30 min, respectively. Considerable reduction in the patulin and HMF values was found while there is a dramatic improvement in the colour and clearness of apple juice. However, AC did not cause a significant decrease in the fumaric acid level of apple juice. The best result was obtained at 3.0 g/l AC mixed for 5 min. In addition, a negligible reduction in brix and pH values of samples was observed.

1 Introduction

Patulin is a mycotoxin produced by several species of *Aspergillus*, *Penicillium* and *Byssochlamys nivea* [1]. In nature, patulin is found commonly in different products and especially in apples and apple products in which these molds can grow [2, 3]. The results of experiments on animals have shown that patulin is mutagenic, carcinogenic and teratogenic. Consequently, patulin is an important quality parameter of some products in terms of human health [4].

Apple juice plants start buying apples from the middle of September and apple juice processing continues until the end of January. A first part of the apples bought during this period is processed. The other part is stored in an open area in piles. As a result of this storage, apple juice yield is reduced and the amount of patulin in apple juice concentrate increases [3, 5]. The World Health Organisation and many countries have established 50 ppb patulin as the upper recommended concentration in apple juice and apple juice concentrate [6].

Artık et al. [7] worked on the reduction of patulin level by adding dust and granule activated charcoal into the diluted apple juice concentrate with 12 brix value. In their work, it was determined that the patulin amount in apple juice decreased considerably when powdered activated charcoal at the levels of 3–5 g/l was applied for 5 min. The greatest decrease in patulin level was reported as a result of mixing the apple juice sample with 5 g/l of activated charcoal for 5 min.

In another research by *Huebner* et al. [8], ultrafine activated carbon was bonded on granular quartz to produce a composite carbon adsorbent (CCA) with a high carbonaceous surface area, good bed porosity, and increased bulk density. CCA in fixed-bed adsorption columns was evaluated for efficacy in reducing patulin levels in aqueous solution and apple juice columns containing 1.0, 0.5 and 0.25 g of CCA were continuously loaded with a patulin solution (10 µg/ml) and eluted at a flow rate of 1 ml/min. The results indicated that 50% breakthrough capacities for patulin on 1.0, 0.5, and 0.25 g CCA columns were 137.5, 38.5 and 19.9 µg, respectively. *Sands* et al. [9] determined that granular activated carbon (GAC) used in batch and fixed-bed adsorption experiments was also effective in reducing patulin levels in apple cider.

The objective of this study was to determine patulin, HMF, fumaric acid and the colour of apple juice when AC in different levels and periods were applied. It also aims to help the apple juice manufacturing industry for determining the level of AC in the production of apple juice and apple juice concentrate.

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2 Materials and methods

2.1 Materials

In this research, apple juice and AC (Granucol FA) were used as the materials. The apples used for the production of apple juice were obtained from a well-established local factory (Denizli/Turkey). The AC was provided from Erbslöh Geisenheim Inc., Germany, and it has good suspensibility when directly stirred into the solution. It was found that the AC 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.

2.1.1 Production of apple juice

The apples were smashed and pressed in a cloth bag to obtain cloudy (unclarified) apple juice. The cloudy apple juice was heated for 3–5 min at 80 °C and cooled down to 45–50 °C, and sufficient amount of pectolytic enzyme was added for the hot enzymatic reaction. Following the pectolytic treatment, measured quantities of gelatine and bentonite were added. After letting it rest for about 1–2 h, the clear apple juice was obtained by filtering the mixture.

For each treatment, 500 ml of the produced apple juice was used. The AC was added into a glass beaker at 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g/l concentrations and the experiments were performed in batch with stirring. In each case, the AC 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 AC during different periods.

2.2 Methods

2.2.1 Determination of patulin

The determination of patulin in the samples was carried out by using a Shimadzu VP series high pressure liquid chromatography apparatus, as suggested by ISO 8128 [10]. Patulin in the crystalline form was obtained from Sigma (Chemical Co). Patulin was extracted from samples of the apple juice with three times the equivalent volume of ethyl acetate. The mobile phase employed was a 10% aqueous acetonitrile solution with a flow rate of 1.0 ml/min. For the analysis, a Nucleosil 100-7 C18 250 × 4.6 mm column, a UV-VIS diode array detector (Shimadzu, model SPD-M10 Avp) set at 272 nm, a LC-10AT-VP Shimadzu HPLC pump and a Hewlett Packard 3396 Series II integrator were used.

Apple juice samples containing known amounts of patulin were spiked with different levels (25, 50, 75, 100, 200 µg/l) of patulin to determine the recovery of the extraction procedure. The average

percentage recovery of patulin in apple juice was found to be 95.6% for added levels of 25, 50, 75, 100 and 200 ppb. The levels of patulin in apple juice samples were corrected for the average percent recovery of 95.6%.

2.2.2 Determination of HMF

The analysis of HMF (Sigma, H 9877) was carried out by spectrophotometric measurement of the colour intensity at 550 nm [11] formed by HMF with p-toluidine (Sigma, T 8766) and barbituric acid (Sigma, B 0625).

2.2.3 Determination of fumaric acid

For the fumaric acid analysis, the chromatographe was equipped with a ODS 2 4.6 × 250 mm column, a UV-VIS detector diode array detector (Shimadzu, model SPD-M10 Avp) set at 210 nm and a Hewlett Packard 3396 Series II integrator [12]. Standard fumaric acid was obtained from Sigma (Chemical Co). The apple juice samples were vacuum-filtered through 0.45 µm membranes (Millipore Co.). A 5 ml aliquot was pipetted onto the Bio-Rex 5 resin bed and allowed to run through freely. The organic acids were desorbed from the resin with 5 ml 10% (v/v) H_2SO_4, followed by addition of deionized water to a final volume of 25 ml. The mobile phase employed was a phosphate buffer (0.02 M KH_2PO_4) at a flow rate of 1.0 ml/min.

2.2.4 Further determinations

Measurement of *colour* and *clearness* of 12 brix-regulated apple juice compared to distilled water was carried out by a spectrophotometer (Shimadzu UV-1201V). To measure the colour, the spectrophotometer was adjusted at 440 nm (transmittance value), whereas, to measure the clearness, the spectrophotometer was set at 625 nm (transmittance values) [13].

The *water soluble matter* (brix) was determined by using the digital refractometer (RFM 340) [14]. The *pH* (potentiometric) was measured with pH meter (WTW, pH 537) [14]. The *moisture* was determined by drying until getting a fixed weight [15] and the *ash* amount was determined by burning at 500 ± 25 °C [16]. All results were expressed as average of duplicate samples. *Total surface area* [m^2/g], *molasses factor* and *methylene blue adsorption* [%] of AC was provided from the manufacturing company.

2.2.5 Statistical analysis

Statistical analysis of the data was performed using the system developed by SAS Institute, Inc. [17]. 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.

3 Results and discussion

The effect of various mixing periods and different doses of AC on the patulin, HMF, fumaric acid, colour, clearness, pH and brix values are shown in Tables 1 and 2, respectively. Variations of patulin, HMF, fumaric acid, colour and clearness in apple juice samples treated with different doses and mixing periods of AC are presented in Figure 1–5.

The patulin content of control sample was decreased from 62.3 ppb to 26.7 ppb by the treatment of 30 min with AC at the level of 3 g/l. In general, it was observed that increasing the amount of AC decreases the patulin level in the apple juice. However, it was observed that 5 min of treatment of the apple juice with AC was sufficient with respect to other treatments. *Artık* et al. [7] determined that the application of 3–5 g/l AC was sufficient to get optimal improvement in the reduction of patulin. Treatments with AC for a longer time period have only a little effect on the reduction of patulin level (Fig. 1).

Table 1. Effect of various mixing periods with AC on patulin, HMF, fumaric acid, colour and clearness of apple juice samples.

Mixing period [min]	Patulin[z] [ppb]*	HMF[yz] [ppm]	Fumaric acid[z] [ppm]*	Colour[xz] (440 nm)*	Clearness[xz] (620 nm)**
Control	62.3 a	15.6	0.46 a	62.8 d	96.8 e
5	47.6 b	14.8	0.43 b	73.5 bc	97.7 c
10	44.2 d	14.4	0.42 c	74.0 b	97.9 b
20	47.2 c	13.8	0.41 c	74.5 a	98.0 a
30	43.4 e	12.7	0.43 b	74.0 b	97.6 d

[x] Transmittance; [y] Not significant; [z] Results are the mean of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 g/l AC doses treatments.
*, ** Values with different letters in the same column are significantly different at $p < 0.05$ and $p < 0.01$, respectively.

Table 2. Effect of different doses of AC with various mixing periods on patulin, HMF, fumaric acid, colour and clearness of apple juice samples.

AC Dose [g/l]	Patulin[y] [ppb]**	HMF[y] [ppm]*	Fumaric acid[y] [ppm]**	Colour[xy] (440 nm)**	Clearness[xy] (620 nm)**
Control	62.2 a	15.6 a	0.46 a	62.8 g	96.8 g
0.5	56.4 b	15.2 b	0.44 b	67.9 f	97.3 f
1.0	47.8 c	14.1 d	0.42 c	71.6 e	97.7 e
1.5	45.5 d	14.5 c	0.42 d	75.0 d	97.9 d
2.0	40.6 e	13.7 e	0.41 d	77.4 c	98.1 c
2.5	35.8 f	12.5 f	0.41 d	80.4 b	98.4 b
3.0	30.8 g	11.8 g	0.39 e	82.9 a	98.4 a

[x] Transmittance; [y] Results are the mean of 0, 5, 10, 20, 30 min mixing periods.
*, ** Values with different letters in the same column are significantly different at $p < 0.05$ and $p < 0.01$, respectively.

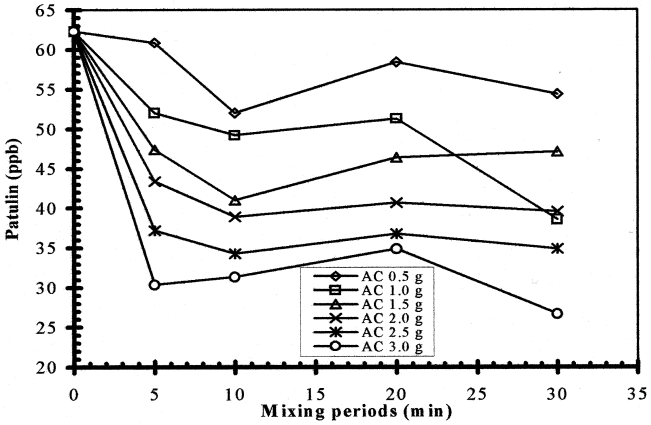

Figure 1. Variations of patulin contents of apple juice samples treated with different AC doses and mixing periods.

The HMF content of apple juice decreased with the amount of AC applied. Approximately, a maximum reduction in the HMF value was observed in the first 5 min and little reduction was observed after that. *Artık* et al. [5] determined that the HMF content of the apple juice decreased from 80.4 mg/l to 24.9 mg/l by applying AC (Fig. 2).

The fumaric acid contents of all samples were decreased by the treatment with AC of the apple juice. However, this decrease is insignificant for the apple juice factories. As for the patulin and HMF levels, the fumaric acid content decreased with the treatment by AC of 5 min. In general, the level of

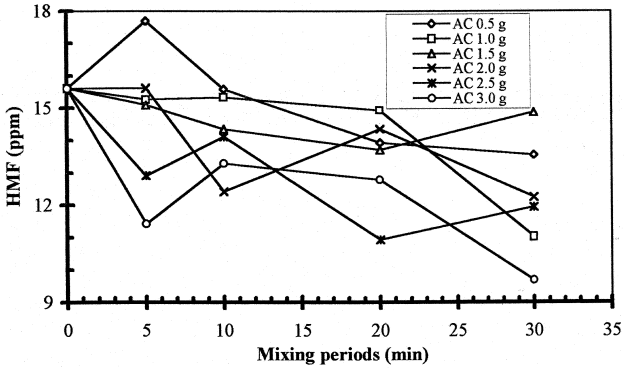

Figure 2. Variations of HMF contents of apple juice samples treated with different AC doses and mixing periods.

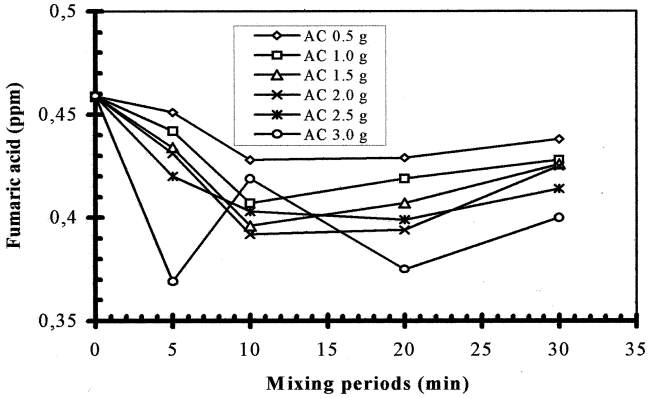

Figure 3. Variations of fumaric acid contents of apple juice samples treated with different AC doses and mixing periods.

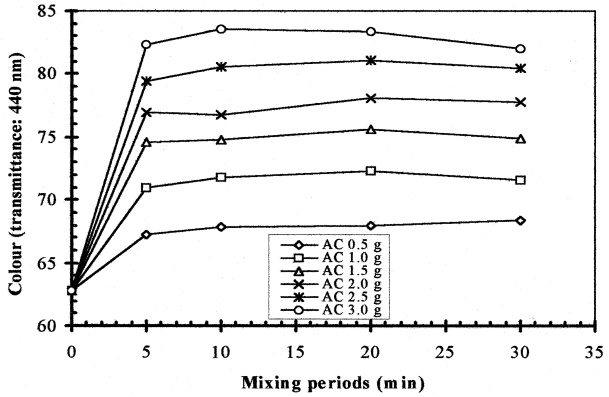

Figure 4. Variations of the colour of apple juice samples treated with different AC doses and mixing periods.

fumaric acid does not change when the treatment with AC is carried out for a longer period (Fig. 3).

The transmittance value measured in 12 brix-diluted apple juice on the spectrophotometer at 440 nm wavelength should not be under 40% for some marketing reasons. The best improvement in colour was observed with the treatment by AC of 10 min at the level of 3 g/l. The transmittance values of the samples increased and absorbance values decreased with the treatment of AC. A linear increment on the transmittance value and a linear decrease of the absorbance value were determined as the dosage of the AC was increased (Fig. 4).

Transmittance and absorbance values of the control sample at 625 nm were 96.8% and 0.014, respectively. The best improvement of the clearness of apple juice was obtained with

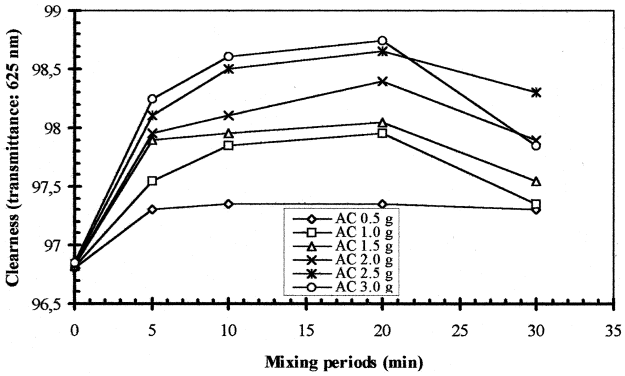

Figure 5. Variations of the clearness of apple juice samples treated with different AC doses and mixing periods.

the treatment of 10 min with AC at the level of 3 g/l. Transmittance and absorbance values were 98.8% and 0.005 respectively, with the treatment of 20 min with AC at the level of 3 g/l (Fig. 5). Furthermore, there is a linear relationship between colour and clearness and the amount of AC. However, *Artık* et al. [5] expressed a linear relationship between the concentration of powdered activated charcoal and colour improvement but non-linear relationship with the degree of clearness of the apple juice concentrate. But the length of the mixing period did not seem to have any effect on the colour and clearness. The pH ranged from 3.92 to 3.86 and brix from 34.6 to 35.0.

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