OCHRATOXIN A CONTAMINATION IN VINEGAR[1*]

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin that is often produced by mold species such as *Aspergillus ochraceus, A. carbonarius, A. niger* and *Penicillium verrucosum*. Ochratoxin-producing molds widely grow up and form toxins in raisins, figs, coffee beans and grains. In this study, the presence of OTA in home-made vinegars was examined with high performance liquid chromatography (HPLC). 88% of vinegar samples were OTA negative. In one of the samples examined, the amount of OTA is above 2 µg/L, which is the limit value. OTA is a mycotoxin with nephrotoxic properties. OTA, which enters the body, is absorbed from the intestines and enters the blood and accumulates by transporting blood to tissues and organs. The organ most affected by OTA is the kidneys, which may lead to a kidney disease called nephropathy. The results obtained in this study showed that there was a low amount of OTA in vinegars made at home. Since the presence of OTA in vinegar may pose a health risk, the necessary precautions should be taken by examining the factors affecting the formation of OTA.

Keywords: *Ochratoxin, Vinegar, Health, Mycotoxin, Disease*

INTRODUCTION

Ochratoxin A (OTA) is a toxic compound produced by certain species of mold. In the genus *Aspergillus, A. ochraceus, A. carbonarius* and *A. niger* are the most well-known OTA producers. In the genus *Penicillium*, *P. verrucosum* and *P. nordicum* is the major species producing OTA **[1]**. OTA is an isocoumarin pentaacetate, chlorine-containing mycotoxin. Ochratoxin B (OTB), other derivatives of ochratoxin, does not contain chlorine, while ochratoxin C (OTC) is the ethyl ester of OTA **[2].** Figure 1 shows the chemical structure of OTA. It is well soluble in polar organic solvents such as methanol, ethanol, chloroform, very slightly soluble in water however, insoluble in petroleum and saturated hydrocarbons. It has a weak acidic property. In people who eat food and beverages contaminated with OTA, the kidneys are especially affected more than other organs. It can lead to kidney disease, which is known as lethal. Long time exposure to OTA with food and beverages can increase its nephrotoxic effect. This condition has been common, especially in Balkan countries, and this disease has been called Balkan Endemic Nephropathy (BEN) **[1]**. As a result of storing grain in improper conditions in villages, it was determined that the amount of OTA increased and led to disease **[3].** But rec ent studies have shown that this condition encountered in the Balkans is related to exposure to toxins other than OTA

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[4]. Studies on the toxic properties of OTA have shown that OTA suppresses the immune system (immunotoxin), affects the liver (teratogenic) and is a potential carcinogen **[5].** OTA can be found in body fluids such as blood, urine and breast milk. Because of this property, body fluids are used as biomarkers in people's exposure to OTA **[6, 7].**

OTA has been found to be positive in many foods, solid and liquid. It has been proven to exist in various cereals **[8].** Moreover, the presence of OTA was determined in fruits such as dried figs, raisins; in beverages such as fruit juices, beer and wine; in products such as cocoa and coffee **[9, 10].** The amount of OTA in raisins is more than 50% of the amount of OTA in fresh grapes. For example, the highest amount of OTA in fresh grapes is 1.5µ/kg, while in raisins this amount is 98 µg/kg **[11, 12].** In general, the amount of OTA in wine was also very low compared to raisins when the wines produced in various countries were examined. The highest OTA amounts found in wine were 0.815 µg/L in Turkey; 0.09 µg/L in Greece; and 0.144 µg/L in Spanish wines **[13, 14, 15].**

Vinegar is used to give flavor to food, salads and sauces and is not considered as basic foodstuff. However, it is known that making vinegar at home is common due to the use of excessive fruits on the one hand, and the health benefits of vinegar on the other. In addition, vinegar is used as a beverage by some consumers. In our country, there is no study on the presence of OTA in vinegar. The aim of this study was to examine the presence of ochratoxin A in home-made vinegar from different fruits.

MATERIAL

Vinegar samples were supplied from various provinces in their original containers and kept in the refrigerator till analyzed.

METHOD

Determination of OTA in Vinegar

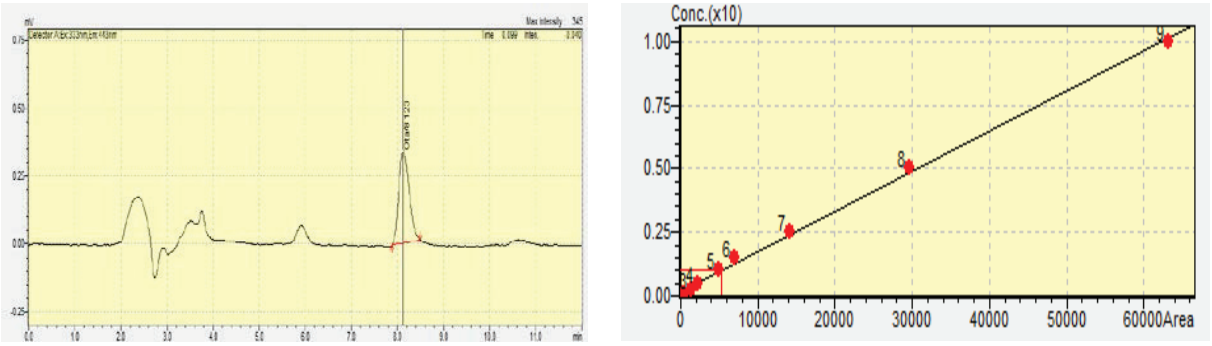
OTA analysis in vinegar samples was determined by High Performance Liquid Chromatography (HPLC) system (Shimadzu serial no: L20225219693, model LC-20A) as specified in R-Biopharm application guide. HPLC requirements for OTA determination are given below. Mobile phase: acetonitrile:water:acetic acid (51: 47: 2 v /v/v); column: C-18 ODS-2 column (25cm x 4.6 mm x 5µm); fluorescence detector: excitation: 333 nm, emission: 443 nm; flow rate: 1 mL/min; injection volume: 100 µL; pressure: minimum 0 bar, maximum 300 bar and column temperature: 40 °C.

Vinegar samples are filtered and 10 mL samples are taken. Samples are adjusted to 7,8 with 2M NaOH. It is centrifuged at 1600 rpm for 10 min by adding 10 mL phosphate buffered saline (PBS) on it. 10 mL of supernatant (upper phase) is taken and passed through the Ochraprerp immunoaffinite column. With 20 mL of PBS, the flow rate is 5 mL per minute so that the immunoaffinity column is washed. 1.5 mL of methanol: acetic acid (98:2 v/v) mixture is added to the column and OTA is recovered. 1.5 mL of water is added to it, vortexed, passed through a 0.45 µm filter, and 1.5 ml of mixture is taken to a glass vial. 100µl is injected into the HPLC device. For OTA analysis, the retention time in the column is approximately 12 minutes. OTA recovery value in vinegar was determined as 86% (dilution factor=0.3).

RESULTS AND DISCUSSION

There is no legal regulation for OTA in vinegar in the Turkish Food Codex. Therefore, the results obtained from the study were evaluated by taking into account the limit value (2 µg/l) for vine and fruit

vines in Turkish Food Codex **[16].** 88% of vinegar samples analyzed by HPLC were found to be OTA negative. In one of the OTA-positive samples, the amount of OTA is higher than 2 µg/L, which is the limit value. Figure 1 shows the chromatogram (1 µg/L) and calibration curve of the OTA standard, and Figure 2 shows the chromatogram of the OTA positive vinegar sample.



a) **b)**

Figure 1. Chromatogram of OTA standard (a), Calibration curve of OTA standard (b)

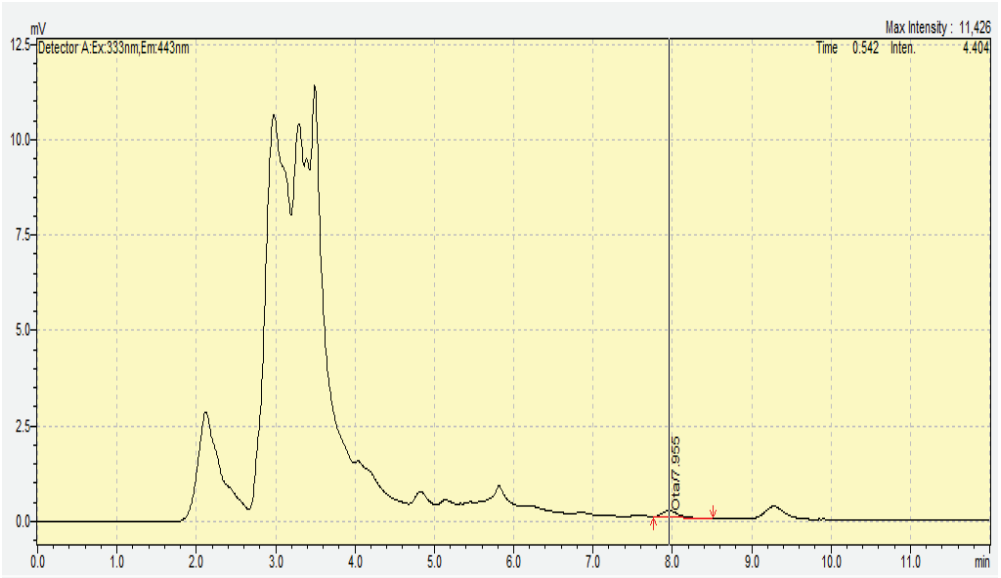


Figure 2. HPLC chromatogram of OTA positive vinegar sample

Ochratoxin A is a nephrotoxic, genotoxic, teratogenic, immunotoxigenic and possible carcinogenic mycotoxin. Although vinegar is not a basic foodstuff, it is often used to give flavor to soups, salads, sauces and meals. In addition, it is also known that today, especially home-made vinegar is considered as a drink. In this study, a large proportion (88%) of home-made vinegars

were found to be OTA negative and only in one sample were found to be higher than the OTA limit value. However, since the presence of OTA in vinegar can pose a risk to public health, the factors affecting the formation of toxins should be examined. The raw materials and the equipment used during the production of vinegar at home, and environmental factors should be taken into account. In this study, people from whom vinegar samples were provided with positive results were contacted and it was determined that these people used fruits that were not suitable for table use, fell from trees and remained on moist soils in hot climate conditions. Moldy and damaged fruits should not be used in vinegar production. After the vinegar production is completed, the fruits should be separated and the vinegar should be kept in a cool environment in this way.

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