WHICH ONE MAY BE THE BEST QUALITY PARAMETER OF RISKY FOODS IN TERMS OF MOLD: AFLATOXINS, PATULIN, OCHRATOXIN A AND ERGOSTEROL

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Abstract

Agricultural products can be exposed to mycotoxins produced by several kinds of molds as seconder toxic compound, as a result of the development of various toxigenic molds during the stages of preharvest, harvest and storage. Therefore, it is important to determine the presence of mold in foods quickly because of the deterioration of aroma, flavor, appearance and textural structure as well as the mycotoxins. Due to infection of 25% of the agricultural products with mycotoxins in the world wide and global population rising increasingly, researchers indicate importance of safety and adequate food production in the future. Some important mycotoxins are aflatoxins (AFs), patulin and ochratoxin A (OTA). Aflatoxins, which are mycotoxins produced by molds, causes significant quality and economic loss and have unfavorable effects on human health. Patulin, often detected in apple and apple products, is a mycotoxin produced by several species of Aspergillus, Penicillium and Byssochlamys. OTA is a nephrotoxic and nephrocarcinogenic mycotoxin which is predominantly produced by the two ubiquitous fungal genera, Aspergillus and Penicillium. Additionally, the other chemical component for determination of mold growth is ergosterol, a constituent of cell membrane of molds, provides a quick assay for presence of molds. The aim of this study is to compare the aflatoxins, patulin, ochratoxin A and ergosterol in risk foods in terms of mold as a microbiological quality parameter and to state the most effective metabolite as quality parameter.

Key words: *aflatoxin, ergosterol, ochratoxin, patulin, quality parameter*

1. INTRODUCTION

Edibility of foods may decrease dependent on many reasons from farm to fork. As molds are resistant to various environmental conditions, they have great significance within these reasons. Molds (fungus) naturally grow in many agricultural products; especially red berries, figs, nuts, corn, peanuts, black pepper, bread, cheese and many fatty seeds in the field, in the gardens, after the harvest, during storage, or during processing of these products as food and animal feed (Karapınar, 2013). For instance, it is unavoidable mold growing in hazelnuts in consequence of inapropriate drying, storing, processing and harvest (Özçakmak & Dervişoğlu 2007).

Some molds produce secondary toxic compounds called mycotoxins in foods and cause spoilage of taste and flavor (Günaydın & Karaca 2015). The word, mycotoxin, is derived from the combination of "mykes", which means mold in Greek, and "toxicum", which means poison in Latin. Agricultural products can be exposed to mycotoxins produced by several kinds of molds as seconder toxic compound, as a result of development of various toxigenic molds (Bakırcı 1995; Hopmans 1997; Özmenteşe 2002). Fusarium, Aspergillus and Penicillium are common producers of mycotoxins infecting humans and animals through consumption of foodstuffs. Common mycotoxin producer molds can synthesize aflatoxins, cyclopiazonic acid, zearelenon, fumonisin B1, patulin, ochratoxin A, secalonic acid D, T-2 toxins, deoxynivalenol and nivalenol (Sudakin 2003; Keser & Kutay 2009; Bakırcı 2014; Kadakal & Tepe 2018). Hazelnut, pistachios, dried figs, black olives, red powder and pulp pepper, as well as other grain products, especially corn are regarded as foods contaminated with mycotoxins that are hazardous for human health (Oruç 2005). The presence of mycotoxins in animal products such as milk and dairy products, meat, eggs results from consumption of animal feed containing mycotoxin by animals (Karagözlü & Karapınar 2000). In addition, as a result of consumption of animal feeds containing mycotoxins by animals, humans are indirectly exposure to mycotoxins by consuming animal origin food

(Karapınar 2013). Mycotoxins are one of the most important issues which is supposed to be controlled in terms of food safety (Oruç 2005).

It is estimated that 25% of world's food crops, including cereals, nuts, fruit-vegetable, milk, cheese and meat, overall, are affected by mycotoxins. Consumers are mostly exposed to mycotoxins throughout vegetable-sourced foods (Tsitsigiannis et al. 2012). Mold growing causes significant organoleptic disorders such as color, odor and taste as a result of chemical and enzymatic reactions as well as mycotoxin formation in foods and feeds. Furthermore, content of water and dry matter reduce, carbohydrates and fats degrade, nutrients and energy values decrease and products lose their commercial value (Oguz & Kurtoğlu 2000; Hedayati et al. 2016).

Mycotoxins have several negative effects on humans and animals, including being carcinogenic, mutagenic and teratogenic (Karaca & Nas 2006; Stanisz et al. 2015). As a result of consumption of mycotoxin-contaminated foods by humans, poisonings can occur called "mycotoxicosis" (Jahed Khaniki 2007; Oruç et al. 2005). Mycotoxins cause different levels of poisoning based on the duration of exposure in humans and animals, the amount of mycotoxin and the physiological state and sensitivity of the organism (Salwa et al. 2000; Oruç 2005; Iqbal et al. 2012). The toxicity of mycotoxin especially depends on the molecular character of the subject mycotoxin, on the frequency of exposure and on the amount absorbed (Quillien 2002). Chronic diseases occur once low doses are taken for longer periods. These are diseases in organs (liver and kidney), degenerations, disorders of the immune system, imperfect and incomplete organ formations, skin necrosis, decrease in uraemia and weight loss. The individual's sensitivity to acute toxic effects is influenced by the individual's genetic, physiological characteristics and environmental factors (Anonymous 2006). In consequence of high doses mycotoxin intake, acute toxic effects occur and death may shortly occur after consumption of food (Karapınar 2013).

2. AFLATOXIN

Aflatoxins, which are derivated from difuranocoumarin, are generally produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Juan et al. 2008). Aflatoxin B1, B2, G1 and G2 (AFB1, AFB2, AFG1 and AFG2) are the main aflatoxins naturally forming in foodstuffs. Among the types of aflatoxin, AFB1 is the most common and has the highest toxicity (Saleemullah et al. 2006). Besides, it has been reported that AFB1 undergoes hydroxylation by the microsomal cytochrome P450 enzyme system in the liver and converts to aflatoxin M1 (AFM1), which excretes to the milk. After ingestion of AFB1-contaminated foodstuffs by lactating mothers, it takes 12–24 hour for AFM1 to appear in breast milk (Williams et al. 2004; Fallah 2010; Fallah et al. 2014; Kamkar et al. 2014). It is reported that AFs have high toxic, immunosuppressive, mutagenic, carcinogenic and teratogenic effects (Castells et al. 2008). Numerous researches have proved that aflatoxins have the highest carcinogenic effect among mycotoxins (Davis & Diener 1978). AFs have been classified as carcinogenic compounds to humans. AFB1 has been classified as a group 1 human carcinogen (International Agency for Research on Cancer (IARC) 1993). Likewise, IARC (2002) has categorised AFM1 as a potent carcinogenic compound (group 1) for humans. The most toxic and most frequently seen mycotoxins in foods are aflatoxins (Salwa et al. 2000; Oruç 2005; Iqbal et al. 2012). Aflatoxins cause serious economic losses as well as negative effects on human health.

Aflatoxin is mostly found in nuts (groundnut, hazelnut, pistachio, almond, pine nuts, various walnuts), cereals (wheat, rye, barley, oats and rice) and legumes (soybeans, beans, peas, cowpeas and lentils). Oil seeds such as cotton, sunflower, cocoa beans, sesame and rape seeds, spices especially red pepper, black pepper and dried fruit such as figs are also risky products in terms of aflatoxins (Uylaşer et al. 2005; Imperato et al. 2011). Milk and dairy products are other risky products in terms of aflatoxins. AFM1, derivative of AFB1, is present aflatoxin type in these products as addressed above (Kim et al. 2000; Galvano et al. 1998, Bakırcı 2001). It has been reported that amount of aflatoxin M1 determined in cheese is related with cheese type, process and the amount of whey removed (Applebaum et al. 1982; Galvano et al. 1998). Exposure to aflatoxins is generally considered resulting from mainly imported materials from countries having warm and humid climates (Imperato et al. 2011). European Union

countries have established the maximum level for AFB1 and total AF in peanuts, hazelnuts, dried fruits, grains and processed products as 2 ppb and 4 ppb, respectively (EC 2007).

Several methods have been developed for assay of aflatoxin. Immunoaffinity column is the most frequently used method. In this method, prepared sample for aflatoxin analyze is purified by passing through immunoaffinity columns. Purified sample is analyzed by methods such as HPLC (high performance liquid chromatography), TLC (thin layer chromatography) and MS (mass spectroscopy). Since AFB1 and AFG1 have weak fluorescence, they are derivatized with potassium bromide in HPLC analysis (Meçik 2007). Post-column derivatization and immunoaffinity column liquid chromatography has been accepted as the standard method for the determination of aflatoxin level by the European Committee for Standardization (CEN) (Stroka et al. 2000). In this regard, it is stated that in the confirmation studies the determination limits of the HPLC method are 2 ppb for AFB1, 4 ppb for total aflatoxin and 0.05 ppb for AFM1 (Gilbert & Anklam 2002). Additionally, AFs can be analyzed by using ELISA (Enzyme-Linked Immunosorbent Assay) as immunological, although not frequently for quantitative determination (Meçik 2007).

3. PATULIN

Patulin [4-hydroxy-4H-furo(3, 2-c)pyran-2(6H)-one], is produced by many fungal species. *Penicillium patulum* and *Penicillium expansum* are the most important patulin producer molds in foods. Aspergillus (*A. clavatus, A. terreus, A. giganteus*), Byssochlamys (*B. nivea* and *B. fulva*) and Penicillium (*P. roqueforti, P. melinii, P. equinum, P. calavrforme* etc.) can also produce patulin. Patulin-producing molds infect to fruits that are damaged by various causes in transportation and short-term storage (Artık et al. 1992).

Apple, apple juice and concentrate, apple jam, pear, apricot, peach, tomato may contain patulin (Hohler 1998), but patulin cannot be synthesized or remain stable in orange, orange juice, vegetables such as cabbage, radish, celery and onions (Artık et al. 1995; Kadakal & Nas 2000). Patulin frequently causes problems in pome fruit products especially in apple juice. The apples naturally decay as a result of keeping apples in a heap for a long time before process, so patulin levels were reported to be found high in apple juice and apple juice concentrate produced from decayed apples (Kadakal et al. 2003). Besides, it is reported that patulin may also be found in various foods such as shellfish, cereals and cheese (Pottono et al. 2013; Wright 2015; Blaiotta et al. 2017). It has been stated that the presence of patulin in the indicated products is closely related to the composition of fruit and vegetables and that the -SH groups in these products affect patulin synthesis or impair their stability (Artık et al. 1995; Kadakal & Nas 2000). Absence of patulin content in dairy products such as cheeses and meat products such as sausage and salami is associated with the same reason (Lieu & Bullerman 1977; Scott & Kanhere 1979; Kokkonen et al. 2005).

Although there is no evidence for negative effects of patulin on humans, the Joint Food and Agriculture Organization–World Health Organization (WHO) Expert Committee (1995) on Food Additives has suggested a limit value (maximum tolerable daily intake for patulin of 0.4 µg/kg of body weight) based on the results of studies on animals. Due to patulin has a mutagenic, teratogenic and carcinogenic effect on rats stated in some studies (Ciegler et al. 1976; Özçelik 1979), patulin has become an important quality parameter in some products, especially in apple juices.

The maximum allowed amount of patulin by World Health Organization (WHO) in foods is 50 µg/kg (Woller & Majerus 1982). European countries have set limits of patulin content in various foodstuff, 50 µg/kg for fruit juices and spirit drinks,10 µg/kg for infant's food by Commission Regulation No. 1881/2006. U.S. Food and Drug Administration (FDA) and China limits the patulin concentration to 50 µg/kg in apple products (CPG Sec.510.150 and GB 2761-2017) (Li et al. 2018).

For identification and quantitative analysis of patulin, several methods are suggested to be used. These methods are high-performance liquid chromatography with ultraviolet detection (HPLC-UV or HPLC-DAD), thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography tandem mass spectrometry (HPLC-MS), fluorescence polarization,

chemiluminescence assay, quantitative PCR assay, capillary electrophoresis (CE), surface plasmon resonance (SPR), electrochemical reduction techniques and quartz-crystal microbalance (QCM) (Li et al. 2017). Among these methods, for individual patulin analysis in food the single-residue liquid chromatography methods using mass spectrometry ultraviolet or DAD detection have been proposed (Sadok et al. 2018). HPLC-DAD has been reported to be found more suitable for routine analysis because of no requirement derivatization compared to GC-MS (Moukas et al. 2008; Li et al. 2017). However, HMF resulting from sugars and phenolic compounds behave similar to patulin in HPLC analysis (Li et al. 2017). So, interference occurs between HMF or phenolic compounds between patulin. In addition, patulin peak occurs at 12-13 minutes (Kadakal et al. 2003). This eluation time is longer when compared with assay of ergosterol, another marker chemical component for determination of mold growth.

4. OCHRATOXIN A

Ochratoxin A (OTA) (2S)-2-[[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4 dihydroisochromene-7-carbonyl] amino]-3-phenylpropanoic acid, is produced by many different species of Aspergillus (*A. ochraceus*, *A. niger*, *A. alliaceus*, *A. auricomus*, *A. carbonarius*, *A. glaucus*, *A. melleus*) and Penicillium (*P. nordicum*, *P. verrucosum*) (Kuruoğlu et al. 1999; Dall'Asta et al. 2008; Bakırcı 2014). International Cancer Research Agency (IARC 1993) categorized OTA as a possible human carcinogen (category 2B). OTA has been reported to cause various types of tumors in the urinary tract and kidney diseases called "Balkan Endemic Nephropathy" in some Balkan countries such as Bulgaria, Greece and Romania (Battilani 2002). OTA-producing molds can easily grow in fruits and cause issues in case of infection of fruits by pathogenic microorganisms or damaged by any physical / chemical action (Delage et al. 2003). OTA is mostly found in grains (barley, corn, wheat, rye), fruits (figs, raisin) and some seeds (coffee, cocoa seeds) (Kuruoğlu et al. 1999; Dall'Asta et al. 2008; Bakırcı 2014). Foods containing ochratoxins are barley, wheat, corn, rye, oats, figs, beans, olives, raisins, nuts, spices, coffee, beer, medicinal herbs, herbal teas and grapefruit juice (Akpınar 2015). According to results of Pattono et al. (2013), ochratoxin A may be found at high levels in dairy products such as cheese. Dall'Asta et al. (2008) reported that some commercial mold-ripened blue cheeses may contain ochratoxin A at low levels.

The suggested limits for OTA are 1-5 ppb for infant foods and children's foods, 3-50 ppb for other foods and 5-1000 ppb for feeds (Karagözlü & Karapınar 2000). It has been reported that in the European Union, the OTA level should be 1 ppb for baby foods and 5 ppb for cereals (Van Egmond 1996), whereas the level of OTA for all foods in the European Community has been established as 4-5 ppb (Anon 1997). Any limit value of OTA for animal foods has not been established by European Commission (EC) (Pattono et al. 2013; Keyvan and Yurdakul 2015). Nevertheless, OTA has been limited for wines as 2 µg/L by EC (2006).

The most frequently used method for OTA analyzes is LC/FLD (florescence detector liquid chromatography). Several methods based on fluorescence detector liquid chromatography coupled with immunoaffinity column cleanup or solid phase extraction cleanup have been verified and accepted as official method for OTA determination for various foods such as cereals, coffee, wine and beer. In addition, other methods of analysis for OTA determination are; thin layer chromatography (TLC), gas chromatography (GC-MS), laser-induced fluorescence combined with capillary electrophoresis and enzyme immunoassay (EIA) (Medina et al. 2006). LC/FLD analyze is easier, less costly and more precision compared to LC/MS (Frenette et al. 2008).

5. ERGOSTEROL

In contrast to bacteria and yeast, it is difficult to assay growth of molds since they do not have a single cell during growth of molds (Taniwaki et al. 2006). When a fungal biomass cannot be separated from a solid substrate, fungus growth can be monitored by quantification of a chemical component such as ergosterol (Ekinci et al. 2014). Ergosterol, a precursor of vitamin D and found in two forms of free and

esterified, is found in fungus (Kadakal & Artık 2004; Yuan et al. 2007). Ergosterol turns into water soluble matter through boiling with an alkaline component such as potassium hydroxide and is included in saponifiable lipid groups (Kadakal & Artık 2004). Ergosterol, called provitamin D2 and overwhelmingly found in the membrane of fungi, is commercially produced from yeast (Lee 1983; Olsson et al. 2003; Thevissen et al. 2003; Mille-Lindblom et al. 2004).

Ergosterol is found at high levels because it is part of the membranes of the molds. However, it is a small component of the sterol mixture of various animals and plants. Content of sterols in bacterium is also insignificant, since they constitute less than 0.01% of dry weight and very little of this belongs to ergosterol (Kadakal et al. 2005). Ergosterol is commercially produced by yeasts (Bocchi et al. 1995), although molds are considered primary producers because of their greater biosynthetic capacity when compared to yeast. Besides, ergosterol biosynthesis depends on age, mold species, energy source and oxygen. (Ghiretti et al. 1995). Ergosterol plays significant role in regulation of selective permeability and is a necessary component for healthy fungal cells (Kadakal 2003). For that reason, ergosterol has recently been accepted as an indicator for the determination of fungus growth in food, and detection of formation has been accepted as an important quality parameter for the determination of fungus growth level (Saldamlı 2001). Ergosterol assay has been reported to be more sensitive and quicker compared to chitin, a cell wall component of molds (Seitz et al. 1977; Seitz et al. 1979; Matcham et al. 1985). Ergosterol are more resistant to environmental conditions compared to chitin (Bjurman 1994). So, ergosterol has been used as a microbiological quality indicator in tomato and its products in recent years. For instance, a limit value of 15 mg of total ergosterol/kg total solids has been proposed as an index of acceptable quality for tomato products (Bocchi et al. 1995; Ghiretti et al. 1995; De Sio et al. 2000; Bertoni et al. 1994).

Individual sterol assays consist of lipid extraction, saponification, unsaponifiable matter extraction or seperation/partial purification of sterols. Unsaponifiable compounds such as squalene and other linear hydrocarbons may interfere in chromatographic analysis, e.g thin layer chromatography or solid phase extraction, formation of sterol derivatives and their analysis by capillary gas chromatography (Lagarda et al. 2006) or gas–liquid chromatography (GLC), in which a seperation stage is supposed to be carried out. A suitable resolution might also be obtained with high performance liquid chromatography (HPLC), which is faster than GLC analysis and operates under milder column temperatures and non-destructive detection conditions (Manzi et al. 1996; Bada et al. 2004). Reversed-phase HPLC and ultraviolet detection (HPLC-UV) is optimized and validated method for sterols extraction and analysis. The developed methodology was afterwards applied to quantify ergosterol (Barreiare et al. 2014).

6. CONCLUSIONS

Molds can grow under certain conditions in foods. As a result of mold growth, mycotoxins may form. However, mycotoxins formed in foods are indigenous to variety of food and different from each other. The contamination of food with mycotoxin-producer molds does not mean that mycotoxin could form in foods. Because toxin production depends on many factors such as environmental conditions, variety, composition of the food, microbial quality and mold strains. It should not be understood that conditions supporting fungal growth are responsible for formation of mycotoxin. However, ergosterol is a component which can be detected in all foods contaminated with molds.

In order to determine level of mycotoxins, mycotoxins are supposed to be analyzed separately. In addition, assays of mycotoxin such as aflatoxins, OTA take long time and are high cost because of long-time extraction phases, expensive equipments such as immunoaffinity column and valuable chemical expenditure in most frequently used, validated and optimized HPLC methods. Low detection limits of mycotoxins (ppb level) are another negativeness being supposed to be taken into account. On the other hand, ergosterol can be detected in foods, in which molds grow, regardless of type of food as ergosterol is compulsory component for living and healthy fungus cells and found in membrane of mold's cell wall. In addition, ergosterol analysis can be considered to be more advantageous than mycotoxin analysis because it is chromatographically fast, low cost, highly accurate and has high detection limits (ppm

level). When comparing all of these, assay of ergosterol may be regarded as most effective metabolite for determination of mold growth in foods.

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