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REVIEW ARTICLE

Food Safety Risk: Ochratoxin A in Türkiye

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin produced by fungi of the genus *Aspergillus* and *Penicillium*. OTA causes damage to the kidneys and liver in experimental animals and is even classified as a possible human carcinogen. As an occasional phenomenon, many foods in a particular region may be contaminated with OTA. Regardless of whether it is a developed or developing country, OTA contamination poses both public health and economic risks. We have compiled studies on OTA in food and humans (milk and blood) in Turkey over the last 25 years and discussed what needs to be done to reduce the public health risk.



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1. Ochratoxin A (OTA)

Ochratoxin A is a mycotoxin produced mainly by fungi of the *Aspergillus* and *Penicillium* genera and contaminates foods in which these fungi grow in it (Malir et al., 2016; Wang et al., 2016). The effects of OTA exposure (up to 2 years) on experimental animals have been studied, and it causes damage to many tissues, especially the kidney and liver, in experimental animals (IARC, 1983; NTP, 1989). While kidney tumors were observed especially in male rats, an increase in kidney and liver tumors was observed in mice. Based on the data obtained from

these animal experiments, OTA has been classified as a possible human carcinogen.

It is frequently observed that exposure to OTA causes fibrosis, oxidative stress and apoptosis in cells/tissues (Atroschi et al., 2000; Palabiyik et al., 2012; Chae et al., 2022). The harmful effects of OTA have been reported to occur through many pathways such as inhibition of macromolecule synthesis, oxidative stress, lipid peroxidation, genotoxicity, mitochondrial dysfunction, alteration of Ca²⁺ homeostasis, disruption of glucose homeostasis, cellular differentiation, and apoptosis. These mechanisms of action have been discussed in various

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reviews (Kószegi & Poór, 2016; Khoi et al., 2021). There is disagreement on whether OTA creates DNA adducts (Delatour et al., 2008; Mantle et al., 2010). It is thought that the disagreements on whether OTA can be classified as a genotoxic toxin that forms a DNA adduct or not will not remain an inconclusive disagreement in the long term, as it will be important in determining the legal limit of OTA that can be found in foods. It is reported that a non-genotoxic carcinogen is 10 times less risky for human health compared to a carcinogen that has the ability to form defined DNA adducts (Mantle et al., 2010). Therefore, if it is definitively proven that OTA is genotoxic, the permissible limits in foods will need to be further reduced. However, if OTA does not have a genotoxic effect, the measures to be taken to keep the OTA level in foods much lower will be costly for society and will cause serious economic losses by causing many harmless foods to be classified as unsafe for consumption.

OTA can be found in many agricultural foods (coffee, tea, grapes, apricots, figs, wheat, etc.) regardless of developed or developing countries (within the legally permitted limits) and in some cases, it can exceed the legal limits. Apart from agricultural foods, OTA can reach the consumer through the meat, milk and eggs of animals that eat OTA-contaminated feed. Moreover, OTA can directly reach babies through the milk of mothers exposed to it. OTA can be found in human blood for a long time (plasma half-life ~36 days) after exposure (Studer-Rohr et al., 2000), and it has also been observed that OTA can cross the placenta in OTA exposure in pregnant animals, which increases OTA exposure (Hallén et al., 1998; Minervini et al., 2013). This makes OTA exposure more concerning. To evaluate the risk posed by this alarming toxin for Türkiye, it was aimed to compile studies showing OTA contamination in foods frequently consumed by Turkish people over the last 25 years.

2. OTA in Turkish Teas

Türkiye is one of the world's largest tea producers (FAO, 2015). Türkiye first tried to grow tea with seeds purchased from Japan in 1838, but no successful results were obtained under the conditions of those days (Honda, 2012). However, positive results were obtained from the studies carried out in 1924-1937 and tea cultivation started in Türkiye. The first tea factory in Türkiye was established in 1947 (Çaykur, 2023). As tea production increases in Türkiye, tea has become the most preferred beverage. Türkiye is the country with the highest per capita tea consumption in 2019 in the world (Yildirim & Karaca, 2022). Between 2017 and 2022, Türkiye consumed 14-15 kg of tea per person (Erkal, 2023). Therefore, the presence of OTA in tea has become a more important issue for Türkiye.

Gazioglu and Kolak (2018) found that the OTA concentration in Turkish tea could reach 139.5 µg/kg, but this was only a measurement obtained from a single tea sample.

This amount is at least approximately 14 times higher than the permitted limit for dried herbs in the Turkish Food Codex (TOB, 2023). In another study (Toman et al., 2018), OTA levels were investigated in 46 tea samples taken from Türkiye and the Czech Republic, also OTA transfer to the drinking part of the tea was examined. OTA concentration in 36 tea samples from the Czech Republic was observed to vary between 0.35 ng/g and 56.7 ng/g, but OTA was not detected in any of 14 different (9 teapots and 5 filtered teabags type) Turkish teas. There may be many reasons for the difference in OTA levels in Turkish tea in the studies of Gazioglu and Kolak (2018) and Toman et al. (2018). The storage conditions in the markets, the duration and conditions of tea storage starting from harvesting to packaging, can directly affect OTA formation and accumulation. Taking tea samples from homes as well as products purchased from markets is important in observing the OTA exposure of the population through tea. Because it is highly possible for various fungi to grow and produce fungal toxins in tea that is not consumed for a long period after it is opened at home. Taking samples to determine OTA concentration from tea stored at home in Türkiye can be a tool to investigate the public's exposure to OTA.

It was observed that 41.5±7% of the toxin in the tea passed into the drinking part during the tea brewing/preparation (150 ml of boiling water was added to 5 grams of tea and left to brew for 15 minutes) process (Toman et al., 2018). The tolerable weekly OTA intake has been reported as 112 ng/kg (WHO, 1991; Micco et al., 1995), and based on this information, the tolerable weekly OTA amount for a 70 kg person is 7.84 µg. If OTA risk is ignored, the 'tea-loving' population in Türkiye may consume more than 25% of this limit directly from tea. As seen in the study of Toman et al. (2018) when 5 g of tea with an OTA concentration of 55 µg/kg is taken and 150 ml of tea is brewed, 0.275 µg passes into the liquid part of the tea. An individual who consumes this much tea every day consumes 1.925 µg of OTA in 7 days. This means approximately 25% of the tolerable limit for a 70 kg person. OTA concentration in consumed tea and 'tea addiction' of person increase, a significant portion of the tolerable limit is wasted only through tea.

Since there is a serious transfer of OTA from the tea leaves to the drinking part of the tea through Turkish brewing and the society has a high tea consumption habit, OTA contamination of teas poses a serious public health risk for Turkish society. It is important for Turkish public health that keep lower the permitted OTA level in tea than the legal OTA limits allowed in Europe and other countries.

3. OTA in Grapes and Products Originated from Grapes

In Türkiye, grapes are produced at a significant level in the Aegean, Mediterranean, Southern and Western Anatolia and Marmara Regions, and it has been reported that 3 650 thousand

tons of grapes are produced in 461 thousand hectares of land throughout the country (Bashimov, 2017). In Türkiye, the grape itself and its leaves are used in various ways. While grapes can often be eaten directly, grapes can be dried and made into pekmez (grape molasses) or wine. Depending on the region, period and facility, OTA can be found in different concentrations in grapes and grape-derived products.

Grapes can be eaten or used as raw material for other products. The OTA concentration in the starter grapes may increase or decrease at the end of the production process. OTA amounts in these products vary according to the process. No detectable levels of OTA were found in the raisin samples (17 samples) taken from the market in Burdur province in 2015 (Yurdakul et al., 2019). However, OTA was detected in 50 raisins, 10 grape juice and 25 pekmez samples collected from İstanbul between 2008 and 2009 for OTA analysis (Akdeniz et al., 2013). It was reported by Akdeniz et al. (2013) that OTA concentration exceeded the limit of quantification in 8% of 50 raisin samples, 2% of 10 grape juice samples and 92% of 25 pekmez samples (limit of quantification 0.15 µg/kg for raisins and 0.3 µg/kg for grape juice and pekmez samples). OTA concentration ranged between 0.19-2.59 µg/kg (Average 1.15 µg/kg) in raisins, 0.90-1.90 µg/kg (mean 1.4 µg/kg) in grape juice and 0.44-5.32 µg/kg (mean 2.04 µg/kg) in pekmez samples.

Pekmez is a sweet traditional food made by boiling and condensing grapes. This boiling process does not reduce the OTA level in grapes; in fact, it is observed that the OTA concentration increases as condensation occurs (Arici et al., 2004). Therefore, the OTA concentration in grapes used in pekmez production must be below legal limits. Other studies also show that pekmez contains more OTA than other grape products. Tosun et al. (2014) analyzed 82 grape pekmez samples (55 homemade, 7 organic and 20 commercial) from Niğde, Manisa, Çanakkale, Denizli, Sivas, Yozgat, Mardin, İzmir and Kayseri. There were 37 OTA positive pekmez samples (73% of homemade pekmez, 71% of organic pekmez and 35% of commercial pekmez). The average OTA amounts were 9.2 µg/L in organic pekmez, 3.5 µg/kg in homemade pekmez and 1.4 µg/kg in commercial pekmez. The maximum OTA level was found to be 31.2 µg/L in organic pekmez, 15 µg/L in homemade pekmez and 12 µg/L in commercial pekmez. However, OTA was detected in only one of the 16 grape pekmez samples taken from markets in Burdur province in 2015, and the detected OTA concentration was 20.48 µg/kg (Yurdakul et al., 2019). Although OTA cannot be detected in some pekmez samples or the average OTA concentration is below the legal limits, it is observed that the OTA concentration can reach high levels in some samples.

Wine is an alcoholic beverage produced by the fermentation of grapes and is widely preferred around the world. OTA levels of 95 wine samples (2005-2006) originating from various

provinces of Türkiye were investigated (Var & Kabak, 2007). The average OTA concentration in these wines was determined to be 158 ng/L in wines of Thracian origin (44 samples), 60 ng/L in wines of Aegean origin (28 samples) and 27 ng/L in wines of Eastern Anatolia origin (8 samples). In total, OTA at the level of 500-815 ng/L was found in only 3 samples. The samples in this study are below the limit specified in the Turkish Food Codex (2.0 µg/kg). In addition, when evaluated together with wine samples from other countries, it is seen that Turkish wines with low OTA concentration are better positions among EU originated wines (Czerwiecki et al., 2005).

4. OTA in Bread

White bread has 8.7 grams of protein, 58.3 grams of carbohydrates and 1.5 grams of fat per 100 grams (Karağağoğlu et al., 2008) and is consumed as a common food in Türkiye. Bread is produced daily or even hourly, and is consumed by the consumer immediately or within two days after purchase. Since it is a popular food, investigating the OTA level in bread is important for Türkiye. Various types of bread produced from different flours are consumed in Türkiye, the most common of which is white bread. The OTA concentration in flour affects the OTA concentration of the final product. A total of 132 flour and bread samples (34 white flour, 14 whole wheat flour, 10 corn flour, 36 white bread, 28 whole wheat bread and 10 corn bread) taken from different points of Bursa in 2005-2006 were analyzed (Cengiz et al., 2007). OTA was detected in 83% of the samples. The average OTA concentrations seen in this study were determined: 6.89±0.46 µg/kg in white flour, 9.30±1.33 µg/kg in whole wheat flour, 6.39±1.10 µg/kg in corn flour, 7.84±0.39 µg/kg in wholemeal bread, 4.94±0.46 µg/kg in corn bread and 6.38±0.48 µg/kg in white bread purchased from civilian markets and bakeries.

Although bread fermentation and baking processes reduce the amount of OTA in bread, the OTA level in bread is related to the amount of OTA in flour. It was studied how the bread making process affects the OTA level. By contaminating OTA-free white flour with OTA at 5 and 10 µg/kg, the effect of the bread preparation process on this contamination level was investigated (Var et al., 2018). This flour was mixed with α-amylase, cellulase, glucose oxidase mixture, salt, water and fresh yeast and kneaded for about 20 minutes to prepare the dough. An average OTA concentration of 2.76 µg/kg was found in dough made with flour containing 5 µg/kg OTA and 7.36 µg/kg in dough made with flour containing 10 µg/kg OTA. This decrease in concentration is related to the added water. The divided dough was shaped into bread and incubated at 30 °C for 75 minutes for fermentation. After fermentation, 1.35 µg/kg OTA was detected in the dough made from flour containing 5 µg/kg OTA and 4.69 µg/kg OTA was detected in the dough made from flour containing 10 µg/kg OTA. They suggest that the decrease in OTA concentration observed after fermentation is a result of *S. cerevisiae* activity. After fermentation, bread was

produced by baking the dough at 240 °C for 30 minutes. OTA concentrations of 0.63 µg/kg and 2.37 µg/kg were detected in bread made with flour containing 5 µg/kg and 10 µg/kg OTA, respectively. Unlike the pekmez production process, where OTA concentration increases, this study does not provide information about other OTA derivatives, but it has been reported that cooking at 240 °C for 30 minutes reduces the OTA level by approximately 50%. If the amount of OTA in the raw material flour is not excessive, the risk of OTA exposure will decrease as the bread-making process reduces the amount of OTA.

5. OTA in Seafoods

Fish feed consisting of fishmeal, wheat, soybean and corn can be used to feed the fish in aquaculture (Pietsch, 2020). With the increase in vegetable ingredients in fish feed, the exposure of fish to OTA has increased (El-Sayed et al., 2009). It has been shown that a significant amount of OTA can form in fish feed during a one-month storage period (Pietsch et al., 2020). Eating OTA-contaminated feed causes loss of growth and/or survival rate in fish and tissue damage in fish embryos (Manning et al., 2003; Srour, 2004; Wu et al., 2016). However, exposure of fish to OTA does not only create economic risks. Through the food chain, OTA in the body of fish can reach human consumption. Various studies have reported that OTA is found in seafood in markets. OTA of 0.36-1.51 µg/kg was found in 33% of 40 dried seafood products (including fish, shrimp and mussel) purchased from the Zhanjiang seafood market (Deng et al., 2020). In another study, 27 seafood samples (muscles, entrails of fresh fish and dried seafood) were taken from a local supermarket in Shanghai, and OTA was detected in 4 of these samples at a concentration of 0.5-1.9 µg/kg (Sun et al., 2015).

No study has been found that determined OTA level in fish or fish feed sold in the market in Türkiye. However, the presence of other mycotoxins has been investigated. Between 1998 and 2000, 85 fish food samples (only 20 of which were outside Türkiye) were taken, and aflatoxin was detected in the range of 21.2-42.4 ppb in 20 of these samples (Altug & Beklevik, 2003). The presence of mycotoxin in a feed indicates that this environment is suitable for fungal growth, and this indicates the possibility of OTA presence in the feed. OTA and aflatoxin can co-occur in the same samples (Kara et al., 2015). Therefore, determining the amount of OTA in fish feed and the amount of OTA in the edible parts of fish bought from farms will contribute to elucidating the risk of OTA in fish in Türkiye.

6. OTA in Milk and Dairy Products

Milk and dairy products (such as yoghurt, ayran and cheese types) have an important place in the diet of children and adults. While cheese is preferred for breakfast, yoghurt and ayran (a drink made from yoghurt) is a traditional food and beverage consumed with many foods. The amount of OTA in this

traditional beverage food is dependent on the amount of OTA in milk. The presence of OTA in the range of 2-270 ng/L was detected in 37 of the samples from 40 milk collection tanks in Burdur (Keyvan et al., 2018). In another study, an average of 119±9 ng/L OTA was found in 105 cow milk samples (Turkoglu & Keyvan, 2019). It did not make a significant difference whether the milk was raw, pasteurized or UHT. The presence of low levels of OTA in cow's milk may be due to microbial activity in their digestive tract and/or exposure to low doses of OTA. Undoubtedly, this is a desired situation for later products.

Additionally, yoghurt making production reduces the OTA concentration. Škrinjar et al. (1996) prepared skimmed milk powder culture medium and contaminated with OTA (at concentrations of 50-1500 µg/L) in order to investigate the effect of fermentation with yoghurt culture and various bacteria on OTA concentration. Fermentation significantly reduces OTA in OTA-contaminated culture media (No OTA residues were detected between 50-1000 µg/L OTA contaminated culture). This indicates that yoghurt produced under appropriate fermentation conditions and ayran produced from this yoghurt will be reliable in terms of OTA.

Surf cheese is a traditional dairy product produced especially in Hatay province. Various spices and herbs are added to the cottage cheese, kneaded and shaped, then dried in the air for 3-4 days and surf cheese is produced. A total of 30 surf cheese samples were taken from random sellers in Hatay province. OTA was detected in 28 of the cheese samples taken (between 0.058 and 5.04 µg/kg) and its mean concentration was 0.615 ± 0.228 µg/kg (Sakin et al., 2018). However, the mean OTA concentration seems to be compatible with the acceptable limits in other products. The amount of OTA found in this cheese may be due to the OTA concentration in milk, added spices and herbs, or the drying process.

7. OTA in Baby Supplementary Foods

Baby supplementary foods have an important role in baby nutrition. Since they may contain wheat and various fruits, they are likely to contain OTA. In 2011, 62 baby formula products (6 infant formula, 36 follow-on formula and 20 toddler formula) from 8 different brands from Çorum was investigated for the presence of mycotoxins (Kabak, 2012). Although OTA was detected in 12 of 62 samples, OTA concentrations are 0.017-0.184 µg/kg (average 0.103 µg/kg) and do not exceed the limit (0.5 µg/kg) according to the European Commission and the Turkish Food Codex. Although high amounts of OTA contamination were not found in the supplemented infant formulas investigated in this study, we cannot say that babies can be completely protected from OTA exposure. Because OTA is found even in breast milk.

8. OTA in Breast Milk

OTA is found in the meat, eggs and milk of animals that consume foods contaminated with OTA. Humans are not to be exceptions. OTA is found in the milk of mothers who consume foods containing OTA, and in this way, OTA is passed to newborn babies. It has been reported that experimental animals exposed to OTA in utero and throughout development exhibit more renal lesions compared to adults (Bondy et al., 2021). Therefore, since breast milk is the primary nutritional source for human infants, OTA concentration in breast milk is of critical importance.

OTA may be found in breast milk taken from mothers. Milk samples were taken from 75 mothers in Ankara and OTA was detected in 100% of the samples (Gürbay et al., 2010). It was observed that the OTA level was approximately between 0.62–13.11 µg/L. In this study, it was reported that the OTA concentration was between 0.6-1.5 µg/L in 28 samples, the OTA concentration was between 1.5-3 µg/L in 34 samples, and the OTA concentration was higher than 3 µg/L in 13 samples (3 µg/L is the upper limit allowed for many beverages). In another study, OTA was found at concentrations between 0.40 µg/L and 2.72 µg/L (OTA concentration above 0.5 µg/L in 97.5% of the milk) in 122 breast milk samples taken from mothers living in Ankara in 2017-2018 (Memiş & Yalçın, 2021). In addition to breast milk samples collected from Ankara, OTA has also been detected in breast milk samples in various countries; 20% of samples from Italy have OTA concentration of 0.1-12 µg/L (Micco et al., 1995), 36% of samples from Egypt have OTA concentration of 5-45 µg/L (El-Sayed et al., 2002), 35% of samples from the Republic of Sierra Leone 0.2-337.0 µg/L OTA concentration was observed (Jonsyn et al., 1995).

A study conducted in Egypt evaluated OTA levels in breast milk and infant kidney functions. In this study, OTA concentration in the serum and milk of 50 mothers and OTA levels in the serum of the babies of these mothers (who were breastfed for 4 months and did not receive any additional nutrition) were measured and the kidney functions of the babies were evaluated (Hassan et al., 2006). The mean OTA concentrations were found to be 4.28 µg/L in mothers' serum samples and 1.87 µg/L in the milk samples (Hassan et al., 2006). Considering the mean values, it is seen that there is 2.2 times as much OTA in serum as the OTA value in milk. Therefore, it can be assumed that mothers are also exposed to OTA. It was observed that the serum of the babies in the Egyptian study had 1.26 ± 1.1 µg/L OTA. In this study, they stated that microalbuminuria and $\beta 2$ microglobulinuria were found to a greater extent in babies exposed to OTA, compared to those not exposed. This indicates that kidney functions may be affected by OTA exposure. The study did not include any short or long-term observations of these babies. Therefore, we cannot comment further. However, such observation studies need to be carried out for longer periods. These studies will be useful in showing the impact of OTA exposure on society.

9. Conclusion

Contamination of agricultural products with OTA can occur in many different steps. OTA production may be the cause of fungal activity in many plant-based products, in the field, in production, factory, storage and distribution processes, on market shelves and even while keeping the product at home (Figure 1). The mean OTA contamination level of some products is given in Table 1, and there are some samples in some products where the OTA level exceeds the permissible limits. That indicates that OTA is produced independently in many steps.

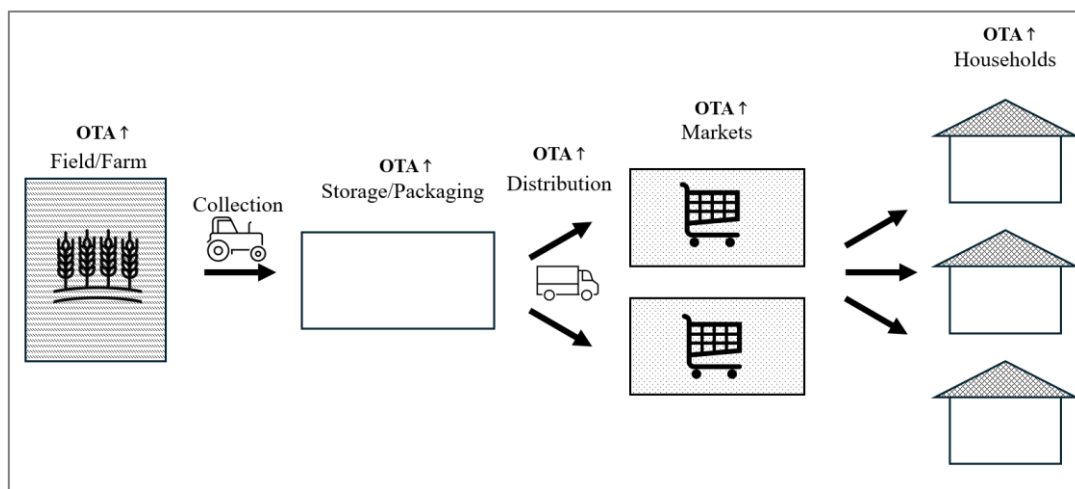


Figure 1. Possible steps that increase the OTA level in food from production to consumption.

Table 1. Mean OTA concentration in some frequently consumed foods and beverages in Türkiye.

Product	Sample	OTA Concentration	Reference
Tea (İstanbul)	1	139.5 µg/kg	Gazioglu and Kolak (2018)
Tea	14	-	Toman et al. (2018)
Raisins (Burdur)	17	-	Yurdakul et al. (2019)
Raisins (İstanbul)	50	1.15 µg/kg	Akdeniz et al. (2013)
Grape juice (İstanbul)	10	1.4 µg/kg	Akdeniz et al. (2013)
Commercial pekmez (İstanbul)	25	2.04 µg/kg	Akdeniz et al. (2013)
Commercial pekmez (Multiple Cities)	20	1.4 µg/kg	Tosun et al. (2014)
Organic pekmez (Multiple Cities)	7	9.2 µg/L	Tosun et al. (2014)
Homemade pekmez (Multiple Cities)	55	3.5 µg/kg	Tosun et al. (2014)
Wine (Thrace)	44	158 ng/L	Var and Kabak (2007)
Wine (Aegean)	28	60 ng/L	Var and Kabak (2007)
Wine (Eastern Anatolia)	8	27 ng/L	Var and Kabak (2007)
White flour (Bursa)	12	6.89±0.46 µg/kg	Cengiz et al. (2007)
Whole wheat flour (Bursa)	14	9.30±1.33 µg/kg	Cengiz et al. (2007)
Corn flour (Bursa)	10	6.39±1.10 µg/kg	Cengiz et al. (2007)
Whole wheat bread (Bursa)	28	7.84±0.39 µg/kg	Cengiz et al. (2007)
Cornbread (Bursa)	10	4.94±0.46µg/kg	Cengiz et al. (2007)
White bread (Bursa)	11	6.38±0.48 µg/kg	Cengiz et al. (2007)
Milk from collection tank (Burdur)	40	2-270 ng/L	Keyvan et al. (2018)
Raw, pasteurized or UHT milk (Burdur)	105	119±9 ng/L	Turkoglu and Keyvan (2019)
Surf cheese (Hatay)	30	0.615 ± 0.228 µg/kg	Sakin et al. (2018)
Baby supplementary food (Çorum)	62	0.103 µg/kg	Kabak (2012)

Therefore, by tracking the products leaving the factory by government officials or company officials or researchers, identifying and publishing the hot spots where OTA formation is maximum, it will be possible to take special measures on the basis of these points. By reducing fungal activity in these hot spots, the amount of OTA in more products can be kept at acceptable levels. In addition, informing not only the state administration but also the public about fungal toxins will contribute to reducing the risk of OTA in foods at home or will ensure that risky foods are not consumed. Thus, by reducing the OTA level in foods, people can keep their OTA exposure below the tolerable weekly OTA intake level.

Conflict of Interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

Biogenic Amines in Seafood

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ABSTRACT

Biogenic amines (BAs) are organic compounds naturally formed in foods through the microbial decarboxylation of amino acids. These compounds are particularly significant in seafood due to the high susceptibility of these products to microbial contamination and improper storage conditions. Biogenic amines, including histamine, tyramine, putrescine, and cadaverine, are often indicators of food freshness and spoilage. Their presence at high levels can pose serious health risks to consumers, making the study of their formation, types, and control methods crucial for food safety. This comprehensive review examines the mechanisms underlying the formation of biogenic amines in seafood. The primary sources of these compounds are bacteria from the families Enterobacteriaceae, Lactobacillaceae, and Pseudomonadaceae. Factors such as temperature, pH, and oxygen availability significantly influence bacterial activity and, consequently, biogenic amine production. Histamine is highlighted as one of the most toxic biogenic amines, capable of causing histamine poisoning, which presents symptoms such as flushing, headaches, nausea, and vomiting. Control and prevention strategies for biogenic amine formation are critically discussed, including the maintenance of the cold chain, hygienic processing techniques, salting, fermentation, and the use of starter cultures. Additionally, innovative microbial inactivation methods such as high hydrostatic pressure and UV radiation are explored for their efficacy in reducing biogenic amine levels. The review also addresses current regulations and standards set by international health authorities, particularly focusing on histamine levels in fish and fish products. These regulations ensure regular monitoring and compliance to protect consumer health. Future research directions are proposed, emphasizing the need for developing new detection methods for rapid and sensitive identification of biogenic amines, improving bacterial inactivation techniques, and advancing genetic and molecular biology studies to better understand the production mechanisms of these compounds. Furthermore, raising consumer awareness about the health impacts of biogenic amines through educational programs is highlighted as a critical area for public health promotion. In conclusion, the review underscores the importance of biogenic amines in seafood quality and safety. Effective control and prevention measures, combined with ongoing research and regulatory compliance, are essential to minimize health risks associated with these compounds. Ensuring proper handling, storage, and processing conditions, along with increasing consumer awareness, will contribute significantly to managing and mitigating the risks posed by biogenic amines in seafood.



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1. Introduction

In recent years, food safety and quality have become critical issues in public health, driven by increased consumer awareness and the globalization of food markets. Seafood, a significant component of human diet worldwide, is particularly prone to quality and safety concerns due to its perishable nature

and susceptibility to microbial contamination. Among the various chemical hazards associated with seafood, biogenic amines (BAs) stand out due to their potential to cause adverse health effects and their role as food spoilage indicators.

Biogenic amines are low molecular weight organic bases formed by the decarboxylation of amino acids through the enzymatic action of microorganisms. This process can occur

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during food processing, storage, and fermentation. In seafood, biogenic amines are primarily formed due to bacterial activity, which is influenced by various factors such as temperature, pH, and oxygen availability. The most commonly found biogenic amines in seafood include histamine, tyramine, putrescine, and cadaverine. These compounds are of particular concern because they can reach toxic levels if seafood is not properly handled and stored.

Histamine, often associated with scombroid fish poisoning, is one of the most toxic biogenic amines. It is formed predominantly in fish of the Scombridae family, such as tuna and mackerel. Consumption of histamine-contaminated seafood can lead to histamine poisoning, characterized by symptoms such as flushing, headaches, nausea, vomiting, and in severe cases, cardiovascular disturbances. Tyramine, another significant biogenic amine, can cause hypertensive crises in susceptible individuals, particularly those on monoamine oxidase inhibitors (MAOIs). While putrescine and cadaverine are not directly toxic, they can potentiate the effects of histamine and are often used as indicators of microbial spoilage.

The formation of biogenic amines in seafood is a complex process influenced by several factors. Bacterial contamination is the primary source, with specific bacteria such as those from Enterobacteriaceae, Lactobacillaceae, and Pseudomonadaceae families playing key roles. Environmental factors such as temperature, pH, salt concentration, and the presence of oxygen also significantly affect the formation and accumulation of biogenic amines. For instance, improper refrigeration can accelerate bacterial growth and enzymatic activity, leading to higher levels of biogenic amines.

Given the health risks associated with biogenic amines, their control and prevention in seafood is of paramount importance. Effective management strategies include maintaining the cold chain from capture to consumption, employing hygienic processing techniques, and utilizing preservation methods such as salting and fermentation. Additionally, the use of starter cultures that inhibit or reduce biogenic amine formation has shown promise. Recent advancements in microbial inactivation techniques, such as high hydrostatic pressure and UV radiation, offer additional tools for controlling these compounds.

Regulatory frameworks have been established by international health authorities to limit the levels of biogenic amines in seafood, particularly histamine. These regulations mandate regular monitoring and compliance to ensure consumer safety. Despite these measures, incidents of biogenic amine poisoning still occur, underscoring the need for continuous research and improvement in detection methods, prevention strategies, and public awareness.

Future research should focus on developing rapid and sensitive detection methods for biogenic amines, improving the

efficacy of bacterial inactivation techniques, and gaining a deeper understanding of the genetic and molecular mechanisms underlying biogenic amine production. Moreover, consumer education programs are essential to raise awareness about the risks associated with biogenic amines and promote safe handling and storage practices.

Biogenic amines represent a significant challenge in ensuring the safety and quality of seafood. Understanding their formation, health effects, and control measures is critical for mitigating their risks. This comprehensive review aims to provide an in-depth analysis of biogenic amines in seafood, highlighting the latest research, regulatory standards, and future directions for enhancing food safety.

2. Formation and Sources of Biogenic Amines

Biogenic amines (BAs) are formed primarily through the microbial decarboxylation of amino acids in seafood. This process is catalyzed by specific enzymes known as decarboxylases, produced by certain bacteria. The formation of biogenic amines in seafood is influenced by several intrinsic and extrinsic factors, including the type of microorganism present, temperature, pH, salt concentration, and oxygen availability. Understanding these factors is crucial for developing effective strategies to control BA levels in seafood.

2.1. Microorganisms Responsible for Biogenic Amine Formation

The primary microorganisms involved in BA production in seafood belong to several bacterial families. Key species include:

i. **Enterobacteriaceae**: This family includes genera such as *Enterobacter*, *Klebsiella*, and *Escherichia*, which produce significant amounts of histamine, putrescine, and cadaverine. Recent studies, such as those by Rivas et al. (2023), have highlighted the prevalence of these bacteria in improperly stored seafood and their role in histamine formation.

ii. **Lactobacillaceae**: Lactic acid bacteria (LAB) from this family, particularly *Lactobacillus* and *Pediococcus* species, are commonly associated with fermented seafood products. While LAB are often beneficial in food fermentation, certain strains can decarboxylate amino acids, leading to the production of tyramine and other BAs (Garai et al., 2007).

iii. **Pseudomonadaceae**: Members of this family, especially *Pseudomonas* spp., are psychrotrophic bacteria that can grow at low temperatures, making them significant contributors to BA formation in refrigerated seafood. Research by Arulkumar et al. (2023) has demonstrated the ability of *Pseudomonas* spp. to produce putrescine and cadaverine even under cold storage conditions.

2.2. Factors Influencing Biogenic Amine Formation

Several factors affect the growth of BA-producing bacteria and the subsequent formation of BAs in seafood:

i. **Temperature:** Temperature is a critical factor influencing the bacterial growth rate and enzymatic activity. Higher temperatures generally accelerate the production of BAs. Studies by Biji et al. (2020) have shown that histamine formation in fish increases significantly at temperatures above 15°C. Proper refrigeration (below 4°C) is essential to slow bacterial activity and BA formation.

ii. **pH Levels:** The pH of the seafood environment can significantly impact bacterial metabolism. Most BA-producing bacteria thrive in neutral to slightly alkaline conditions (pH 6-8). Research by Ekici and Omer (2020) indicates that adjusting the pH of seafood products can be an effective strategy to inhibit BA production.

iii. **Salt Concentration:** Salt concentration can either inhibit or promote the growth of certain bacteria. High salt concentrations are typically used in fermented seafood products to control spoilage microorganisms. However, some halotolerant bacteria can still produce BAs under these conditions. A study by Gardini et al. (2016) found that specific LAB strains could produce tyramine even at high salt concentrations in salted fish products.

iv. **Oxygen Availability:** The presence or absence of oxygen affects the metabolic pathways of bacteria. While some BA-producing bacteria are facultative anaerobes and can thrive in both aerobic and anaerobic conditions, others are obligate aerobes or anaerobes. Anaerobic conditions can promote the production of certain BAs, such as cadaverine, as shown in the research by Eerola et al. (1993).

2.3. Recent Advances in Understanding BA Formation

Recent advances in molecular biology and genomics have provided deeper insights into the mechanisms of BA formation. High-throughput sequencing and metagenomic analyses have identified specific genes responsible for BA production. For instance, the histidine decarboxylase gene (*hdcA*) in *Morganella morganii* has been extensively studied for its role in histamine production (Oktariani et al., 2022).

Additionally, advances in microbial ecology have shed light on the complex interactions between different bacterial species in seafood matrices. For example, competition and cooperation between bacterial strains can influence the overall BA levels in seafood products. Studies by Kuley et al., (2017) have shown that certain non-BA-producing bacteria can inhibit the growth of BA producers through competitive exclusion or the production of inhibitory substances.

2.4. Mitigation Strategies

Understanding the factors influencing BA formation has led to the development of various mitigation strategies. These include:

i. **Cold Chain Management:** Maintaining a continuous cold chain from harvest to consumption is crucial. Implementing rapid cooling methods and using ice or refrigerated storage can significantly reduce BA formation (Naïla et al., 2010).

ii. **Hygienic Handling and Processing:** Ensuring hygienic conditions during seafood processing and handling minimizes contamination with BA-producing bacteria. Good manufacturing practices (GMP) and hazard analysis and critical control points (HACCP) systems are essential tools in this regard (Abuhlega & Ali, 2022).

iii. **Use of Additives:** Certain food additives, such as organic acids (e.g., lactic acid, acetic acid), can inhibit the growth of BA-producing bacteria. These additives can be used in marinades or during processing to control BA levels (Shim et al., 2022).

iv. **Starter Cultures:** Utilizing starter cultures that do not produce BAs or can outcompete BA producers is an effective strategy in fermented seafood products. Recent studies have identified specific LAB strains that are effective in reducing BA formation during fermentation (Hernández-Jover et al., 1997).

3. Types and Quantities of Biogenic Amines

Biogenic amines (BAs) found in seafood are primarily histamine, tyramine, putrescine, and cadaverine. These compounds are formed by the microbial decarboxylation of specific amino acids. The type and quantity of biogenic amines present in seafood can vary widely based on factors such as the species of fish, the conditions of storage and handling, and the microbial flora present. Understanding the different types of biogenic amines and their concentrations in various seafood products is crucial for assessing potential health risks and implementing effective control measures.

3.1. Histamine

Histamine is the most well-known and toxic biogenic amine associated with seafood, particularly in fish from the Scombridae family (e.g., tuna, mackerel). It is formed from the decarboxylation of histidine by histidine decarboxylase enzymes produced by certain bacteria, such as *Morganella morganii*, *Klebsiella pneumoniae*, and *Hafnia alvei*.

Recent studies have highlighted the variability in histamine levels across different fish species and processing conditions. According to a study by Hungerford (2010), histamine levels in improperly stored tuna can exceed 500 mg/kg, far above the acceptable limit of 50 mg/kg set by the European Union.

Moreover, histamine production is highly temperature-dependent, with rapid increases observed at temperatures above 15°C (Colombo et al., 2018).

3.2. Tyramine

Tyramine is produced from the decarboxylation of tyrosine by the action of tyrosine decarboxylase. It is commonly found in fermented foods but can also occur in fresh and processed seafood. Bacteria such as *Lactobacillus spp.* and *Enterococcus spp.* are known producers of tyramine.

The health impact of tyramine is significant, particularly for individuals taking monoamine oxidase inhibitors (MAOIs), as it can cause hypertensive crises. According to Naila et al. (2010), tyramine levels in some fermented fish products can reach up to 200 mg/kg, posing a potential health risk if consumed in large quantities.

3.3. Putrescine and Cadaverine

Putrescine and cadaverine are formed from the decarboxylation of ornithine and lysine, respectively. These amines are commonly associated with spoilage and are not directly toxic but can potentiate the effects of histamine by inhibiting the enzymes responsible for histamine detoxification.

Recent research by Shim et al. (2022) has shown that putrescine and cadaverine levels can serve as reliable indicators of spoilage in seafood. In a study of various fish species, putrescine levels were found to range from 10 to 300 mg/kg, while cadaverine levels ranged from 20 to 500 mg/kg, depending on the storage conditions and duration.

3.4. Other Biogenic Amines

Other biogenic amines, such as serotonin, spermidine, and spermine, are also present in seafood, albeit in lower concentrations. Serotonin, while primarily known for its role in neurotransmission, can also be found in certain fish species. Spermidine and spermine are involved in cellular growth and function but can contribute to the overall biogenic amine load in seafood.

4. Quantitative Analysis and Acceptable Limits

Quantitative analysis of biogenic amines in seafood is typically performed using chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). These methods allow for precise measurement of biogenic amine concentrations and are essential for monitoring compliance with regulatory standards.

The acceptable limits for biogenic amines in seafood vary by region and regulatory body. For instance, the European Union has set a maximum allowable histamine level of 50 mg/kg for fresh fish and fish products (Commission Regulation

(EU) No 2073/2005). The US Food and Drug Administration (FDA) has established a defect action level of 50 mg/kg for histamine in fish (Florida Sea Grant, 2011).

5. Recent Studies on Biogenic Amine Levels in Seafood

Recent studies have provided valuable insights into the levels of biogenic amines in various seafood products. For example, a study by Kuley et al. (2017) reported that histamine levels in improperly stored mackerel reached 400 mg/kg within 24 hours at 25°C. Another study by Mah et al. (2002) found that tyramine levels in salted anchovies could exceed 150 mg/kg, highlighting the need for careful monitoring and control.

In addition to these findings, research by Doeun et al. (2017) has demonstrated the effectiveness of certain preservation methods in reducing biogenic amine levels. For instance, the use of organic acids and vacuum packaging significantly decreased histamine and putrescine levels in refrigerated fish.

6. Health Effects of Biogenic Amines

Biogenic amines (BAs) in seafood can pose significant health risks to consumers. The severity of these health effects depends on the type and concentration of the biogenic amines, as well as individual susceptibility. Understanding the health implications of different biogenic amines is essential for public health and for developing effective control measures.

6.1. Histamine

Histamine is the most well-known biogenic amine associated with foodborne illness, particularly scombroid poisoning. Histamine poisoning occurs when individuals consume fish with high levels of histamine, typically resulting from improper storage. The symptoms of histamine poisoning can occur within minutes to hours after consumption and include:

- Flushing
- Headache
- Nausea
- Vomiting
- Abdominal cramps
- Diarrhea
- Palpitations
- Hypotension or hypertension

In severe cases, histamine poisoning can lead to respiratory distress and cardiac complications. According to Petrovic et al. (2022), histamine levels above 50 mg/kg in seafood are considered hazardous, and levels exceeding 500 mg/kg are associated with severe toxic reactions.

Recent epidemiological studies, such as those by Hungerford (2010), indicate that histamine poisoning is more common in warmer climates where inadequate refrigeration of fish is more prevalent. The study highlights the need for improved monitoring and handling practices to reduce the incidence of histamine poisoning.

6.2. Tyramine

Tyramine, while less commonly associated with seafood, can cause adverse health effects, particularly in individuals taking monoamine oxidase inhibitors (MAOIs). Tyramine ingestion can lead to hypertensive crises, characterized by:

- Severe headache
- Hypertension
- Palpitations
- Sweating
- Nausea
- Vomiting

Hypertensive crises occur because tyramine can induce the release of norepinephrine from nerve endings, leading to vasoconstriction and increased blood pressure. Naila et al. (2010) emphasize that even moderate levels of tyramine (above 6 mg per meal) can trigger hypertensive reactions in sensitive individuals.

6.3. Putrescine and Cadaverine

Putrescine and cadaverine, while not directly toxic, can enhance the toxicity of histamine by inhibiting histamine-N-methyltransferase and diamine oxidase, the enzymes responsible for histamine detoxification. This synergistic effect can exacerbate the symptoms of histamine poisoning.

Recent studies by Shim et al. (2022) have shown that the presence of putrescine and cadaverine in seafood can increase the severity of histamine poisoning symptoms. In their research, fish samples with high levels of putrescine and cadaverine showed significantly higher histamine toxicity compared to samples with histamine alone.

6.4. Other Biogenic Amines

Other biogenic amines, such as spermidine and spermine, are involved in cellular metabolism and growth. While generally less toxic, high levels of these amines can contribute to overall toxicity and spoilage. For instance, spermidine and spermine can decompose into putrescine and cadaverine under certain conditions, potentially increasing the biogenic amine load in seafood (Doeun et al., 2017).

6.5. Chronic Health Effects

Long-term exposure to low levels of biogenic amines has been less extensively studied, but there is growing concern

about their potential cumulative effects. Chronic ingestion of biogenic amines could potentially lead to:

- Gastrointestinal disturbances
- Headaches
- Allergic reactions
- Increased risk of chronic inflammatory conditions

Khora (2016) suggest that continuous exposure to low levels of biogenic amines, particularly in populations with high seafood consumption, could contribute to subtle but significant health impacts over time. Their review calls for more research into the chronic effects of biogenic amine exposure and the mechanisms underlying these effects.

6.6. Preventive Measures and Recommendations

To mitigate the health risks associated with biogenic amines, several preventive measures are recommended:

i. **Proper Storage and Handling:** Maintaining the cold chain from harvest to consumption is crucial. Rapid cooling and proper refrigeration (below 4°C) can significantly reduce biogenic amine formation.

ii. **Hygienic Practices:** Implementing good manufacturing practices (GMP) and hazard analysis and critical control points (HACCP) systems can minimize contamination with biogenic amine-producing bacteria.

iii. **Consumer Education:** Educating consumers about the risks associated with biogenic amines and proper handling of seafood can help reduce the incidence of biogenic amine poisoning.

iv. **Regulatory Compliance:** Adhering to regulatory standards for biogenic amine levels in seafood is essential. Regular monitoring and testing can ensure compliance and protect consumer health.

v. **Research and Innovation:** Continued research into new preservation methods, such as modified atmosphere packaging and the use of bioprotective cultures, can provide additional tools for controlling biogenic amine levels.

7. Control and Prevention of Biogenic Amines

Controlling and preventing the formation of biogenic amines in seafood is crucial for food safety and quality control. Various methods have been developed for this purpose:

i. **Maintaining the Cold Chain:** Keeping seafood at low temperatures slows down the proliferation of biogenic amine-producing bacteria and minimizes amine formation.

ii. **Hygienic Processing Techniques:** Ensuring hygienic conditions during production reduces contamination and prevents biogenic amine formation.

iii. **Salting and Fermentation:** These methods can control microbial activity and reduce biogenic amine production.

iv. **Use of Starter Cultures:** Employing starter cultures that inhibit or reduce the formation of biogenic amines helps maintain acceptable amine levels.

v. **Microbial Inactivation Techniques:** Techniques such as high hydrostatic pressure, UV radiation, and irradiation can be used to inactivate biogenic amine-producing microorganisms.

7.1. Mechanisms of Biogenic Amine Formation

Recent research has elucidated the intricate pathways involved in biogenic amine formation during food processing and storage (ten Brink et al., 1990). Microbial decarboxylases, particularly those produced by certain species of bacteria, play a crucial role in amino acid metabolism and biogenic amine production. Understanding these mechanisms is essential for developing targeted interventions to inhibit biogenic amine formation.

7.2. Innovative Detection Methods

Advancements in analytical techniques have facilitated the development of rapid and sensitive methods for detecting biogenic amines in food samples (Stratton et al., 1991). Chromatographic techniques, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), coupled with mass spectrometry detection, offer enhanced sensitivity and specificity for biogenic amine analysis. Additionally, emerging technologies, such as biosensors and spectroscopic methods, show promise for on-site monitoring of biogenic amines in realtime.

7.3. Preventive Strategies

Effective control and prevention of biogenic amines require a multifaceted approach that addresses various stages of food production, processing, and distribution. Recent studies have explored the use of natural antimicrobial agents, including plant-derived compounds and bacteriocins, to inhibit the growth of biogenic amine-producing microorganisms (Ko et al., 2015). Additionally, innovative packaging technologies, such as active and intelligent packaging systems, can help extend the shelf life of foods and prevent biogenic amine formation.

8. Regulatory Considerations and Future Directions

Regulatory agencies worldwide have established guidelines and maximum limits for biogenic amines in food products to ensure consumer safety (EFSA, 2011). Future research directions may focus on the development of risk assessment models to predict biogenic amine formation in specific food matrices and the exploration of synergistic effects between

antimicrobial agents and other food additives. Furthermore, interdisciplinary collaborations between food scientists, microbiologists, chemists, and regulatory authorities are essential to address the complex challenges associated with biogenic amine control and prevention.

8.1. Regulations and Standards

International health authorities have established acceptable levels for biogenic amines and implemented regulations for their control. For example, the European Union has issued directives to limit histamine levels in fish and fish products. These regulations mandate regular monitoring of biogenic amine levels and ensuring they remain within legal limits.

8.2. Future Research Areas

Research on the formation and control of biogenic amines in seafood continues to evolve. Future research areas include:

i. **Development of New Detection Methods:** Creating new analytical methods for the rapid and sensitive detection of biogenic amines.

ii. **Improvement of Bacterial Inactivation Techniques:** Investigating new techniques for more effective inactivation of biogenic amine-producing microorganisms.

iii. **Genetic and Molecular Biology Studies:** Understanding the genetic structures and enzymes involved in the production of biogenic amines by bacteria.

iv. **Increasing Consumer Awareness:** Developing educational programs to raise consumer awareness about the health effects of biogenic amines.

9. Conclusion

Biogenic amines are critical compounds in terms of quality and safety in seafood. Controlling the formation of these amines and ensuring they remain within legal limits is vital for food safety. Ensuring hygiene and appropriate temperature conditions in food processing and storage processes is essential to minimize the formation of biogenic amines. Additionally, further research on the health effects of biogenic amines and increasing consumer awareness will contribute to managing the risks associated with these compounds.

Conflict of Interest

The authors declare no conflict of interest.

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Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, 43(10), 1325-1330. <https://doi.org/10.1111/jfd.13229>

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