Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the effect on the Spanish population of the derogation of national regulation on maximum allowed limits for aflatoxins B_1, B_2, G_1 and G_2 in food

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Abstract

Aflatoxins are toxic metabolites produced by some species of molds belonging to the genus *Aspergillus* which grow on plants and vegetable-origin foods. Among the aflatoxins that can be found (B_1, B_2, G_1, G_2, M_1 and M_2), from a food safety point of view the most remarkable is aflatoxin B_1, because it is the most prevalent in foods and toxic for humans.

Due to the toxicity of these substances and to protect consumers' health, the European Union has stated maximum residue limits (MRL) for aflatoxins in foods, in the Regulations (EC) No 1881/2006 and (EU) No 165/2010. MRL have been established for aflatoxin B_1 and the sum of B_1, B_2, G_1 and G_2 in different foods, including those in which contamination with these kind of toxins is more frequent and can be more dangerous for human health.

The Spanish Royal Decree 475/1988 (Real Decreto, 1988), approved before the Regulation (EC) No 1881/2006, sets MRL for the aflatoxins in food for human consumption; 5 µg/kg for aflatoxin B_1 and 10 µg/kg for the sum of aflatoxin B_1, B_2, G_1 and G_2.

Despite the Regulation (EC) No 1881/2006 includes food which most could usually pose a risk for human health, specific papers have demonstrated the presence of total aflatoxins and aflatoxin B_1 in foods not included in the Regulations (EC) No 1881/2006 and (EU) No 165/2010, even at higher concentrations than those set up by the Spanish Royal Decree 475/1988.

For this reason, notwithstanding the management measures that are pertinent, the Scientific Committee considers that, while there are no more data about maximum limits for these substances other food samples different than those included in the previously mentioned legislations (as tiger nuts or other doubtful foods regarding their producing conditions) the Royal Decree 475/1988 offers a significant protection for consumers.

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Key words

Aflatoxins, aflatoxin B_1, aflatoxicosis, foods, hepatic damage, prevention, residues, cereals, tiger nut.

Introduction

Aflatoxins are toxic metabolytes produced by several species of fungus of the *Aspergillus* genus, which grow on plants and food of vegetable origin. The main aflatoxin-producing species are *Aspergillus flavus* and *Aspergillus parasiticus*, although other species that produce aflatoxins in food have also been identified such as *A. nomius* (Kruztman et al., 1987), *A. tamarii* (Goto et al., 1997), *A. pseudotamarii* (Ito et al., 2001) or *A. australis* (IARC, 2002).

These compounds were discovered at the end of the 1950s and beginning of the 1960s as a result of an investigation conducted to evaluate the high mortality rate in poultry and other food-producing animals as a consequence of ingesting feed containing peanuts originating from South America (Blount, 1961) (Sargeant et al., 1961). Aflatoxins are fluorescent compounds with a condensed coumarinic structure with one bifurane and one pentanone (aflatoxins B) or one lactone (aflatoxins G) group. The letters B and G refer to the colour of the aflatoxins under ultraviolet light (Blue and Green), and the numbers 1 and 2 refer to their position in chromatographic separation.

The more relevant aflatoxins in terms of food safety are B_1 and B_2 (produced by *A. flavus* and *A. parasiticus*), G_1 and G_2 (produced by *A. parasiticus*) and M_1 and M_2 (metabolytes of aflatoxins B_1 and B_2 which are excreted in milk). Among them, aflatoxin B_1 stands out from a health safety point of view as it is the most common in food as well as the most toxic for humans (Deng et al., 2010).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated aflatoxins B and G on several occasions since 1987 (JECFA, 1987, 1997, 1999, 2002, 2007) and has recommended that, due to their carcinogenic potential, dietary exposure to aflatoxins should be minimised as much as possible. In this way, the 2007 report by the Panel on Contaminants of the European Food Safety Authority (EFSA) indicated that exposure to aflatoxins from any food source should be kept as low as reasonably as possible, due to their genotoxic and carcinogenic properties (EFSA, 2007).

Recently, *Codex Alimentarius* has established maximum limits for total aflatoxins (the sum of aflatoxins B_1, B_2, G_1 and G_2) in some nuts (almonds, peanuts, hazelnuts, pistachios and Brazil nuts) intended for further processing, at 15 µg/kg in comparison to the 10 µg/kg allowed for the same ready to eat products, based on the information provided by JECFA (*Codex Alimentarius*, 2008).

In the European Union, due to the toxicity of these compounds, and in order to ensure the efficient protection of public health, the Regulation (EC) No 1881/2006 (EU, 2006a) established maximum content of, among other contaminants, aflatoxin B_1 and total B_1, B_2, G_1 and G_2 in various types of foods including tree nuts, cereals, corn, rice, peanuts, oilseeds and in their derived or processed products, in some spices, processed cereal products, in foods for infants and young children, infant formulae and follow-on formulae, dietary foods for special medical purposes intended to be specifically for infants (Table 2).

As a result, the products specified in the regulations which contain aflatoxins in levels exceeding the established maximum content must not be marketed as such, even after being combined with other food products, and must not be used as an ingredient in other foods. Also, this Regulation considers that, given that selection or other physical treatments allow the aflatoxin content of various foods, like peanuts and other oilseeds, nuts, rice and corn, to be reduced, and as a way of minimising the repercussions on the market, it is advisable to allow greater aflatoxin content in products that are not intended for direct human consumption or as ingredients in food products. In these cases, the maximum aflatoxin content has been established taking into account the effectiveness of the treatments mentioned in reducing aflatoxin content to levels below the maximum content established for the products intended for direct human consumption or to be used as ingredients in food products.

Because of this, peanuts and other oilseeds, tree nuts, dried fruit, rice and corn that fail to comply with the maximum aflatoxin content can be marketed under specific conditions which include, among others, that before they are intended for direct human consumption or used as ingredients in food products, they undergo a physical treatment that successfully reduces the aflatoxin content to the maximum permitted levels for those products treated.

In the same context, Regulations (EC) No 401/2006 (EU, 2006b) and (EU) No 178/2010 (EU, 2010b) have established rules for sampling and analysis for the official control of mycotoxins in food products, while Regulation (EC) No 1152/2009 (EU, 2009) has regulated the specific conditions for the import of the products in question, owing to the risk of contamination by aflatoxins of certain food products from some third countries.

In Spain, prior to the approval of Regulation (EC) No 1881/2006, Royal Decree 475/1988 (Real Decreto, 1988) established maximum permitted limits of aflatoxins B_1, B_2, G_1 and G_2 in food for human consumption of 10 µg/kg for total aflatoxins B_1, B_2, G_1 and G_2, and 5 µg/kg for aflatoxin B_1. Unlike the Regulation (EC) No 1881/2006, the Royal Decree 475/1988 does not specify certain foods that this is applicable for, meaning that it can be applied to all food intended for human consumption which is not specifically regulated by the European Union regulations.

Faced with the existence of the two regulations, both controlling maximum permitted limits of aflatoxins B_1, B_2, G_1 and G_2 in food, the question arises of whether the national regulation covers aspects that are not included in the European Union regulation, and, in consequence, provides a certain level of consumer protection, or whether, on the other hand, it is redundant and does not offer greater levels of safety.

For this reason, the Executive Director of the Spanish Agency for Food Safety and Nutrition (AESAN) has questioned the Scientific Committee in relation to the effect that the derogation of Royal Decree 475/1988, which regulates the maximum permitted levels of aflatoxins B_1, B_2, G_1 and G_2 that are not covered by Regulation (EC) No 1881/2006, would have on the Spanish population.

Biological activity and toxicity of aflatoxins for humans

Aflatoxins B and G are genotoxic and carcinogenic mycotoxins (SCF, 1996), having been classified by the International Agency for Research on Cancer as belonging to group 1 (substances that are carcinogenic for humans) (IARC, 1993). A potential immunosuppressant and nutritional interference effect has also been reported (Williams et al., 2004), as have mutagenic, teratogenic and hepatotoxic effects (Kensler et al., 2011). The most powerfully carcinogenic aflatoxin is considered to be aflatoxin B_1 (JECFA, 1999) which is also the aflatoxin commonly found in the greatest concentrations in contaminated food and feed (Sweeney and Dobson, 1998).

The biological effects produced as a response to aflatoxin consumption depend on variation between species, age, gender, nutritional status, the components of the foods in which they are present, and interaction with chemical substances. Additionally, the dose and exposure period of the organism to the toxin are also very important. With regard to this, it should be taken into account that although the foods in which they appear most prevalently are the foods of vegetable origin mentioned earlier, aflatoxins can be accumulated in the tissue of food-producing animals after ingestion, and can then reach humans through the consumption of food of animal origin in which they are present including milk and egg (Deng et al., 2010). Their effects on the organism are closely related to their chemical structure, and they can be classified generically as carcinogenic, mutagenic, teratogenic, hepatotoxic and immunosuppressant (Kensler et al., 2011).

The clinical manifestations of acute aflatoxicosis are vomiting, abdominal pain, pulmonary oedema, along with fatty infiltration and necrosis of the liver (Kensler et al., 2011). However, the appearance of these symptoms in humans is extremely rare and the danger of aflatoxins is determined basically by their chronic toxicity. The carcinogenic potential of aflatoxins, fundamentally aflatoxin B_1, has been established strongly in many species of animals, including rodents (which are highly susceptible to these substances), primates and fish. The liver has consistently proven to be the principal organ affected by the toxic action of aflatoxin B_1. However, depending on the species of animal, the dose, the route of exposure and the diet of the exposed subjects, tumours related to the action of the aflatoxins have also been recorded in other organs and parts of the body, such as the kidneys and colon (Kensler et al., 2011).

Factors influencing the development of Aspergillus and aflatoxin production

The factors implicated in the growth of the fungus belonging to the *Aspergillus* genus in foods are as much those relating to the environment in which they develop (pH, composition of the food or water activity) as to extrinsic factors: ambient humidity, storage temperature and microbial competition (Zinedine and Mañes, 2009). *A. flavus* and *A. parasiticus*, the principal aflatoxin-producing fungi, have similar growth and toxinogenesis patterns (Table 1).

Table 1. Limits of aflatoxin growth and production by *A. flavus* and *A. parasíticus*

Growth	***A. flavus***	***A. parasiticus***	***A. flavus***	***A. parasiticus***	***A. flavus***	***A. parasiticus***
	Minimum		**Optimum**		**Maximum**	
Temperature ºC	10-12	12	33	32	43	42
Water activity	0.8	0.80-0.83	0.98	0.99	>0.99	>0.99
pH	2	2	5-8	5-8	>11	>11
Aflatoxin production	***A. flavus***	***A. parasiticus***	***A. flavus***	***A. parasiticus***	***A. flavus***	***A. parasiticus***
	Minimum		**Optimum**		**Maximum**	
Temperature ºC	13	12	16-31	25	31-37	40
Water activity	0.82	0.86-0.87	0.95-0.99	0.95	>0.99	>0.99
pH	–	2	–	6	–	>8

Source: (ICMSF, 1996).

Although the conditions described vary slightly depending on the bibliographical source, some authors report that *A. flavus* or *A. parasiticus* grow in a temperature range between 10-12 and 42-43 ºC, and optimally between 32 and 33 ºC. They can grow in a wide pH range (2.1 to 11.2), with optimal growth between 3.5 and 8. In terms of water activity (a_W), the minimum values for growth are between 0.80 and 0.83, and the optimum is 0.99 (Sweeney and Dobson, 1998).

With regard to toxinogenesis, the temperature range in which toxins are produced is from 12 to 40 ºC, with the optimum between 25 and 30 ºC. In *A. parasiticus* the proportion of production of aflatoxins B compared to G is greater at high temperatures (ICMSF, 1996). They are produced in a pH range between 3.5 and 8, with an optimum pH of 6. In terms of water activity (a_W), the minimum for production is 0.82 for *A. flavus* (Sweeney and Dobson, 1998) with an optimum of 0.99 (Cousin et al., 2005).

The typical climate of Mediterranean countries, characterised by high temperatures and high humidity levels, is ideal for both the growth of the fungi and the ability of the fungi to produce aflatoxins (optimum between 25 and 30 ºC) (Sweeney and Dobson, 1998). This type of climate could be linked to the fact that in some of these countries, as is the case in Morocco, there is a high prevalence of hepatic carcinoma, as well as other pathologies of unknown etiology which it is believed may be related to the ingestion of aflatoxins (Zinedine and Mañes, 2009).

The production of aflatoxins also depends on the sources of carbohydrates and nitrogen, phosphates, lipoperoxides and trace elements (Luchese and Harrigan, 1993). As a result, this production is favoured by an environment rich in carbohydrates, although some substrates rich in fat and protein like peanuts also allow aflatoxin production (Marth, 1990). In some cases, the presence of other fungi may reduce aflatoxin synthesis (Wicklow et al., 1980) (Tsubouchi et al., 1981) and some components of various foods also reduce aflatoxin production. Thus, for example, oleuropein, a phenolic iridoid compound contained in olives, reduces aflatoxin production by *A. flavus* and *A. parasiticus*. Caffeine inhibits *Aspergillus* growth and aflatoxin production (CAST, 1998) and the same occurs, for example, with oregano and cinnamon essential oils (García-Camarillo et al., 2006).

It has also been reported that some vitamins, such as vitamin K_5, which present antimicrobial activity, slow down the growth of aflatoxin-producing *Aspergillus* species, as well as aflatoxin production by those fungi (Miranda et al., 2011).

It is also a well-known fact that some lactic acid bacteria produce compounds with anti-microbial and antimycotic properties, and can be used to control the growth of pathogenic bacteria, spoilage bacteria and fungi. Lactic acid bacteria with the greatest capacity to inhibit or reduce the growth of toxin-producing fungi belong to the geni *Lactococcus*, *Lactobacillus*, and, to a lesser degree, *Pediococcus* and *Leuconostoc* (Dalié et al., 2010). It has also been demonstrated that, as well as inhibiting fungal growth, certain lactic acid bacteria have the capacity to capture aflatoxins, thus reducing their bioavailability. This process is rapid and reversible (Bueno et al., 2006), and is dependent as much on the dose as on the strain studied (Kankaanpää et al., 2000). Studies recently carried out have shown that this capacity to capture aflatoxins (particularly aflatoxin B_1) is due to the peptidoglycans in the bacterial wall of some lactic acid bacteria (Lahtinen et al., 2004). This mechanism has also been observed in other micro-organisms, such as *Saccharomyces cerevisiae* (Bueno et al., 2006).

Moreover, it is known that certain substances have an inhibitory effect on the toxicity caused by aflatoxins, whether through metabolising them or inhibiting their oxidising effects. One example of this is saffron (*Crocus sativus*), the aqueous extract of which is capable of preventing lipid peroxidation induced by various substances such as aflatoxins, as well as of promoting detoxifying enzymatic systems, and whose tests on mice have verified it contains protective substances against diverse hepatotoxic agents, among them aflatoxin B_1 (Premkumar et al., 2003). Furthermore, it has been verified that the administration of green tea capsules, due to their polyphenolic content, protects against aflatoxin-induced cellular damage, not only because of their antioxidant properties but also because they activate metabolisation (Kensler et al., 2011).

We must also take into account that *Aspergillus* is an aerobic micro-organism, meaning that vacuum packaging or modified atmospheres packaging reduce oxygen availability and inhibit aflatoxin production (Ellis, 1993).

Aflatoxin presence in foods

Aspergillus contamination can occur in crops themselves, as with peanuts and corn, sometimes assisted by insect action, or it can occur during transport or storage, as with cereals (ICMSF, 1996). The toxin can remain in the food even when the toxigenic fungus has disappeared, and, as this is the case, aflatoxins have been detected in almost all parts of the world and in almost all food staples, to a greater or lesser extent. In addition, the fact that these toxins present an elevated thermal stability is also a key factor, enabling them to remain in some cooked foods, and meaning that freezing has very little effect on their presence in foods (Rasch et al., 2010).

The most important tools for the prevention and limiting of the appearance of aflatoxins in foods are good practice in agricultural production and food storage. Foods should be stored at relative temperatures and humidities that discourage both fungal growth and aflatoxin production by potentially aflatoxin-producing fungi. It is also extremely important to avoid cross-contamination between different batches of foods. While foods are being processed, the objective of reducing the exposure of the foods to aflatoxins can be achieved through selection or other physical processes prior to direct human consumption or use as ingredients in food products. For example, hulling rice and eliminating the chaff through grinding reduce the aflatoxin content, meaning that ground white rice has a lower aflatoxin content than unhulled rice. In addition, it has also been demonstrated that pressure cooking can reduce the aflatoxin content of uncooked rice by as much as 83% (Park and Kim, 2006).

These treatments reduce aflatoxin content and their effectiveness is taken into account when setting the maximum content in specific foods (Table 2). For this reason, in peanuts and other oilseeds, tree nuts, dried fruit, rice and corn which will be subject to processing before direct human consumption, higher aflatoxin levels are permitted since the treatment will bring the final content to values below the maximum content established for the products in question if they are intended for direct human consumption or for use as ingredients in food products.

Additionally, when establishing the aflatoxin levels for oilseeds and products derived from them, it has been taken into consideration that the production process of refined vegetable oils eliminates aflatoxins almost completely. As a consequence, oilseeds, including peanuts, which are going to be crushed for the production of refined vegetable oil, have been excluded from Regulation (EC) No 1881/2006, as refined vegetable oil itself.

Ammonia treatment reduces aflatoxin content in animal feed (ICMSF, 1998) and with regard to heat treatment, although aflatoxins are relatively thermostable, some studies have indicated that their content in contaminated foods can be reduced through a heat treatment of above 100 ºC (ICMSF, 1996). However, in order to eliminate them completely, the temperature would have to reach their melting point, which is unacceptable in food processing (Marth, 1990).

The foods most susceptible to aflatoxin contamination, and which are at greater risk of exposure are included in Regulation (EC) No 1881/2006 (corn, rice, cereals in general, hazelnuts, almonds, Brazil nuts, pistachios, peanuts and other oilseeds, dried fruits like dried figs or raisins, and spices like paprika, nutmeg, ginger or turmeric). Maximum limits have been established for them (Table 2).

Table 2. Maximum permitted aflatoxins B and G contents in food products (µg/kg) according to Regulation (EC) No 1881/2006

	Maximum aflatoxin contents (µg/kg)		
Section	**Food products**	**B_1**	**Total of B_1, B_2, G_1 and G_2**
2.1.1	Groundnuts (peanuts) and other oilseeds, to be subjected to sorting or other physical treatment, before direct human consumption or use as ingredient in foodstuffs, with the exception of: -groundnuts (peanuts) and other oilseeds to be crushed for refined vegetable oil production	8.0	15.0
2.1.2	Almonds, pistachios and apricot kernels to be subjected to sorting, or other physical treatment, before direct human consumption or use as an ingredient in foodstuffs	12.0	15.0
2.1.3	Hazelnuts and Brazil nuts, to be subjected to sorting, or other physical treatment, before direct human consumption or use as an ingredient in foodstuffs	8.0	15.0
2.1.4	Tree nuts, other than the tree nuts listed in 2.1.2 and 2.1.3, to be subjected to sorting, or other physical treatment, before direct human consumption or use as an ingredient in foodstuffs	5.0	10.0
2.1.5	Groundnuts (peanuts) and other oilseeds and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs, with the exception of: -crude vegetable oils destined for refined vegetable oils. -crude vegetable oils	2.0	4.0
2.1.6	Almonds, pistachios and apricot kernels, intended for direct human consumption or use as an ingredient in foodstuffs	8.0	10.0
2.1.7	Hazelnuts and Brazil nuts, intended for direct human consumption or use as an ingredient in foodstuffs	5.0	10.0
2.1.8	Tree nuts, other than the tree nuts listed in 2.1.6 and 2.1.7, and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
2.1.9	Dried fruit to be subjected to sorting, or other physical treatment, before direct human consumption or use as an ingredient in foodstuffs	5.0	10.0
2.1.10	Dried fruit and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0	4.0
2.1.11	All cereals and all products derived from cereals, including processed cereal products, with the exception of foodstuffs listed in 2.1.12, 2.1.15 and 2.1.17	2.0	4.0
2.1.12	Maize and rice to be subjected to sorting or other physical treatment before direct human consumption or use as an ingredient in foodstuffs	5.0	10.0
2.1.14	Following species of spices: -*Capsicum* spp. (dried fruit thereof, whole or ground, including chillies, chilli powder, cayenne and paprika), -*Piper* spp. (fruit thereof, including white and black pepper), -*Myristica fragrans* (nutmeg),-*Zingiber officinale* (ginger), -*Curcuma longa* (turmeric), -Mixtures of spices containing one or more of the above mentioned spices	5.0	10.0
2.1.15	Processed cereal-based foods and baby foods for infants and young children	0.10	–
2.1.17	Dietary foods for special medical purposes intended specifically for infants	0.10	–

Along with these foods, aflatoxin presence has been reported in other food products, including products of animal origin such as liver (Mahmoud et al., 2001), hamburgers and other processed meat products, probably due to the addition of spices (Aziz and Youssef, 1991). Aflatoxin presence has also been recorded in poultry meat (Bintvihok et al., 2002) (Hussain et al., 2010), aquacultured fish like tilapia (*Oreochromis niloticus x O. Aureus*) (Deng et al., 2010) or sea bass (*Dicentrarchus labrax L.*) (El-Sayed and Khalil, 2009), or milk (Zinedine and Mañes, 2009). In the case of the latter, the presence of aflatoxins B and G is much less relevant than the presence of aflatoxin M_1, metabolite of aflatoxin B_1 (Ayar et al., 2007).

The contamination of foods of animal origin occurs through the diet of the food-producing animals (Rasch et al., 2010). In the case of aquacultured fish, the presence of this type of toxin has increased in recent years, owing to the increasing use of vegetable flour and feed to feed these fish (Deng et al., 2010), although up to the present date the data available in the scientific bibliography comes mainly from experimental studies and not from market studies. It is important to point out that in the case of fish as much as mammals, these toxins are accumulate fundamentally in the liver (Deng et al., 2010), meaning that it is in this tissue that the greatest aflatoxin concentration will be found if the animal has been exposed. The elimination of this organ before human consumption through evisceration significantly reduces the risk from ingesting aflatoxins as a result of consuming these foods.

Experiments carried out on poultry showed that food contaminated with aflatoxin B_1 (3 mg/kg) caused concentrations greater than 7 μg/kg in the liver and 0.38 μg/kg in the meat of quails that had consumed it (Bintvihok et al., 2002). In another study, Hussain et al. (2010), determined that by feeding chickens with feed contaminated with 6.4 mg/kg of aflatoxin B_1 for 7 days, concentrations of aflatoxin B_1 were reached of close to 7 μg/kg in the liver and greater than 3 μg/kg in muscle, although these concentrations fell to undetectable levels a few days after discontinuing the use of contaminated feed in their diet.

In the case of aquacultured fish, Deng et al. (2010) verified that by feeding tilapias with feed contaminated with various doses of aflatoxin B_1, hepatic concentrations in the fish reached up to 40 μg/kg of aflatoxin B_1 without the mortality rate increasing. On the other hand, another study showed that, although symptoms of illness in the animals undergoing the study were evident, a contamination of 18 mg/kg of aflatoxin B_1 in the feed of sea bass (*Dicentrarchus labrax L.*) over prolonged periods of time caused the appearance of remnants of this toxin in the muscles of the fish in values close to 5 μg/kg, at the same time as evident symptoms of illness appeared in the animals undergoing the study (El-Sayed and Khalil, 2009).

Aflatoxin contamination has also been found in products of vegetable origin other than those included in the Regulation (EC) No 1881/2006, which are shown in Table 3. Although in the case of some foods, such as spices, the potential aflatoxin exposure carried by these foods is low, as they are consumed in small amounts, in other cases like coffee and chickpeas, ingestion could become a potential risk taking into account the aflatoxin concentrations found by some authors.

Table 3. Examples of foods not included in Regulation (EC) No 1881/2006, in which concentrations of aflatoxin B_1 or total aflatoxins greater than those limits established in Royal Decree 475/1988 have been found

Food	Maximum content detected (µg/kg)	Type	Source
Chickpeas	205	Aflatoxin B_1	(Ahmad and Singh, 1991)
Various spices and aromatic herbs	35	Aflatoxin B_1	(El-Kady et al., 1995)
Paprika	20	Aflatoxin B_1	(Martins et al., 2001)
Roasted coffee	16	Total aflatoxins	(Soliman, 2002)
Decaffeinated coffee	24	Total aflatoxins	(Soliman, 2002)
Ginseng root	16	Total aflatoxins	(D'Ovidio et al., 2006)

In alcoholic drinks, such as wine and beer, aflatoxin B_1 can also occasionally be found. In the case of wine, this toxin can be found most frequently in red wine, followed by rosé and finally white wine (Rasch et al., 2010). However, the principal mycotoxin present in alcoholic drinks is ochratoxin A (Mateo et al., 2007), with aflatoxins of groups B and G not reaching elevated levels.

With regard to tiger nuts (*Cyperus esculentus*), in 1999, in response to a question in the European Parliament, the European Commission claimed that maximum limits in this product had not been set because data on aflatoxin contamination in the product was not available (Parliament, 1999). Subsequently, in 2002, three notifications were made in the European Union about imported tiger nuts contaminated with aflatoxins (SCIRI, 2002) and, in April 2004, a notification was made regarding tiger nuts imported from Mali with a contamination level of 300 µg/kg of aflatoxin B_1 (RASFF, 2004). Aflatoxins have been detected in tiger nuts stored for 150 days, with an average level of 454 µg/kg of aflatoxin B_1 and 80 µg/kg of aflatoxin G_1 (Adebajo, 1993). With regard to *horchata* (a drink made from tiger nuts), various scientific essays have been published in which the presence of aflatoxin B_1 in *horchata* drinks marketed in Spain is reported (Arranz et al., 2006) (Rubert et al., 2011). In both essays a small proportion of contaminated *horchata* drinks is reported, furthermore with a low contamination index, below 2 µg/kg.

Recent modifications to legislation concerning aflatoxin contamination in foods

The findings and the changes that are occurring in the field of nutrition make it necessary to rethink nutritional policies. In the case in question, both the introduction into the diet of foods originating from other countries which were not previously a habitual part of the Spanish diet, and the changes in environmental conditions, can lead to changes in fungal development or the production of mycotoxins. On this point, the European Food Safety Authority has recently initiated the process for studying the potential increase in aflatoxin B_1 presence in cereals as a consequence of climate change, using predictive models to indicate an emerging potential food contamination due to mycotoxins (EFSA, 2009).

In addition, in 2007, EFSA's panel on contaminants (CONTAM), in response to a question about the potential increase in the risk to the health of consumers that a possible increase in the maximum aflatoxin limits in almonds, hazelnuts and pistachios might cause, indicated that an increase in the maximum total aflatoxin content from 4 to 8 or 10 µg/kg in these foods would have a minimal effect on the estimations regarding dietary exposure, the risk of cancer and the calculated exposure ranges. This criterion has been taken into account in Regulation (EU) No 165/2010 (EU, 2010a), which modified the limits initially established in Regulation (EC) No 1881/2006.

Conclusions of the Scientific Committee

In light of all the information explained previously, it is a verified fact that the maximum contents of aflatoxins B and G in the foods which may most frequently be carriers of aflatoxins of this type are expressly regulated by Regulation (EC) No 1881/2006.

However, the Scientific Committee considers that there are various facts that must be taken into account for correct consumer protection against exposure to these toxic agents:

- There are other foods of vegetable origin in which these toxins have been detected in similar or greater quantities (Table 3) than the limits established in Royal Decree 475/1988, such as coffee, chickpeas, ginseng root and certain varieties of aromatic herbs and spices.
- In experimental studies these toxins are shown to be able to accumulate in various tissues of food-producing animals, such as the liver and muscle of poultry or farmed fish, with levels of contamination occasionally found in some tissues that are greater or similar to those established in Royal Decree 475/1988. Although farmed fish is usually consumed once eviscerated, the livers of poultry intended for human consumption can habitually be found on the market.
- The fact should not be avoided that tiger nuts, which are not included in Regulation (EC) No 1881/2006, as well as being the raw material for the production of *horchata*, are also consumed directly. In recent years, very high concentrations (in one case reaching 300 µg/kg) of aflatoxins have been found in some cases in batches of tiger nuts imported from various African countries. In the case of *horchata*, the levels found are below the limits established in Royal Decree 475/1988, meaning that with the data available up to the present time, this food product would not represent a significant risk to the population.
- It is also a fact that, owing to globalisation and the intense immigration that Spain has experienced in recent years, it is increasingly frequent to find food products on the market that are not habitual in the Spanish diet. Some of these products could be susceptible to containing aflatoxins, despite not figuring in Regulation (EC) No 1881/2006, meaning that it would be advisable to carry out further studies on aflatoxin content in specific foods not included in Regulation (EC) No 1881/2006, such as tiger nuts or species of farmed fish in which there are indications that these mycotoxins may be present, and to stress the usefulness of aflatoxin controls in animal feed in order to avoid the contamination of products of animal origin.

For all these reasons, and without prejudice to any pertinent management measures, the Scientific Committee considers that, at this time and until representative data becomes available on aflatoxin presence in foods such as those previously cited, or others for which production conditions are uncertain, Royal Decree 475/1988 which regulates the maximum permitted limits for aflatoxins B_1, B_2, G_1 and G_2 offers a significant level of consumer protection with regard to specific foods not regulated by Regulation (EC) No 1881/2006.

References

Adebajo L.O. (1993). Survey of aflatoxins and ochratoxin A in stored tubers of Cyperus esculantus L. Appelgren LE, Arora RG. *Mycopathology,* 124 (1), pp: 41-46.

Ahmad, S.K. and Singh, P.L. (1991). Mycofloral changes and aflatoxin contamination in stored chickpea seeds. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure and Risk Assessment,* 8 (6), pp: 723-730.

Arranz I., Stroka, J. and Neugebauer, M. (2006). Determination of aflatoxin B_1 in tiger nut-based soft drinks. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 23 (3), pp: 305-308.

Ayar, A., Sert, D. and Çon, A.H. (2007). A study on the occurrence of aflatoxin in raw milk due to feeds. *Journal of Food Safety*, 27, pp: 199-207.

Aziz, N.H. and Youssef, Y.A. (1991). Occurrence of aflatoxins and aflatoxin-producing moulds in fresh and processed meat in Egypt. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessmen*t, 8 (3), pp: 321-331.

Bintvihok, A., Thiengnin, S., Doi, K. and Kumagai, S. (2002). Residues of aflatoxins in the liver, muscle and eggs of domestic fowls. *Journal of Veterinary Medical Sciences*, 64, pp: 1037-1039.

Blount, W.P. (1961). Turkey "x" disease. *Journal of British Turkey Federation*, 9, pp: 55-58.

Bueno, D.J., Casale, C.H., Pizzolitto, R.P., Salano, M.A. and Olivier, G. (2006). Physical adsorption of aflatoxin B_1 by lactic acid bacteria and *Saccharomyces cerevisiae*: A theoretical model. *Journal of Food Protection*, 70, pp: 2148-2154.

CAST (1998). Council for Agricultural Science and Technology. Antimicrobial Plant substances. Naturally occurring antimicrobial in food. Task force report, 132. April 1998, pp: 35-40.

Codex Alimentarius (2008). Codex General Standard for contaminants and toxins in food and feed. Codex Stan 193-1995. pp: 9. Available at: http://www.codexalimentarius.net/download/standards/17/CXS_193e.pdf. [accessed: 5-4-2011].

Cousin, M.A., Riley, R.T. and Pestka, J.J. (2005). Capítulo 10: Foodborne micotoxins: Chemistry, biology, Ecology and Toxicology. En libro: *Foodborne pathogens: microbiology and molecular biology*. Ed. Fratamico, P.M., Bhunia, A.K. y Caister J. L. S. Academic Press, pp: 163-273.

Dalié, D.K.D., Deschamps, A.M. and Richard-Forget, F.R. (2010). Lactic acid bacteria-potential for control of mould growth and mycotoxins: A review. *Food Control*, 21, pp: 370-380.

Deng, S.X., Tian, L.X., Liu, F.J., Jin, S.J., Liang, G.Y., Yang, H.J., Du, Z.J. and Liu, Y.J. (2010). Toxic effects and residue of aflatoxin B_1 in Tilapia (Oreochromis miloticus x O. aureus) during long-term dietary exposure. *Aquaculture*, 307, pp: 233-240.

D'Ovidio, K., Trucksess, M., Weaver C., Horn, E., Mcintosh, M. and Bean, G. (2006). Aflatoxins in ginseng roots. *Food Additives & Contaminants*. 23 (2), pp: 174-180.

EFSA (2007). The European Food Safety Authority. Opinion of the scientific panel on contaminants in the food chain related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. Question number: EFSA-Q-2006-174. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/446.htm. [accessed: 6-4-2011].

EFSA (2009). Modelling, predicting and mapping the (re)emergence of aflatoxin B_1 in cereals in the EU due to climate change. Available at: http://www.efsa.europa.eu/en/art36grants/article36/1211902566559.htm. [accessed: 5-4-2011].

El-Kady, I.A., El-Maraghy, S.S.M. and Mustafa M.E (1995). Natural occurrence of mycotoxins in different spices. *Folia Microbiologica,* 40, pp: 297-300.

Ellis, W.O, Smith, J.P., Simpson, B.K., Khanizadehy, S. and Oldham, J.H. (1993). Control of growth and aflatoxin production of Aspergillus flavus under modified atmosphere packaging (MAP) conditions. *Food Microbiology*, 10 (1), pp: 9-21.

El-Sayed, Y.S. and Khalil, R.H. (2009). Toxicity, biochemical effects and residue of aflatoxin B_1 in marine water-reared sea bass (*Dicentrarchus labrax* L.). *Food and Chemical Toxicology*, 47, pp: 1606-1609.

EU (2006a). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20 of December 2006, pp: 5-34.

EU (2006b). Commission Regulation (EC) No 401/2006 of 23 February 2006

laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. OJ L 70, 09.03.2006, p. 12.

EU (2009). Commission Regulation (EC) No 1152/2009 of 27 November 2009 imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by aflatoxins and repealing Decision 2006/504/EC. OJ L 313, 28 of November 2009, p: 40.

EU (2010a). Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins**.** OJ L 50, 27 of February 2002, p: 8.

EU (2010b). Commission Regulation (EU) No 178/2010 of 2 March 2010 amending Regulation (EC) No 401/2006 as regards groundnuts (peanuts), other oilseeds, tree nuts, apricot kernels, liquorice and vegetable oil. OJ L 52, 03 of March 2010, p: 32.

García-Camarillo, E.A., Quezada-Viay, M.Y., Moreno-Lara, J. and Sánchez-Hernández, G. (2006). Actividad antifúngica de aceites esenciales de canela (*Cinnamomum zeylanicum Blume*) y Orégano (*Origanum vulgare L.*) y su efecto sobre la producción de aflatoxinas en nuez pecanera. *Revista Mexicana de Fitopatología* A.C., 24 (001), pp: 8-12.

Goto, T., Ito, Y., Peterson, S.W. and Wicklow, D. (1997). Mycotoxin producing ability of *Aspergillus tamarri*. *Mycotoxins*, 44, pp: 17-20.

Hussain, Z., Khan, M.Z., Khan, A., Javed, I., Saleemi, M.K., Mahnood, S. and Asi, M.R. (2010). Residues in aflatoxin B_1 in broiler meat: Effect of age and dietary aflatoxin B_1 levels. *Food and Chemical Toxicology*, 48, pp: 3304-3307.

IARC (1993). International Agency for Research on Cancer. IARC Monographs, 56, pp: 362. Available at: http://monographs.iarc.fr/ENG/Monographs/vol56/mono56-14.pdf. [accessed: 5-4-2011].

IARC (2002). International Agency for Research on Cancer. Aflatoxins. Available at: www.inchem.org/documents/iarc/vol82/82-04.html. [accessed: 5-4-2011].

ICMSF (1996). International Commission on Microbiological Specifications for Food. Toxigenic fungi: Aspergillus. Microorganism in foods, 5, pp: 347-381. Characteristics of Food Pathogens. Academic Press, London.

ICMSF (1998*).* International Commission on Microbiological Specifications for Food*.* Microoganisms in foods, 6, pp: 348. Microbial Ecology of Food Commodities. Aspen Publishers, INC, Gaithersburg, Maryland.

Ito, Y., Peterson, S.W., Wicklow, D. and Goto, T. (2001). *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *flavi*. *Mycology Research*, 105, pp: 233-239.

JECFA *(*1987)**.** Joint FAO/WHO Expert Committee on Food Additives. Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 759 and Corrigendum.

JECFA (1997). Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868.

JECFA *(*1999). Forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 884.

JECFA (2002). Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 906.

JECFA (2007). Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947.

Kankaanpää, P., Tuomola, E., El-Nezami, H., Ahokas, J. and Salminen, S.J. (2000). Binding of aflatoxin B_1 alters the adhesion properties of *Lactobacillus rhamnosus* strain GG in Caco-2 model. *Journal of Food Protection*, 63, pp: 412-414.

Kensler, T.W., Roebuck, B.D., Wogan, G.N. and Groopman, J.D. (2011). Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. *Toxicological Sciences*, 120 (S1), pp: S28-S48.

Kurtzman, C.P., Horn, B.W. and Hesseltine, C.W. (1987). Aspergillus nomius, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii. Antonie Van Leeuwenhoek*. 53 (3), pp: 147-58.

Lahtinen, S.J., Haskard, C.A., Ouwehand, A.C., Salminen, S.J. and Ahokas, J.T. (2004). Binding of aflatoxin B_1 to cell wall components of *Lactobacillus rhamnosus* strain GG. *Food Additives and Contaminants*, 21, pp: 158-164.

Luchese, R.H. and Harrigan, W. (1993). Biosynthesis of aflatoxin - the role of nutritional factors. *Journal of Applied Microbiology*, 74, pp: 5-14.

Mahmoud, A.L.E., Sayed, A.M. and Abou-El-Alla, A.A. (2001). Mycoflora and natural occurrence of mycotoxins in some meat products and livers of poultry and imported bulls. *Pakistan Journal of Biological Sciences*, 4 (5), pp: 611-613.

Marth, E.H. (1990). Mycotoxin, pp: 137-158. In book: *Foodborne diseases.* Ed. Cliver, E. O. Academic Press.

Mateo, R., Medina, A., Mateo, E.M., Mateo, F. and Jiménez, M. (2007). An overview of ochratoxin A in beer and wine. *International Journal of Food Microbiology*, 119, pp: 79-83.

Martins, M.L., Martins, H.M. and Bernardo F. (2001). Aflatoxins in spices marketed in Portugal. *Food Additives and Contaminants*, 18 (4), pp: 315-9.

Miranda, J.M., Jorge, F., Domínguez, L., Cepeda, A. and Franco, C.M. (2011). In vitro growth inhibition of food-borne pathogens and food spoilage microorganism by vitamin K_5. *Food and Bioprocess Technology*, 4, pp: 1060-1065.

Park, J.W. and Kim, Y.B. (2006). Effect of pressure cooking on aflatoxin B_1 in Rice. *Journal of Agricultural and Food Chemistry*, 54, pp: 2431-2435.

Parliament (1999). Written question E-0630/99 by Encarnación Redondo Martínez to the Comission. OJ L 370 of 21 of December 1999, pp: 78-79.

Premkumar, K., Abraham, S.K., Santhiya, S.T. and Ramesh, A. (2003). Protective effects of saffron (*Crocus sativus* L.) on genotoxins-induced oxidative stress in Swiss albino mice. *Phytotherapy Research*, 17 (6), pp: 614-617.

RASFF (2004). Rapid Alert System for Food and Feed. Annual report of the functioning of the RASFF 2004. Available at: http://ec.europa.eu/food/food/rapidalert/report2004_en.pdf. [accessed: 16-5-2011].

Rasch, C., Böttcher, M. and Kumke, M. (2010). Determination of aflatoxin B_1 in alcoholic beverages: comparison of one- and two-photon-induced fluorescence. *Analytical and Bioanalytical Chemistry*, 397, pp: 87-92.

Real Decreto 475/1988 de 13 de mayo de 1988 por el que se establecen los límites máximos permitidos de las aflatoxinas B_1, B_2, G_1 y G_2 en alimentos para consumo humano. BOE 121 de 20 de mayo de 1988, pp: 15329.

Rubert, J., Sebastiá, N., Soriano, J.M., Soler, C. and Mañes, J. (2011). One-year monitoring of aflatoxins and ochratoxin A in tiger-nuts and their beverages. *Food Chemistry*, 127, pp: 822-826.

Sargeant, K., O'Kelly, J., Carnaghan, R.B.A. and Allcroft, R. (1961). The assay of a toxic principle in certain groundnut meals. *Veterinary Record*, 73, pp: 1219.

SCF (1996). Scientific Committee on Food. Opinion on Aflatoxins, Ochratoxin A and Patulin. Reports of the Scientific Committee on Food, 35º Series. European Commision, DG Industry.

SCIRI (2002). Sistema Coordinado de Intercambio Rápido de Información. Colección de Informes Técnicos. Available at:
http://www.aesan.msc.es/AESAN/docs/docs/publicaciones_estudios/memoria/memoria_sciri.pdf. [accessed: 16-5-2011].

Soliman, K.M. (2002). Incidence, level, and behaviour of aflatoxins during coffee bean roasting and decaffeination. *Journal of Agricultural and food Chemistry*, 50 (25), pp: 7477-81.

Sweeney, M.J. and Dobson, A.D.W. (1998). Mycotoxin production by Aspergillus, Fusarium and Penicillium species. *International Journal of Food Microbiology*, 43, pp: 141-158.

Tsubouchi H., Yakamoto, K., Hisada, K., Sakabe, Y. and Tsuchihira, K. (1981). Inhibitory effects of nonaflatoxigenic fungi on aflatoxin production by A. flavus. *Transactions of the Mycological Society of Japan*, 22, pp: 103-111.

Wicklow, D.T., Hesseltine C.W., Shotwell, O. L. and Adams G.L. (1980). Interference competition and aflatoxin levels in corn. *Phytopathology*, 70, pp: 761-764.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. *The American Journal of Clinical Nutrition*, 80 (5), pp: 1106-1122.

Zinedine, A. and Mañes, J. (2009). Occurrence and legislation of mycotoxins in food and feed from Morocco. *Food Control*, 20, pp: 334-344.