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Science and Technology

Meri-Rastilantie 3 C, FI-00980
Helsinki, Finland
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Journal of Food, Agriculture & Environment Vol.5 (1) : 12-16. 2007 *www.world-food.net*

Reduction of patulin content in apple juice concentrate using activated carbon and its effects on several chemical constituents

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Received 03 October 2006, accepted 11 January 2007.

Abstract

Patulin, a mycotoxin produced by various species of *Penicillium* and *Aspergillus* fungi, is often detected in apple juices and ciders. Studies done on laboratory animals have demonstrated that patulin has a broad spectrum of toxicity, including mutagenicity and carcinogenicity. The effect of powder and granular forms of activated carbon in reducing patulin in apple juice concentrate and also on the qualitative characteristics of apple juice was studied by means of factorial design using completely random design (CRD). In powder form, activated carbon resulted in complete reduction of patulin content in apple juice up to concentration of 5 g/l with contact times of 5, 15 and 30 minutes, and with carbon concentration of 3 g/l, the patulin reduction within 5, 15 and 30 minutes was nearly 94, 96 and 97% respectively. Results also showed that use of activated carbon adversely affected the apple juice qualitative characteristics. However, it seems more likely that these effects could be easily solved and would make no remarkable difficulties in apple juice industry. The research revealed that the best results were achieved by treatment with activated carbon in its powder form and at concentration of 3 g/l with a 5 min contact time. In this way the patulin content was reduced to an acceptable level and the negative effect on apple juice qualitative characteristics is negligible.

Key words: Patulin, apple juice concentrate, activated carbon, high performance liquid chromatography (HPLC).

Introduction

Patulin (4–hydroxy–4H-furo(3,2c)pyran–2(6H)–one) is a mycotoxin produced by several species of *Penicillium* and *Aspergillus* fungi, of which *P. expansum* is the most important common contaminant of damaged fruits such as apples [4, 6, 9]. Patulin (structure shown in Fig. 1) is heat resistant, stable in dilute acids and labile in alkali [9]. For first time, the metabolite that was produced by *P. expansum* and *P. patulum* was named patulin [1].

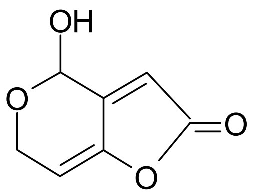

Figure 1. Chemical structure of patulin.

Many fungi found in spoiled food, such as *Aspergillus* spp. and *Penicillium* spp., *A. clavatus*, *A. giganteus*, *A. terreus*, *P. urticae*, *P. expansum* and *Byssochlamys nivea*, produce patulin frequently when they invade apples, pears, peaches and berries. Because it is particularly produced by the apple-rotting fungus *P. expansum*, it is a toxicological concern in apple products such as apple juice [5, 11-14]. According to studies [5] conducted over the past twenty years, only apple products, especially apple juice, were found to be easily contaminated by patulin naturally, therefore the patulin level has been regarded as a quality indicator for fruit used in the processing of apple juice [5, 11-14]. Patulin is quite stable in acidic media at temperature of 125°C with pH levels ranging between 3.5 and 5.5. We are unable to get the toxin-free products by means of industrial processing for apple products through vacuum distillation concentration and high temperature short time (HTST) pasteurization treatment at 90°C for 10 seconds, which reduce 18.4 and 18.8% of patulin in the apple juice. Patulin content decreased with little extent once apple juice being packed and stored. Patulin also disappeared in three weeks with addition of ascorbic acid to patulin-contaminated apple juice, but this could be attributed to some unidentified toxins resulting from this reaction. However, fermented apple juice such as apple cider was less contaminated due to degradation of patulin by yeast (*Saccharomyces* spp.) into primary non-volatile, water-soluble substances [9, 19].

In the United States, the Office of Environmental Health Hazard Assessment has listed patulin as a potential carcinogen [9]. Studies have demonstrated that patulin has a broad spectrum of toxicity, including mutagenicity and carcinogenicity [9]. Regulations for apple juice in several European countries recommended a maximum permitted level of 50 µg/l, as suggested by WHO [1, 9].

In comparison with the other fruit juices, patulin is more stable in the apple juice. The stability is due to low pH and low existence of sulfhydryl groups in it. Therefore, apple juice is more liable to fungal contamination and finally to patulin production [9]. Patulin is produced by *P. expansum* at temperatures of 5-20°C and its production at 30°C is negligible [19]. The production of patulin is generally associated with the damaged surface tissue of fruits. Removal of the decayed portions from raw fruit prior to further processing has reduced patulin concentrations in the final fruit juice significantly [1, 20, 22]. Also past researches have shown that patulin levels in apple juice and cider can be reduced during alcoholic fermentation [9, 19, 20], pasteurization [21], addition of ascorbic acid [2], irradiation treatment [22, 23] and use of activated carbon [1, 8, 17, 22, 23]. Research of Sands *et al.* [15] revealed that use of activated charcoal for the removal of patulin from cider is effective. Also other researchers showed that effects of activated carbon for reduction of patulin is completely favourable [1, 6].

In the present study, activated carbon in two forms (powder and granular) was used for reduction of patulin in apple juice concentrate. The aim of this study was to investigate the possibility of applying activated carbon for the reduction of patulin and its effects on several chemical constituents of apple juice concentrate.

Materials and Methods

Materials: In this study, the apple juice concentrates (from East Azarbaijan, Iran) and chemical materials such as ethyl acetate (GR), acetonitrile (HPLC grade), acetic acid (GR), sodium carbonate (GR) and pure crystalline patulin were obtained from Aldrich Company (Germany). Water used in all the experiments was doubly distilled and deionized. Activated carbon was obtained from Merck Company.

A stock standard solution of patulin was prepared by dissolving 5 mg of pure crystalline patulin in 25 ml of chloroform. A 100 µl volume of this solution was transferred into a 10 ml volumetric flask and evaporated just to dryness under a stream of nitrogen at room temperature. The residue was immediately dissolved in 10 ml of water and acidified with acetic acid. Working standard solutions were prepared by appropriate dilution of the above solution with water.

Use of activated carbon in apple juice concentrates for removing of patulin: The effect of activated carbon in reducing patulin in apple juice concentrates, and also on the qualitative characteristics of apple juice has been studied utilizing both powder and granular forms by means of factorial design with CRD. The samples of apple juice concentrates were diluted to 12°Brix and contacted with concentrations of 1, 3 and 5 g/l activated carbon for 5, 15 and 30 minutes. Apple juice and activated carbon samples were agitated by using a magnetic stirrer at the same mixing rate. The samples were filtrated by Whatman filter paper and kieselguhr filter. Qualitative characteristics of apple juice samples such as Brix (refractometric method), colour [16], pH, acidity [3] and total sugar [16] after treatment with activated carbon were achieved.

Determination of patulin: Patulin was extracted from 10 ml apple juice with three times the equivalent volume of ethyl acetate. The organic phases were combined and extracted with 2 ml of 1.5% sodium carbonate solution by shaking for 15-30 seconds and the lower phase separated. The supernatant was decanted to another flask and 2 drops of acetic acid added to flask, then evaporated to dryness in a water bath at 40°C under a gentle stream of nitrogen. The residue was immediately dissolved in 1 ml of distilled water and injected into column in HPLC apparatus.

The standard curve (y = 117/74 x + 33/189) with a linear regression coefficient (R^2 = 0.9987) (Fig. 2) was obtained. Linearity was observed over concentration range of 1-50 µg/l. The patulin content was determined by using HPLC with UV-detector at 276 nm [4, 7, 9, 18] and the results are shown in Figs 3-4. A 4.6 * 250 mm RP-C18 column and 5% aqueous acetonitrile solution at a flow rate of 1 ml/min were used.

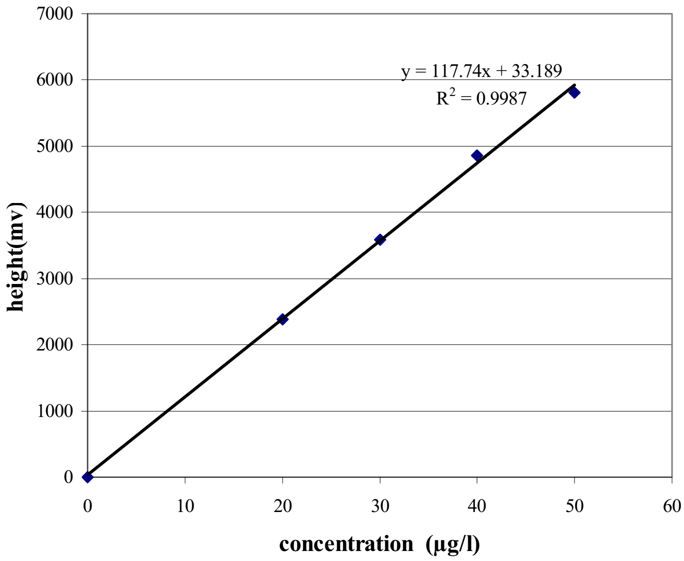

Figure 2. Standard curve of patulin.

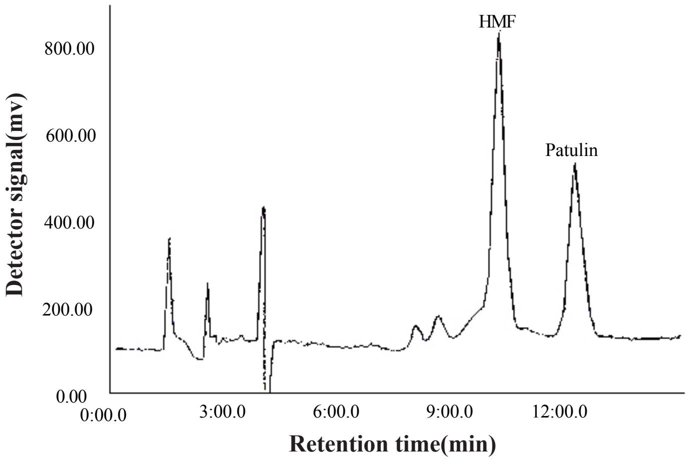

Figure 3. The chromatogram of patulin in apple juice as blank sample that have been measured by HPLC (HMF hydroxy methyl furfural).

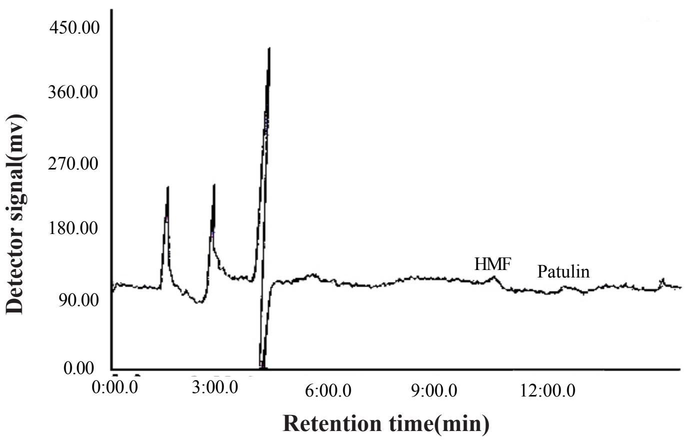

Figure 4. The chromatogram of patulin that have been measured by HPLC in apple juice at treatment combination A1B3C3 (A1 powdered activated carbon, B amount of activated carbon 5 g/l and C contact time duration 30 min).

Results and Discussion

As the results reveal, patulin is produced by several species of *Penicillium*, *Aspergillus* and *Byssochlamys* fungi which often grow on the fruits such as apples, pears and peaches [9]. In this study we used two types of activated carbon for reduction of patulin and different factors have been studied. These factors include the type of activated carbon (Factor A), quantity of activated carbon (Factor B) and contact time duration (Factor C), also other parameters supposed as fixed ones. There were significant differences at 5% level in characteristics of apple juice concentrate treated by activated carbon between different levels of activated carbon, the type of activated carbon, contact time duration and interaction between them. Our results show that the use of powdered activated carbon is very effective in declining the patulin content in the apple juice, but the smallest particles make the filtration difficult from apple juice and for this reason kieselguhr filter is required.

Figs 5-9 show the interaction of activated carbon type and its content at qualitative characteristics according to patulin, total sugar content, Brix, colour decline and decrease of apple juice acidity. As these figures show, treatment combination A_1B_3 has the greatest influence in declining of measured characteristics in apple juice. Significant differences at the level of 5% exist between those characteristics and other treatment combination, also our results confirm that the effect of activated carbon content and type of activated carbon in pH is non-significant. Also interaction between type of activated carbon and contact time duration, and activated carbon content and contact time duration was significant at the level of 5%. Interaction between pH and acidity was not significant. Three-way interaction between type of activated carbon (Factor A) the content of activated carbon (Factor B) and contact time duration (Factor C) was significant at the 5% level except for pH and acidity. Figs 10-13 show interactions between Factors A, B and C on measured characteristics.

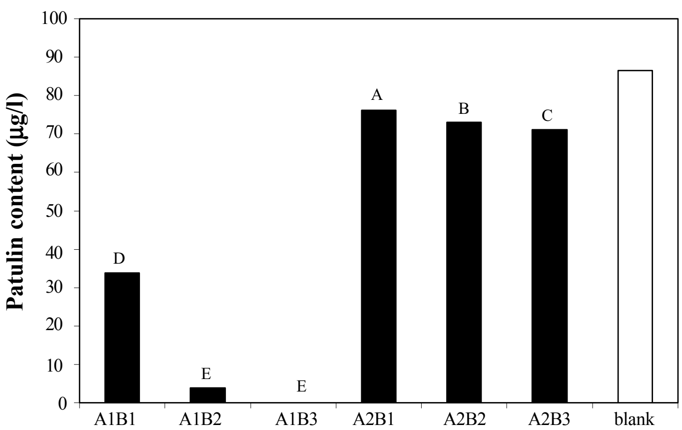

Figure 5. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1 (B1), 3 (B2) and 5 (B3) g/l, on patulin content of apple juice.

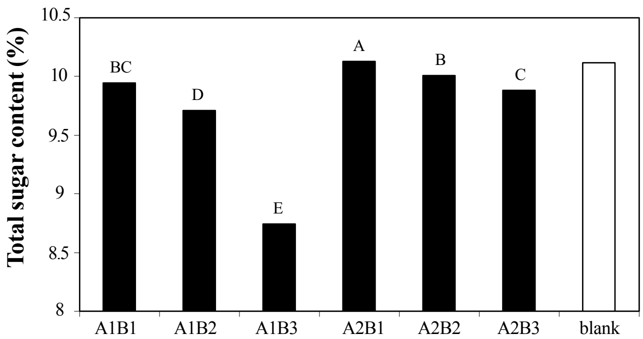

Figure 6. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1(B1), 3(B2) and 5(B3) g/l, on total sugar content of apple juice.

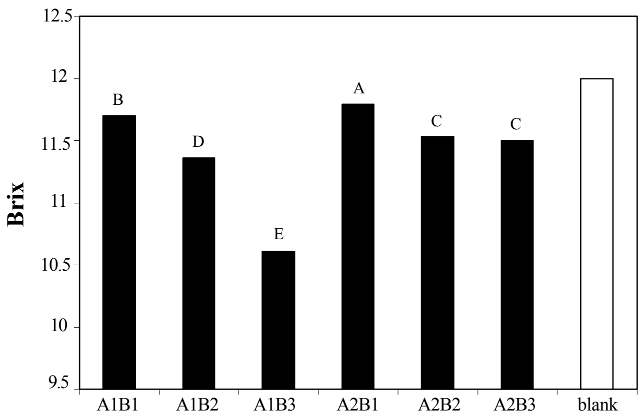

Figure 7. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1 (B1), 3 (B2) and 5 (B3) g/l, on Brix of apple juice.

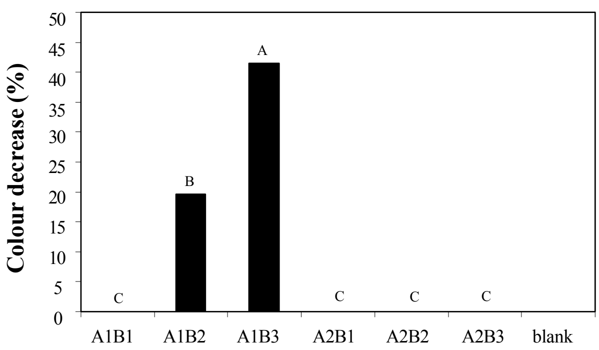

Figure 8. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1(B1), 3 (B2) and 5 (B3) g/l, on colour decrease of apple juice.

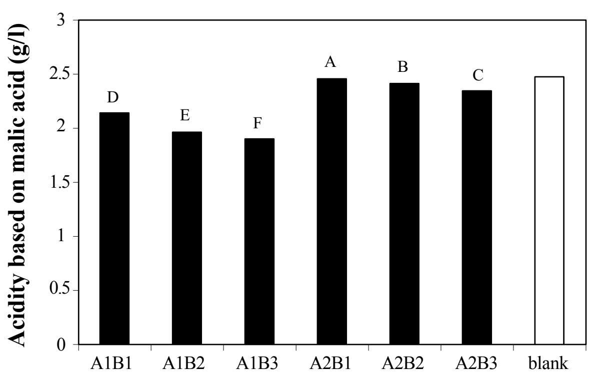

Figure 9. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1 (B1), 3 (B2) and 5 (B3) g/l, on acidity based on malic acid of apple juice.

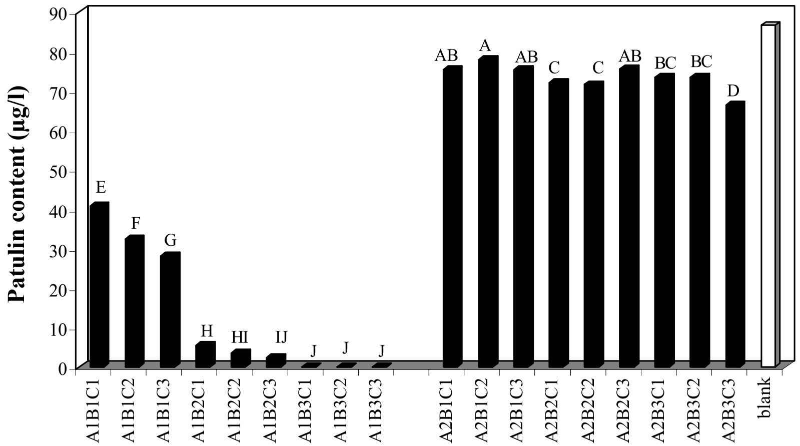

Figure 10. Effect of powdered (A1) and granular (A2) activated carbon, in amount of 1 (B1), 3 (B2) and 5 (B3) g/l applied for 5 (C1), 15 (C2) and 30(C3) min, on patulin content of apple juice.

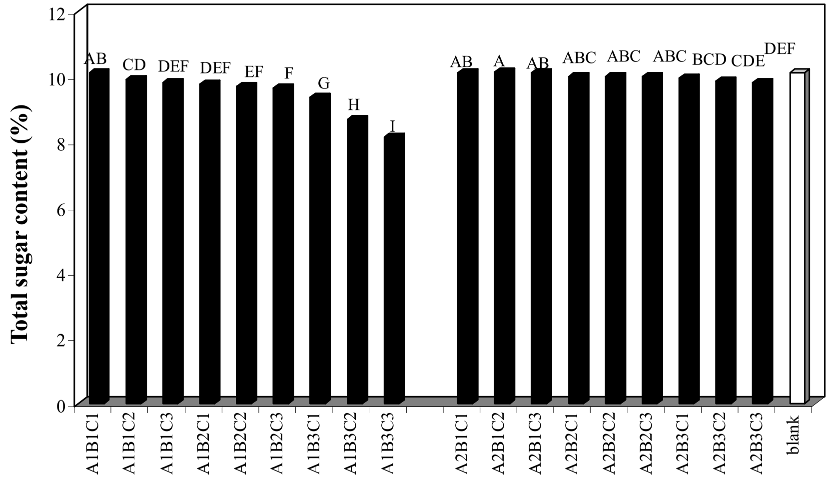

Figure 11. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1 (B1), 3 (B2) and 5 (B3) g/l applied for 5 (C1), 15 (C2) and 30 (C3) min, on total sugar content of apple juice.

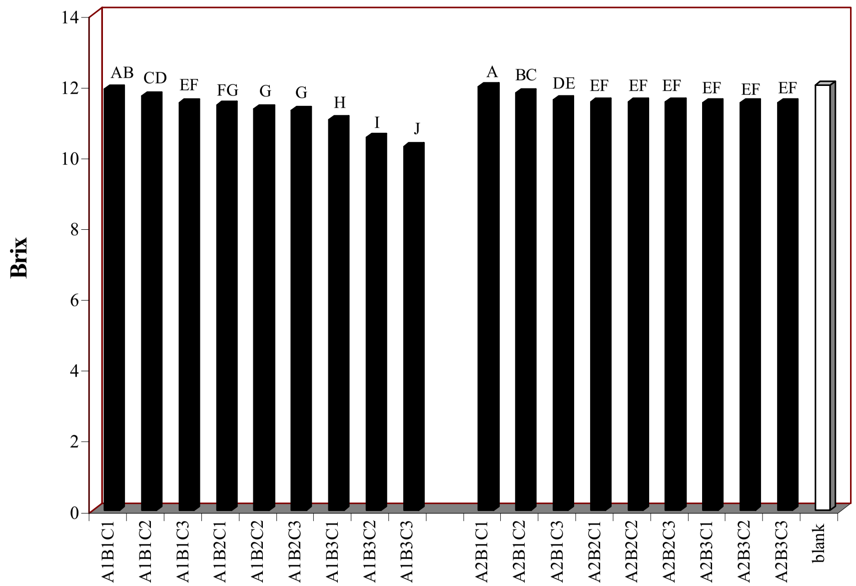

Figure 12. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1 (B1), 3 (B2) and 5 (B3) g/l applied for 5 (C1), 15 (C2) and 30 (C3) min, on Brix of apple juice.

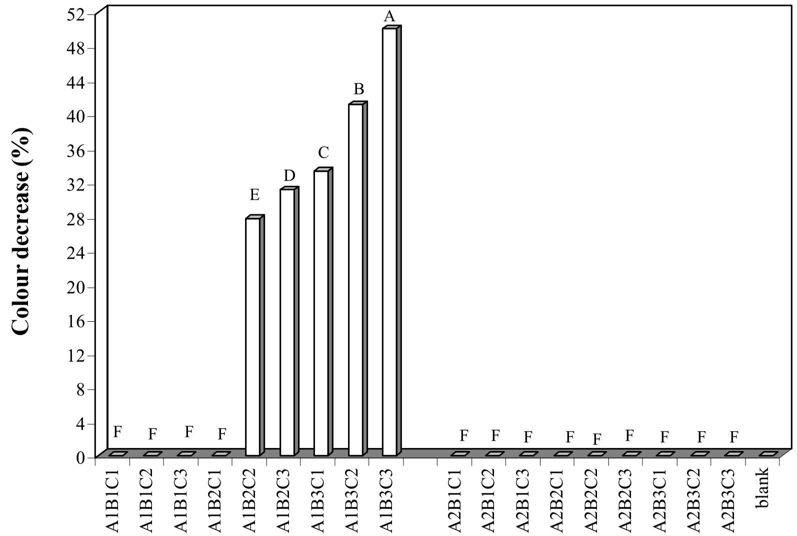

Figure 13. Effect of powdered (A1) and granular (A2) activated carbon, in amounts of 1 (B1), 3 (B2) and 5 (B3) g/l applied for 5 (C1), 15 (C2) and 30 (C3) min, on color decrease of apple juice.

Conclusions

In this research the possibility of decline in the patulin levels by using of activated carbon was investigated. The amount of patulin toxin in apple juice due to bad conditions of apple storing may increase, which is hazardous and illegal. So our aim in this research was to find a good way to keep the product with a maximum level of 50 µg/kg patulin content. In this research after dilution of apple juice concentrate to 12ºBrix the samples were treated with activated carbon to reduce the amount of patulin to an acceptable level. In all samples there was a significant decrease in patulin content, especially when activated carbon was used in the powder form, this reduction was more significant. The maximum concentration of activated carbon was shown to be 5 g/l while its optimum effects occurred in concentrations within 3-5 g/l. Results indicated that using activated carbon is effective on reducing the qualitative characteristics of apple juice such as Brix, color, pH, acidity and total sugar after treatment with activated carbon, but it was more significant when the contact time and activated carbon concentration increased. Using of 5 g/l powdered activated carbon with contact times of 5, 15 and 30 minutes resulted complete remove of apple juice in patulin content, whilst in 3 g/l, the patulin reduction within 5, 15 and 30 minutes was nearly 94, 96 and 97% respectively. The results indicated that use of activated carbon also affected the apple juice qualitative characteristics, but it seems more likely that these effects could be easily solved and would make no remarkable difficulties in apple juice industry. The research revealed that the best treatment with powdered activated carbon was a concentration of 3 g/l with a 5 minute contact time. In this way, the patulin content is reduced to an acceptable level whereas its unfavourable effects on apple juice qualitative characteristics are negligible.

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