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THE QUANTITATIVE EVALUATION OF ALTERNARIA TOXINS IN APPLE AND TOMATO JUICES

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Keywords: Alternariol (AOH), Alternariol monomethyl ether (AME), Tenuazonic acid, (TEA), tomato juice, apple juice.
Abstract: In this study we aimed to quantify the alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TEA) in 10 different brands of tomato and apple juices by HPLC (high pressure liquid chromatography). In tomato juice, it was found that AOH quantity varies in a range of $1{,}95x10^{-3}$ - $8{,}30x10^{-3}$ µg/mL, AME of $1{,}22x10^{-4}$ - $4{,}28x10^{-2}$ µg/mL and TEA between $4{,}0x10^{-2}$ and 2,38 µg/mL. In apple juice, it was determined that the range of AOH quantity is $6{,}11x10^{-4}$ - 0,25 µg/mL, the AME quantity is $2{,}03x10^{-4}$ – $3{,}92x10^{-3}$ µg/mL and the range of TEA is 0,32 -9,62 µg/mL. The differences among tomato juice and apple juice samples, may have resulted from the fruit’s harvest time, harvest type, transport, processing, storage and shelf life in markets.

INTRODUCTION

The effects of mycotoxins on human health can be explained by susceptibility to mycotoxins, the nutritional status of the individual and the individual's resistance. The largest source for Mycotoxicoses is determined by the inappropriate storage and packagıng conditions of foods and nutrients. In Europe compared to Turkey, from many years ago there are rules and strategies to control and prevent mold growth on the seeds, plants or fruits during storage and harvestıng, (Anonymous, 1979).

Microbial secondary metabolites have various biological activities and they are functioning as hormones, antibiotics, toxins, anti-migraine or anti-cancer agents and even as insecticides (Küçük et al, 2003). Because most of them are small molecules with low molecular weights, they can be spread by wind, conducting to extended infected areas. *Alternaria* species can be found in the vegetal decomposing material being normally dispersed in natural ways, affecting the aerial plants organs. Most of the Alternaria species are plant pathogenes that could cause damage; the rest are post-harvest pathogenes of a wide variety of fruits and vegetables (Barkai-Golan, 2001).

Alternaria alternata (Fr.) Keiss recorded among the most widely known agricultural products are generally known as a kind of fungi group remained unstudied, formerly known as *A. tenuis* Neesauct. These genera is known as saprophyte in food and feed products, but it is also recognized as a common pathogen in harvested fruits and vegetables in cold conditions conducting to a decreased plants growing capacity and post-harvest economic losses (Barkai-Golan, 2001). Fruits and vegetables can be contaminated by different pathogens and the many stages of pathogenesis can create a variety of toxic metabolites which has not been identified as *Alternaria spp.* (Panigrahi, 1997). Tenuazonic acid, alternariol, alternariol mono-methyl ether, altenuen and altertoksin-I are toxic metabolites produced by alternaria fungal infection in fruits and vegetables.

A. alternata species is considered the most important mycotoxins producer, as well as many other *Alternaria* species are known to produce these mycotoxins. The most common type of *Alternaria alternata* attack strategy on the harvested products, because cannot penetrate the plant cuticle or epidermis of the host plant, it uses calyx to get inside thru the wound or injured tissue. Such favorable environments for *Alternaria* attack often occur during harvest and collection. The calyx which started the wound infection in tomatoes, is a common pathogen for the *Solanaceae* family representatives like peppers and eggplant. (Barkai-Golan, 2001; Dennis, 1983; Morris et al, 2000)

The fungus can also enter into many fruits from tears formed in the open calyx, including the unharvested cherry fruit (Snowdon, 1990). Even if Alternaria core rot, in apples, is often associated with *A. alternata*, the recent studies conducted in South Africa, showed that Alternaria isolate *A. infectoria*, all types of *A. arborescens* and *A. tenuissi* are associated with this disease (Serdani et al, 2002).

Alternaria alternata is a major pathogenic in Vitis sp. causing infections which usually starts from the apical meristem after grapes harvesting (Hewitt, 1974). Was recorded in Argentina as an important pathogen in 80% of wine grapes and fruits collected (Magnoli et al, 2003). Is not considered an important pathogen for strawberries and raspberries and with an reduced influence on blueberries and gooseberries, remaining the main pathogen for grapes (Smith and Moss, 1985; Wright et al, 2004).

Alternaria is commonly found in fruit and vegetables used for human consumption, inducing a high levels of toxicity, during harvesting and storage. The analysis of the exposure degree to those hazards is required followed by a frequent monitoring of fruit juice and other fruit-derived products (Bottalico and Logrieco, 1993; Stinson et al, 1980).

The purpose of this study is to detect the amount of alternariol, alternariol mono-methyl ether and tenuazonik acid mycotoxins produced by *Alternaria spp*. in apple and tomato juices available on the market.

MATERIALS AND METHODS

In this study, in order to examine Alternaria 1 liter containers of 10 different brands of tomato and apple juice available on the Turkish market, a total of 20 samples were used as experimental material. Test materials were collected from Elazig, Malatya, Antalya, Ankara and Istanbul provinces in their original packaging from different markets in February 2008 and analyzed before the expiration date. During the study, all the samples were kept in the appropriate storage conditions for 7 days.

Analytical grade methanol, chloroform, anhydrous sodium sulfate, and hepta hydrate zinc sulfate and HPLC grade methanol were obtained from *Merck (Darmstadt, Germany)*. The alternariol, alternariol monomethyl ether and Tenuazonic acid standards were purchased from *Sigma (St. Louis, MO, USA)* in February 2008.

The HPLC system consisted of a SPD-10A VP liquid chromatograph (*Shimatzu, Japan*) equipped with an UV detector (model SPD-10AVP). The analytical column was Inertsil ODS-3, 150 mmx4.6mm ID 5µm. The sample and standards solutions were sonicated for 30 seconds before injection into the chromatograph. The mobile phase was methanol/water (80:20) containing 300 mg ZnSO4.H2O/L, 0,7 ml/min. The wavelengths for recording chromatograms were 250nm and 280nm.

A calibration curve was constructed for quantification purposes using the toxin standards and correlating peak-area versus concentration. The peak identity was confirmed by means of comparing the spectrum of the standard with the presumptive positive peak in the sample after normalization. Quantification limits of the method were taken as the minimum amount of the toxin detected in the product that allowed for confirmation by the multiple wavelength detector. The detection limits of the pure toxins by the UV detector were measured as three times the baseline standard variation under the same conditions employed for all the analyzed products.

By using the equation $C_1xV_1 = C_2xV_2$ with dilutions at different concentrations, have been prepared and graphs of study plotted: 50ml of tomato and apple juice mixed in 150ml absolute methanol for 3 minutes and filtered. To the filtered solution, 60ml 10% ammonium sulphate were added and filtered again. In the final filtrate, at 8ºC, 50ml of pure water were added. The final volume of 200ml was divided in two separation funnels and 40ml chloroform were added and shacked for 2 minutes. After the chloroform separation within funnels, the samples were incubated at 35ºC on the shaking unit. 2ml absolute methanol were added on the residues and filtered on the sodium sulphate. The samples were transferred to 2ml HPLC vials for quantitative analysis performed accordıng to the direct comparison method:

The standards were analyzed in 6 µl volumes of 50 ppm transferred in 2ml HPLC vials by HPLC ODS-3 (150 mm X 4.6 mm ID 5µm). The UV detector has a 25°C column temperature, a 280 nm detection wavelength and 1 ml/min of the mobile phase flow rate. The standard peak for Alternariol (AOH) was recorded at 3,7 minutes, for Alternariol mono-methyl ether (AME) at 7,5 minutes and for tenuazonic acid (TEA) at 1,6 minutes (Scott et al, 2001) as shown in the chromatogram presented in Figure 1 .

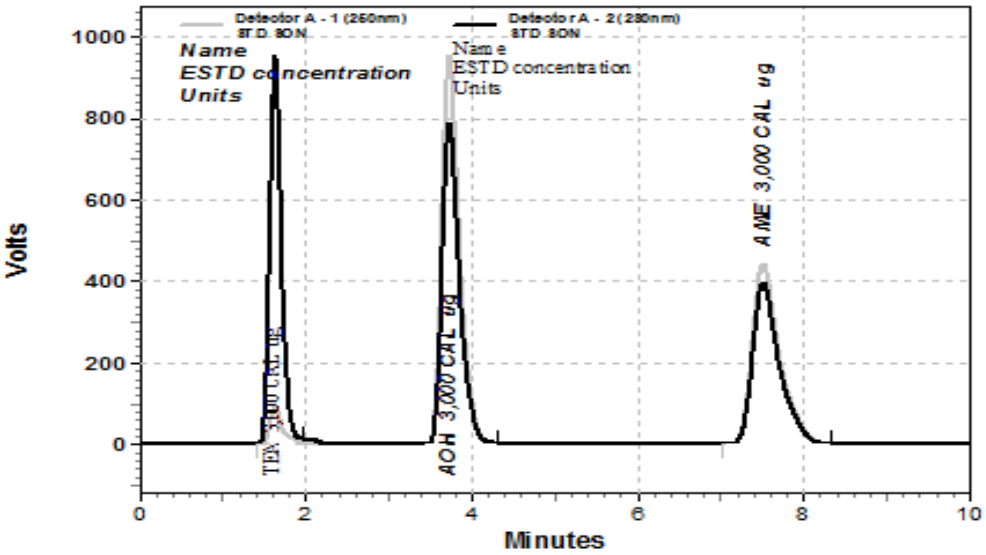

Figure 1. The standard peak for Alternariol (AOH), Alternariol mono-methyl ether (AME) and tenuazonic acid (TEA).

RESULTS AND DISCUSSIONS

The amounts of AOH, AME and TEA isolated from tomato and apple juices, are expressed in µg/ml as follows. The Microsoft Excel 2016 program was used for the data statistics analysis (Figures 2 – 5).

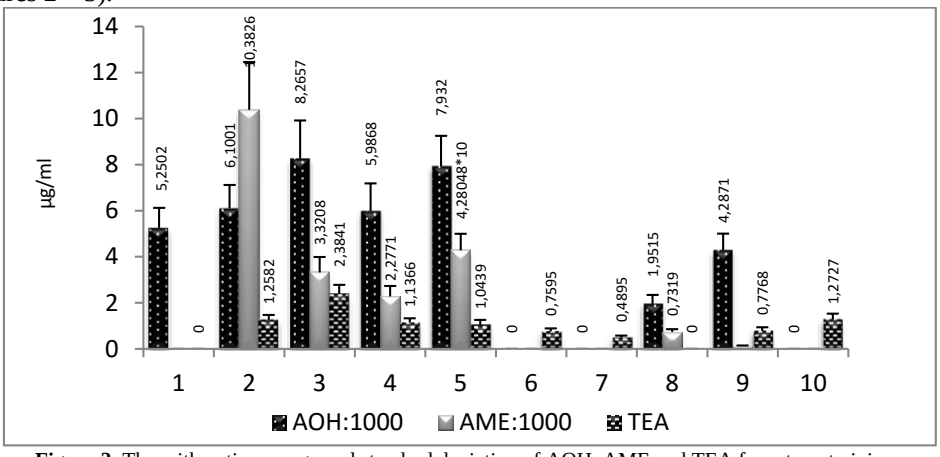

Figure 2. The arithmetic average and standard deviation of AOH, AME and TEA from tomato juices.

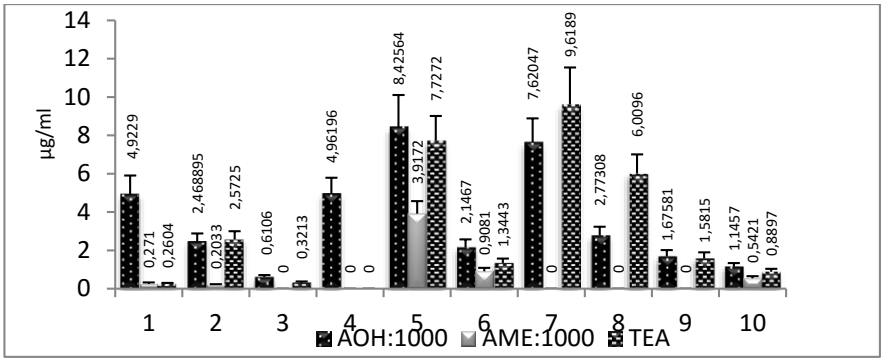

Figure 3. The arithmetic average and standard deviation of AOH, AME and TEA from apple juices.

The arithmetic mean value of mycotoxin concentrations (AOH, AME, TEA) in ten different brands of tomato juice is given in Figure 4.

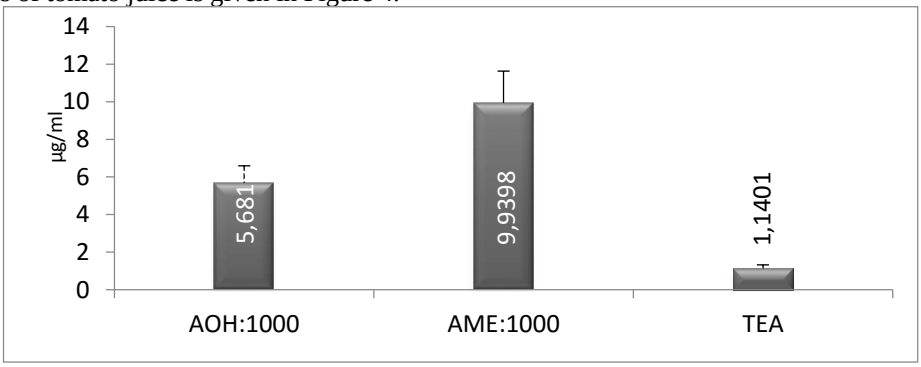

Figure 4. The arithmetic average of mycotoxin (AOH, AME, TEA) in tomato juice.

In tomato juices the AOH was detected in a concentration of 5,6819x10^{-3} ppm, AME of 9,9398x10^{-3} ppm and TEA of 1,1401 ppm (Figure 4.). The mycotoxin average concentrations (AOH, AME, TEA) in ten different brands of apple juice is given in Figure 5.

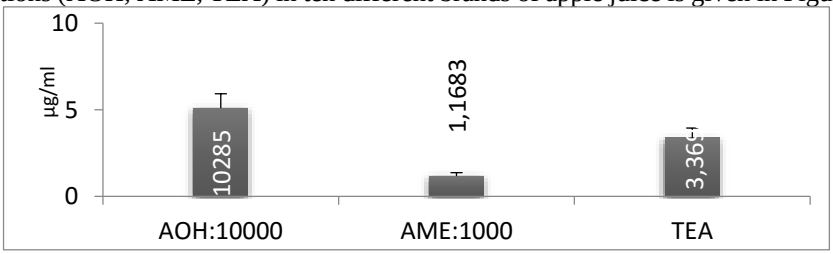

Figure 5. The arithmetic average of mycotoxin (AOH, AME, TEA) in apple juice.

According to our final results, in all 20 juice samples Alternaria toxin (Figures 2-3) was detected. In all these juices samples the concentration of AOH detected in 17 out of 20 samples (85%) has a range of 0,6106-246,8895x10^{-3}µg/ml, the concentration of AME detected in 11 out of 20 samples (55%) has a range of 0,1219-42,8048x10^{-3} µg/ml and also, the concentration of TEA detected in 17 out of 20 samples (85%) has a range of 260,4-9618,9x10^{-3} µg/ml.

Fente et al., in 1998, reported that the linear study range of tomato paste about mycotoxins (AOH) in HPLC is between 5,2-196 $x10^{-3}$ µg/ml. Other study, indicate that the minimum amount for detection of AME and AOH in tomato paste, tomato juice and tomato purees using the HPLC method, is 2,0 $x10^{-3}$ µg/ml for AME and 5,0 µg/ml for AOH (Da Motta et al, 2000).

In Argentina a study conducted on the 80 tomato products (like ketchup, tomato paste, tomato juice), the *Alternaria* toxin was detected in 39 samples (49%): TEA with concentrations in a range of 39-4021 $x10^{-3}$ µg/ml in 23 samples (29%); AOH with concentrations in the range of 187-8756 $x10^{-3}$ µg/ml in 5 samples (6%); and AME with concentrations in a range of 84-1713 $x10^{-3}$ µg/ml in 21 (26%) samples. Six samples were contaminated with TEA and AME, two samples were contaminated with TEA and AOH, other two samples were contaminated with AME and AOH (Da Motta et al, 2000). In the present study, the detected values are, as follows: AOH ($0,6106x10^{-3}$-0,1971 ppm) and AME ($0.1219x10^{-3}$-$42,8048x10^{-3}$ ppm) were found lower compared to previous studies. The TEA concentration (0,3213-10,9377 ppm) was found higher.

In the garden tomatoes the mycotoxin detection during harvest time in Italy, indicate 4750 $x10^{-3}$ µg/ml of TEA, 600 $x10^{-3}$ µg/ml of AOH and 100 $x10^{-3}$ µg/ml of AME (Panigrahi, 1997; Dennis, 1983). In different studies conducted on toxin production, all three toxins (AOH, AME, TEA) were detected in 74 isolates, both AOH and AME were found in 30 isolates, and TEA and AOH in 2 isolates. The TEA was detected in 7 isolates, AME in 3 isolates and AOH 1 out of 11 isolates (Terminiello et al., 2006; Dalcero et al., 1989).

In our study it was found that in tomato juice the AOH quantity is in the range of 1,9515-8,2657 $x10^{-3}$ µg/ml, quantity of AME in the range of 0,1219-42,8048 $x10^{-3}$ µg/ml and the quantity of TEA in range of 489,5-2384,1 $x10^{-3}$ µg/ml. The AOH was detected in 7 samples, AME in 6 samples and AOH was being in 8 samples out of 10 different tomato juices. In Brazil, maximum quantity of TEA in canned tomato juices, tomato essence, tomato puree, tomato paste and cooked tomato have been identified as 178 $x10^{-3}$ µg/ml (Da Motta et al., 2000).

In present research, in all apple juice samples, the detected quantity of AOH is in range of 0,6106-246,8895 $x10^{-3}$ µg/ml, quantity of AME in range of 0,2033-3,9172 $x10^{-3}$ µg/ml, quantity of TEA in range of 321,3-9618,9 $x10^{-3}$ µg/ml. Also, it was detected that AOH was detected in all 10 different apple juices (%100), AME was detected in 5 out of 10 different apple juices (%50) and TEA was detected in 9 out of 10 different apple juices (%90).

Other studies ındıcate that the contamination level of maize with *Alternaria alternata,* is directly correlated with the esophageal cancer incidence (Liu et al, 1989). Also, it has been found that the foods which are mildewed with *A. alternata,* are causing tripe tumors in rats (Ohtsubo et al., 1978; Sauer et al., 1978; Younis and Al-Rawi, 1988).

Alternaria toxins presents an *in vitro* cytotoxicity in mamals and bacteria cells, fetotoxicity and teratogenicity in mice and hamsters (Visconti and Sibilia, 1994). This toxins, although not as active as fumonisin B1, they block the sphingolipids synthesis by inhibiting the rate-limiting enzymes (Gregory et al., 1983; Van der Westhuizen et al., 1998). *A. alternata* group also produce plant-specific phytotoxins (Van der Westhuizen et al., 1998). AAL (*Alternaria alternata* f. sp. *lycopersici*) toxin, is structurally similar to fumonisin B1 and causes necrotic lesions in genetically susceptible tomato lines (Otani et al, 1996; Gilchrist et al, 1992).

The present study results show that when the fungi favorable conditions are provided, fruits can be potentially contaminated with toxins. Hence consumers shouldn’t buy fruits which are rotten or infected with soil. About processed fruits, if they are not cleaned from soil or the rotten ones are not removed before processing and packaging, the mycotoxins also can produce severe toxicity of human food. Thus, it’s believed that people who consume fresh fruits are exposed to less quantity of

Alternaria toxin (Visconti and Sibilia, 1994). Other related studies shown that *Alternaria* toxins can cause dermatologic effects, type I allergy and a weaken immune system (Morison and Weisdorf, 1993). To prevent or minimize the cytotoxic effects, new rules should be implemented about harvesting, transporting, storing and processing stages of food products.

Almost all of the molds are located in water and soil, hence, those two environments are the main sources of plants contamination. In this way, the primary products contact with the irrigation water and soil should be minimized.

Products must be carefully harvested, mainly when the process is not automatized, gloves should be worn. Fruits contaminated with mycotoxin, damaged, decayed or falling into decay shouldn't be collected, they just should be removed from the cultivation area and disposed, also, they shouldn't get in contact with healthy ones, in order to prevent contamination.

If the collected products are stored in the same containers, the risk of contamination in that containers will increase. To avoid this, disposable containers should be used, and sealed usıng stretch. The large scale containers should be disinfected before each use.

The products should be refrigerated during the transport and should be quickly delivered, avoiding any damage.

During storage physical conditions should be fulfilled, according to the type of product. The ıncrease of mycotoxin should be minimized by quantity of heat, air, moisture and stack.

CONCLUSIONS

Alternaria toxin was detected in all 20 analyzed juices samples. In tomato juice were identified concentrations of 0,6106-246,8895x10^{-3}µg/ml AOH, 0,1219-42,8048x10^{-3} µg/ml AME and 260,4-9618,9x10^{-3} µg/ml TEA.In all 10 apple juice samples, the Alternaria toxin it was detected as follows: 0,6106-246,8895 x10^{-3} µg/ml AOH, 0,2033-3,9172 x10^{-3} µg/ml AME, 321,3-9618,9 x10^{-3} µg/ml TEA. The alimentary products processing phases should be strictly controlled by the appropriate rules and measurements according to the Turkish Food Codex. These products which reached the markets should be consumed before the end of shelf life. Further studies are required to detect the levels of contaminants from different alimentary products in order to minimize the risk of toxicity induced by Alternaria sp.

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