International Journal of Agriculture, Environment and Food Sciences

JAEFS

www.jaefs.com

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2021.4.24

Research Article Int J Agric Environ Food Sci 5 (4):640-644 (2021)

# Aflatoxin analysis by LC-MS of local and imported black tea sold in Turkey

**Kasım Takım**[1] **Mehmet Emin Aydemır**[2*]

[1]Harran University, Faculty of Veterinary, Department of Basic Sciences, Şanlıurfa, Turkey
[2]Harran University, Faculty of Veterinary, Department of Veterinary Food Hygiene and Technology, Şanlıurfa, Turkey

*Corresponding author: aydemiremin23@gmail.com

**Abstract**

Tea is a popular drink throughout the world with known health benefits. Although it has been accepted as safe and healthy for centuries, recent research has reported that herbal tea could be contaminated by fungi and mycotoxins. The aim of this study was to investigate the presence of total aflatoxin and aflatoxin B1 in local and imported tea sold in the southeastern and eastern provinces of Turkey. A total of 79 samples were taken from tea originating from Turkey (Mardin; 7, Şırnak; 3, Van; 15, Diyarbakır; 13, Siirt; 9, Batman; 4, Gaziantep; 14, Kilis; 4, and Şanlıurfa; 10), Iran, Sri Lanka, and India. Analysis of the content of the samples was made in respect of total aflatoxin and aflatoxin $B_1$ using the Rapid Common Mass Spectrometry method (2006; 20: 2649-2659) with an LC-MS/MS device. The analyses were performed in an advanced, private, EU-accredited laboratory. According to the results obtained from the LC-MS/MS device, no total aflatoxin or aflatoxin $B_1$ was determined. That no aflatoxins were detected in the tea samples demonstrates that the harvesting, processing, drying and packaging stages of the local and imported teas sold in the southeast Anadolu and South Anadolu regions of Turkey are applied appropriately. These types of analyses should be applied in other regions to determine the presence of aflatoxin in tea in general in Turkey.

**Keywords:** Aflatoxin, Aflatoxin $B_1$, Black tea, Imported tea.

## Introduction

Tea is an aromatic drink made by pouring hot or boiling water on the dried leaves obtained from the plant known as *Camellia sinensis*. After water, tea is one of the most important drinks in the world, with a history of 5000 years, and reported to be drunk by approximately two-thirds of the global population (McKay and Blumberg, 2002; Schwarz et al., 1994; Diby et al., 2017). In a 2017 statistical study from the UK, the USA, and Germany, it was shown that 30%-40% of the participants drank 2-3 cups of tea per day (Sedova et al., 2018). Tea plants are grown in the northern hemisphere at a latitude of approximately 42°, and in the southern hemisphere at 27°, in regions where the climate is hot with abundant rainfall. The countries where tea plants are generally grown are China, India, Sri Lanka, Kenya, Vietnam, Indonesia, Russia, Japan, Myanmar, Turkey, Bangladesh, Iran, Argentina, UgandaTanzania, Malawi, Thailand, Nepal, Rwanda, Burundi and Ethiopia (Kurt and Hacıoğlu, 2013; Takım and Aydemir, 2018). Tea plantations are widespread and tea production is intense in India, China, Sri Lanka, Indonesia, Kenya, Turkey and Japan, with 80% of the world tea produced in these countries (Harman, 2014; Amirahmadi et al., 2013).

The polyphenols found in the tea plant have antioxidant, antimicrobial, anticancer, anti-inflammatory and anti-viral effects on human health. Benefits have also been reported of lowering cholesterol, blood presssure, and the risk of cardiovascular disease, and reducing the risk of osteoporotic fractures in the elderly (Zhang et al., 2013; Khan and Mukhtar, 2007; Shen et al., 2013). However, although tea has many benefits, the presence of harmful polluting substances such as heavy metals, mycotoxins and pesticide remnants, can have a negative effect on human health (Abd El-Atya et al., 2014). Mycotoxins in tea can lead to serious health problems such as immunosuppression, and carcinogenic, genotoxic, hepatotoxic and nephrotoxic effects (Milićević et al., 2010; Santoz et al., 2009).

Aflatoxins are mycotoxins produced by several fungi, the most common of which are *A. flavus* and *A. parasiticus*. At least different aflatoxin types are produced naturally ($B_1$, $B_2$, $G_1$, $G_2$, $M_1$, $M_2$, $Q_1$)

**Cite this article as:**
Takım, K., Aydemir, M.E. (2021). Aflatoxin analysis by LC-MS of local and imported black tea sold in Turkey. International Journal of Agriculture, Environment and Food Sciences, 5 (4), 640-644
**Doi:** https://doi.org/10.31015/jaefs.2021.4.24
**Orcid:** Kasım Takim: https://orcid.org/0000-0003-4631-1982 , Mehmet Emin Aydemir: https://orcid.org/0000-0002-8578-9623
**Received:** 09 May 2021 **Accepted:** 28 November 2021 **Published Online:** 29 December 2021
**Year:** 2021 **Volume:** 5 **Issue:** 4 (December) **Pages:** 640-644
**Available online at :** http://www.jaefs.com - http://dergipark.gov.tr/jaefs

(Murphy et al., 2006). Aflatoxin $B_1$ produced by *A. flavus* and *A. parasiticus* is the strongest liver carcinogen (Lee et al., 1971). Aflatoxins have mutagenic, teratogenic and immunosuppressive activities, and long-term consumption of foods containing aflatoxins increases the risk of liver, stomach and colon cancer (Reiter et al., 2009).

During the harvesting, processing and storage of tea plants, there may be contamination with mycotoxins. Moulds and fungi which can spread to tea during processing and production include *Aspergillus, Penicillium, Pacelomyces, Cladosporium, Alternaria, Mucor, Fusarium, Rhizopus, Absidia* and *Trichoderma* (Abd et al., 2014). Poor farming practices, incorrect processing, drying, packaging and storage, and transportation under inappropriate conditions increase the risk of mycotoxin contamination by promoting fungal growth. Moreover, as the regions where tea is grown are warm, wet and humid, this provides the ideal environment for the development of toxicogenic mould (Sedova et al., 2018).

Mycotoxins are resistant to the traditional heat range of food processing (80°-121°C), so following normal cooking such as boiling or frying, or pasteurisation, there may be very little or no reduction in general toxin levels (Kabak, 2009). Therefore, in the preparation of tea contaminated with aflatoxin, it is not possible for the temperature of the boiling water to impair or decompose the toxins.

Different methods are used to determine the presence of aflatoxins in food and animal feed, such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbance analysis (ELISA). Of these methods, HPLC has been reported to be ideal as it has high sensitivity and specificity (0-320 µg/kg) (Braga et al., 2005).

The aim of this study was to evaluate the presence of total aflatoxin and aflatoxin $B_1$ in local and imported tea sold in the southeastern and eastern provinces of Turkey, using LC-MS/MS analysis.

**Materials and Methods**

**Sample Collection**

A total of 79 samples were taken from tea originating from Turkey, Iran, Sri Lanka, and India. The numbers of samples taken from Turkish provinces were: Mardin; 7, Şırnak; 3, Van; 15, Diyarbakır; 13, Siirt; 9, Batman; 4, Gaziantep; 14, Kilis; 4, and Şanlıurfa; 10 (Figüre 1). The samples were collected from city markets and cafes, placed in sterile sample bags and sent to the laboratory, where they were stored in a cool, dry environment until analysis. The analyses were performed in an advanced, private, EU-accredited laboratory. The analyses were made using the Rapid Common Mass Spectrometry method (2006; 20: 2649-2659) with an LC-MS/MS device.

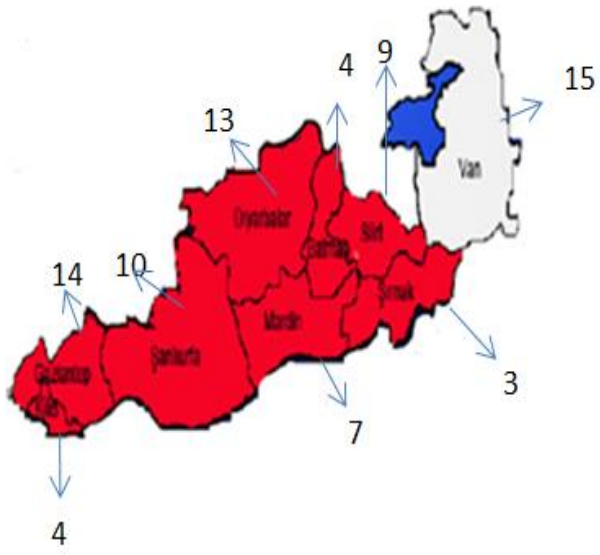


Figure 1. Cities and number of samples

**Sample Preparation.**

The ground tea samples (10g) were homogenised with a 40mL organic extraction solvent mixture (acetonitrile: water:acetic acid, 79:20:1) (Merck, Darmstadt, Almanya) for 60 mins in an orbital shaker (model 711 VDRI, Asal, Milan, Italy). The supernatant part was centrifuged for 10 mins at 3000 rpm (Allegra X-22R centrifuge, Beckman Coulter, Palo Alto, CA, USA). Then with single-stage extraction, 0.5 mL extract with the same amount of acetonitrile: water: acetic acid (20:79:1) was passed through a 0.22 µm filter and was injected into the LC-MS/MS (Sulyok et al., 2006).

**LC – MS / MS device and conditions.**

Determination and the amount determination were performed with a TurboIon Spray ESI source and a QTrap 4000 LC-MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with a 1100 Series HPLC system (Agilent, Waldbronn, Germany). LC analysis was performed using the Finnegan TSQ quantum ultra mass system (Thermo Scientific, CA, USA) formed from a paired pump, a degasser, a column oven and an automatic sampler. The analysis was made on a column over C18 columns of 150 mm, 4.6 and 3 µm fragment size. The column temperature was kept at 30°C. Capillary voltage was 3kV and nitrogen (Merck, Darmstadt, Almanya) was used as the spray gas. Source temperature and desolvation temperature were adjusted to 120°C and 400°C, respectively. Mycotoxins were analysed in selected reaction monitoring (SRM) channels (Sulyok et al., 2006).

Table 1. The results of the total aflatoxin and aflatoxin $B_1$ analyses in the tea samples

| The province from which the sample was collected | Number of samples | Total Aflatoxin | Aflatoxin B1 | Measurement Limit | Unit | Recycling | Analysis method |
|---|---|---|---|---|---|---|---|
| **Mardin** | 7 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Şırnak** | 3 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Van** | 15 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Diyarbakır** | 13 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Siirt** | 9 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Batman** | 4 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Gaziantep** | 14 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Kilis** | 4 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |
| **Şanlıurfa** | 10 | ND | ND | 0.15 | µg/kg | 93 | Rapid Common Mass Spectrometry. 2006; 20: 2649-2659 |

**Results and Discussion**

As seen in the analysis results in Table 1, total aflatoxin and aflatoxin $B_1$ were not determined in any of the 79 samples of tea.

Recently, studies have been carried out on the presence of aflatoxin in products such as spices, nuts and coffee. However, studies on the presence of aflatoxin in black teas have been limited. To the best of our knowledge, there has been no previous study related to aflatoxin analysis in imported tea in Turkey, and there is a limited number of studies of local tea. In a study by Tosun et al. (2016) 48 samples of herbal tea were collected from four local herb shops in the province of Manisa. Of the 48 samples analysed, 43 were determined as aflatoxin positive. The highest aflatoxin concentration (34.18µg/kg) was determined in a chamomile tea sample. In a study by Özden (2018) which was conducted to determine the levels of heavy metals and ochratoxin A in medicinal herbal teas, ochratoxin A was determined in only 1 chamomile tea sample of 21 samples. Omurtag and Yazicioglu (2004) investigated the presence of Fumonisin $B_1$ and $B_2$ in herbal tea and medicinal plants sold in Turkey. Fumonisin $B_1$ was not determined in 54 herbal teas and 61 medicinal plants but Fumonisin $B_2$ was detected in 2 samples. In another study in Istanbul by Hacibekiroglu and Kolak (2013) aflatoxin $B_1$ was determined in 2 of 15 samples of herbal tea.

There are studies in literature reporting aflatoxin determined in tea in other countries. In a 2010 study in Iran, Pouretedal et al. (2013) collected random samples from Tehran markets and showed that 30 of the 40 samples were contaminated with aflatoxins. The mean aflatoxin $B_1$ content of 11 contaminated samples was 10.0 ng/g and the total aflatoxin was mean 12.07 ng/g. In a study of Pu-Erh tea by Haas et al. (2013) 36 samples were examined, no aflatoxin or fumonisin was determined, and ochratoxin A was determined in 4 (11.1%) samples. Carraturo et al. (2018) examined 32 tea samples and reported the most widespread moulds to be *A. niger* strains, followed by *A. tubigensisoli*. Of the 32 samples examined, ochratoxin A was determined in 22 samples. A total of 91 different teas and herbal infusions were analysed by Monbaliu et al. (2010) and mycotoxin was determined in only one sample.

In the potable products, no mycotoxins were determined. In another recent study by Mannani et al. (2020) 76 (58.9%) of 129 herbal tea samples were found to be contaminated with aflatoxins, and 50 of the contaminated samples exceeded maximum levels. Pallarés et al. (2017) reported that they detected AFG1 in only two samples in 12 black tea bag samples they collected in Spain in a study they conducted. Contrary to the results reported in these studies related to the presence of aflatoxin in tea samples, no aflatoxin was detected in any tea sample in our study through Whatman filter paper and then concentrated at 40 °C in a rotary evaporator until dryness. The final obtained extract (4.8 g) was stored at 4 °C until use (Erkan et al., 2008).

**Conclusion**

Imported tea consumption is increasing day by day in Eastern and South Eastern provinces. It is said that imported tea consumption has many negative effects on health. However, these claims have not been scientifically proven. Therefore, the claims on this subject do not go beyond speculation. This study we have conducted has the quality to change the prejudices and prejudices about imported tea. In this study we conducted, a total of aflatoxin and aflatoxin B1 were not detected in imported and domestic tea samples that are popularly consumed by the people in Southeastern Anatolia and Eastern Anatolia in our country. The absence of aflatoxins in tea samples indicates that the harvesting, processing, drying and packaging stages of the local and imported teas sold in the Southeastern Anatolia and Eastern Anatolia regions are carried out properly. In order to determine the presence of aflatoxin in teas throughout Turkey, such analyzes should be made in teas in other regions.

**Compliance with Ethical Standards**

**Conflict of interest**

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest. Author contribution The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

**Author contribution**

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before

**Ethical approval**

Not applicable.

**Funding**

No financial support was received for this study.

**Data availability**

Not applicable.

**Consent for publication**

Not applicable.

**Acknowledgements**

Not applicable.

**References**

Abd El-Aty, A.M., Choi, J.H., Rahman, M.M., Kim, S.W., Tosun, A., Shim, J.H. (2014). Residues and contaminants in tea and tea infusions: a review. *Food Additives & Contaminants: Part A*, *31*(11), 1794-1804 Doi: 10.1080/19440049.2014.958575

Amirahmadi, M., Shoeibi, S., Abdollahi, M., Rastegar, H., Khosrokhavar, R., Hamedani, M. P. (2013). Monitoring of some pesticides residue in consumed tea in Tehran market. *Iranian journal of Environmental Health Science & Engineering*, 10(1), 1-6. Doi: https://doi.org/10.1186/1735-2746-10-9

Braga, S.M.L.F.M., de Medeiros, F.D., de Jesus Oliveira, E., Macedo, R.O. (2005). Development and validation of a method for the quantitative determination of aflatoxin contaminants in Maytenus ilicifolia by HPLC with fluorescence detection. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *16*(4), 267-271. Doi: https://doi.org/10.1002/pca.837

Carraturo, F., De Castro, O., Troisi, J., De Luca, A., Masucci, A., Cennamo, P., Guida, M. (2018). Comparative assessment of the quality of commercial black and green tea using microbiology analyses. *BMC Microbiology*, *18*(1), 1-12. Doi: https://doi.org/10.1186/s12866-017-1142-z

Diby, L., Kahia, J., Kouamé, C., Aynekulu, E. (2017). Tea, coffee, and cocoa. *Encycl. Appl. Plant Sci*, *3*, 420-425.

Hacıbekiroğlu, I., Kolak, U. (2013). Aflatoxins in various food from Istanbul, Turkey. *Food Additives & Contaminants: Part B*, *6*(4), 260264.Doi:https://doi.org/10.1080/19393210.2013.813080

Haas, D., Pfeifer, B., Reiterich, C., Partenheimer, R., Reck, B., Buzina, W. (2013). Identification and quantification of fungi and mycotoxins from Pu-erh tea. *International Journal of Food Microbiology*, *166*(2), 316-322. Doi: https://doi.org/10.1016/j.ijfoodmicro.2013.07.024

Harman C. (2014). Endemic agricultural products in the Black Sea Region: production, marketing and consumption of nuts, tea and kiwi. Giresun University, Social Sciences Institute, Giresun. 1-145s. in Turkish.

Kabak, B. (2009). The fate of mycotoxins during thermal food processing. *Journal of the Science of Food and Agriculture*, *89*(4), 549-554. Doi: https://doi.org/10.1002/jsfa.3491

Khan, N., Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life sciences*, *81*(7), 519-533. Doi: https://doi.org/10.1016/j.lfs.2007.06.011

Kurt G; Hacıoğlu HK. (2013). Examining the statistics of the World Countries with Turkey's Tea Production. In: Eastern Black Sea Industry Trade and Logistics Center 1. Rize Development Symposium Tea - Logistics - Tourism Proceedings Book, 39-64. in Turkish.

Lee, D.J., Wales, J.H., Sinnhuber, R.O. (1971). Promotion of aflatoxin-induced hepatoma growth in trout by methyl malvalate and sterculate. *Cancer Research*, *31*(7), 960-963.

Mannani, N., Tabarani, A., Zinedine, A. (2020). Assessment of aflatoxin levels in herbal green tea available on the Moroccan market. *Food Control*, *108*, 106882. Doi: https://doi.org/10.1016/j.foodcont.2019.106882

McKay, D.L., Blumberg, J. B. (2002). The role of tea in human health: an update. *Journal of the American College of Nutrition*, *21*(1), 1-13. Doi: https://doi.org/10.1080/07315724.2002.10719187

Monbaliu, S., Wu, A., Zhang, D., Van Peteghem, C., De Saeger, S. (2010). Multimycotoxin UPLC− MS/MS for tea, herbal infusions and the derived drinkable products. *Journal of Agricultural and Food Chemistry*, *58*(24), 12664-12671. Doi: https://doi.org/10.1021/jf1033043

Milićević, D. R., Škrinjar, M., Baltić, T. (2010). Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. *Toxins*, *2*(4), 572-592. Doi: https://doi.org/10.3390/toxins2040572

Murphy, P.A., Hendrich, S., Landgren, C., Bryant, C.M. (2006). Food mycotoxins: an update. *Journal of Food Science*, *71*(5), R51-R65. Doi: 10.1111/j.1750-3841.2006.00052.x

Omurtag, G.Z., Yazıcıoğılu, D. (2004). Determination of fumonisins B1 and B2 in herbal tea and medicinal plants in Turkey by high-performance liquid chromatography. *Journal of Food Protection*, *67*(8), 1782-1786. Doi: https://doi.org/10.4315/0362-028X-67.8.1782

Özden, H., Özden, S. (2018). Levels of heavy metals and Ochratoxin A in medicinal plants commercialized in Turkey. *Turkish Journal of Pharmaceutical Sciences*, *15*(3), 376. Doi: 10.4274/tjps.74936

Pallares, N., Font, G., Mañes, J., Ferrer, E. (2017). Multimycotoxin LC–MS/MS analysis in tea beverages after dispersive liquid–liquid microextraction (DLLME). *Journal of Agricultural and Food Chemistry*, *65*(47), 10282-10289. Doi: https://doi.org/10.1021/acs.jafc.7b03507

Pouretedal, Z., Mazaheri, M. (2013). Aflatoxins in black tea in Iran. *Food Additives & Contaminants: Part B*, *6*(2), 127. Doi: https://doi.org/10.1080/19393210.2013.764551

Takım, K., Aydemir, M.E. (2018). Şanlıurfa ilinde tüketilen kaçak çaylarda LC-MS ve GC-MS ile pestisit analizi. *Tarim ve Doga Dergisi*, *21*(5), 650. in Turkish. Doi: 10.18016/ksudobil.402273

Reiter, E., Zentek, J., Razzazi, E. (2009). Review on sample preparation strategies and methods used for the analysis of aflatoxins in food and feed. *Molecular Nutrition & Food Research*, *53*(4), 508-524. Doi: https://doi.org/10.1002/mnfr.200800145

Santos, L., Marín, S., Sanchis, V., Ramos, A.J. (2009). Screening of mycotoxin multicontamination in medicinal and aromatic herbs sampled in Spain. *Journal of the Science of Food and Agriculture*, *89*(10), 1802-1807. Doi: https://doi.org/10.1002/jsfa.3647

Schwarz, B., Bischof, H. P., Kunze, M. (1994). Coffee, tea, and lifestyle. *Preventive Medicine*, *23*(3), 377-384. Doi: https://doi.org/10.1006/pmed.1994.1052

Sedova, I., Kiseleva, M., Tutelyan, V. (2018). Mycotoxins in tea: occurrence, methods of determination and risk evaluation. *Toxins*, *10*(11), 444. Doi: https://doi.org/10.3390/toxins10110444-129.

Shen, C.L., Chyu, M.C., Wang, J.S. (2013). Tea and bone health: steps forward in translational nutrition. *The American Journal of Clinical Nutrition*, *98*(6), 1694S-1699S.Doi: https://doi.org/10.3945/ajcn.113.058255

Sulyok, M., Berthiller, F., Krska, R., Schuhmacher, R. (2006). Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, *20*(18), 2649-2659. Doi: https://doi.org/10.1002/rcm.2640

Tosun, H., Ergönül, P. G., Üçok, E.F. (2016). Occurrence of aflatoxins (B1, B2, G1, G2) in herbal tea consumed in Turkey. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *11*(3), 265-269. Doi: https://doi.org/10.1007/s00003-016-1032-6

Zhang, L., Zhang, Z.Z., Zhou, Y.B., Ling, T.J., Wan, X.C. (2013). Chinese dark teas: Postfermentation, chemistry and biological activities. *Food Research International*, *53*(2), 600-607. Doi: https://doi.org/10.1016/j.foodres.2013.01.016