Chemical Food Safety of Traditional Grains

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Summary

Looking in general at the chemical food safety of traditional grains mycotoxins must be expected to be the major issue. A survey of the scientific literature and of alerts from the EU food safety system demonstrates that the problems to be foreseen for an intensified import into the EU will be more or less the same as for conventional grains. The reported findings for a number mycotoxins in different types of traditional grains (cereals and pseudocereals) are reviewed and held against the current knowledge about toxicity and the existing EU regulation.

Introduction

Materials for food production and processed foods may contain chemical compounds that can impair health. In materials of plant origin these can be grouped into (a) toxic- or antinutritional compounds naturally occurring in the plant species and (b) organic- or inorganic constituents entering into the food chain at different points. An example on an antinutritional substance is phytic acid present in relative high amounts in grain sorghums [1] and which inhibits the absorption of non-haem iron [2]. Chemical substances entering from the outside include heavy metals, pesticides, organic pollutants and toxic compounds formed by micro fungi; so-called mycotoxins.

For traditional grains produced in developing countries possible contamination with mycotoxins currently will be the major problem to address when making a risk assessment for their use in health products or foods. The last ten years the European (EFSA) as well as the US (FDA) food safety authorities have had strong focus on a number of long well known mycotoxins such as the aflatoxins as well as on more recently investigated compounds; such as the group of trichothecenes formed by a number of *Fusarium* spp. [3].

This paper will present a popular scientific overview of the present knowledge about contamination of traditional grains with mycotoxins.

Traditional Grains

Traditional grains of some major importance with respect to the volume of production, and therefore of current interest when looking at newly established - or emerging - imported products on the European market include among others the following traditional African cereal grains [*generally used English name* (*other common names*)]: *sorghum* (*grain sorghum*, *milo*), *finger millet* (*ragi, wimbi*), *teff* (*tef*), *white fonio* (*fonio*, *acha etc.*), *kodo millet* (*kodon, creeping paspalum, ditch millet etc.*) and *pearl millet* (*bulrush millet, babal, bajra/bajira*) [4]. In addition a number of so-called pseudocereals, of which the American crops *amaranth* and *quinoa* are the most important, must be considered [5]. The seeds of sorghums (grain sorghum) may as already mentioned contain high amounts of phytic acid. However, also their content of so-called phenolic and polyphenolic substances (including different types of tannins), which varies with genetic type [6] can contribute to the inhibition of iron uptake [2]; as well as protein utilisation. Among the pseudocereals especially the quinoa whole seed contains up to around 2% of very bitter saponins [5].

Mycotoxins

Mycotoxins are toxic (poisonous) substances formed by micro fungi. A large number of such fungi are pathogens to plants used for the production of food or animal feed or grow on (form mould on) food products. Fungal growth thus may occur while the commodity still is growing in the field and/or after harvest, i.e. during storage, or even after processing (mouldy food/feed products). Not all species of micro fungi are known to form toxins of health concern for humans, however, of those which do, some produce indeed very poisonous substances.

Examples of such toxins include the aflatoxins, a group of four chemically closely related substances named aflatoxin B_1 (the most dangerous), B_2, G_1, and G_2, respectively. The compounds are among others produced by the fungus *Aspergillus flavus*. They have the liver as their main target organ, causing acute poisoning (which may be fatal), chronic liver damage and liver cancer. The compounds are classified as Class I carcinogens by the International Agency for Research in Cancer; IARC). The mechanism behind these effects is a biotransformation of the compounds in the liver to become chemically highly reactive compounds with among others a strong mutagenic effect [3, 7]. If feed to milking cows contains B_1 this will partly be excreted into the milk as the (still) toxic and carcinogenic metabolite aflatoxin M_1.

Another group of mycotoxins of great concern is the so-called trichothecenes produced by a number of species from the genus *Fusarium*. These compounds are on the basis of their molecular structure grouped into tricothecenes of the A-type, such as T-2 toxin, HT-2 toxin, and the B-type compounds including among others deoxynivalenol (DON), nivalenol (NIV) and others [8].

Acute high dose toxicity of trichothecenes is characterised by "radiomimetic" effects such as diarrhoea, vomiting, leukocytosis, haemorrhage, and circulatory shock and death, whereas chronic low dose toxicity is characterised by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes and immunologic effects. Basically, trichothecenes bind to eukaryotic ribosomes and inhibit protein synthesis by blocking translation and inhibiting the elongation of peptide chains [9]. Some species of *Fusarium* also produce the estrogenic mycotoxin zearalenone (known as ZEA or ZEN; from now on ZEA), causing vulvovaginitis in female pigs and feminisation of young male pigs, and the derivaties α- and β-zearalenol (α-, β-ZOL) [8].

As a global incidence of *Fusarium* toxins has been reported for cereals by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) in reports from 2000 and 2001, respectively, a frequent contamination can be expected for grain-based foods [8, 10,11]

Analysing which further known mycotoxins it may be of major importance to look for in cereals and pseudocereals formerly unknown to the Europea market one must add Ochratoxin A (OTA) and the fumonisins (FUM). OTA has for decennia been known to cause severe kidney damage in pigs and are controlled for in slaughter pigs as well as in e.g. cereals for food; in order to protect consumers [3]. The compound is produced by a single *Penicillium* species, *P. verrucosum*, by *Aspergillus ochraceus* and several related *Aspergillus* species, and by *A. carbonarius*, with a small percentage of isolates of the closely related *A. niger* [11]. Like for the trichothecenes, fumonisins (B_1, B_2, and B_3) are mycotoxins formed by fungi of the genus *Fusarium*. However, the only species that produce significant quantities of fumonisins are *Fusarium verticillioides* (= *F. moniliforme*) and the related *F. proliferatum*. The toxins have till now mainly been found in maize (corn) where these fungi cause "kernel rot". Nephrotoxicity, which was observed in several strains of rat, was the most sensitive toxic effect of pure fumonisin B_1 when evaluating the animal studies available [11].

The levels accepted for different mycotoxins (and other undesirable substances) in food and animal feed, in the EU, are at the moment found in "the Commission Regulation setting maximum levels for certain contaminants in foodstuffs" [12] and "the Directive of the European Parliament and of the Council on undesirable substances in animal feed", respectively[13]. For feed only the aflatoxin B_1 content and the content of a fungus (as such) called "rye ergot" are regulated. For food aflatoxins (B_1, sum of B_2, G_1, and G_2, M_1), OTA, Patulin (not mentioned above), DON, ZEA, and fumonisins (sum of B_1 and B_2) are regulated. Further regulations concerning T-2 and HT-2 are announced for 2007/8 [14].

In general the level accepted for a certain mycotoxin differs according to the product type/intended use. An example of this is the maximum level of aflatoxin B_1 which is 8 µg/kg for "groundnuts to be subjected to sorting", 2 µg/kg for "all cereals and products from cereals", and 0.1 µg/kg for "processed cereal-based food for babies and young children [12].

Traditional Grains and Mycotoxins

To get an impression of the risk of mycotoxin contamination of different traditional grains - and their products - two instruments have been used, i.e. a search in the following databases over scientific literature (Agricola, Agris, CAB Abstracts, WEB of Science, PubMed and FSTA) and an analysis of the notifications in the EU "Rapid Alert System" (http://ec.europa.eu/food/food/rapidalert/index_en.htm) for ten consecutive weeks (week 14-24, 2008). The alert system, which for the weekly reports goes back to 2003, informs about the finding of food samples/bulks exceeding one or more maximum limits for contaminants. The information is structured into so-called "Alert Notifications", "Information Notifications" and "Border Rejections".

A high number of notifications dealing with aflatoxins were found as accompanied with a few on DON and OTA, however, non of these had to do with any of the traditional grains (cereals/psedocereals) mentioned in the overviews [4,5]. The literature search gave rise to only ten articles of any apparent significance. This is in accordance with the fact that recent articles for example state that few data are available concerning the occurrence of mycotoxins in African food products [15].

The articles identified can be divided into three categories, namely (1) occurrence of mycotoxins/toxicogenic fungi on crops grown and stored in developing countries [7, 15-21], (2) traditional grains experimentally grown in the USA [22] and (3) grains/grain products imported into the EU [8].

The reported findings may be summarized as followes (by commodity):

Sorghum: samples of sorghum (*S. bicolor*) collected 1999 from traditional storage facilities (underground pits) in Ethiopia showed an average content of aflatoxin B_1 of

10.0 µg kg^{-1} (maximum level found 25.9), OTA 174.8 µg kg^{-1} (max. 2106) and were shown to contain both DON, NIV, ZEA and FUM too [15]. Freshly harvested grain samples grown at different locations in West Africa 2005 showed an average content of total aflatoxins of 8.8 µg kg^{-1} [7]. Two out of five isolates of *Aspergillus flavus* isolated from grains of "Dura" (*Sorghum* sp.) collected in Sudan around 1997 proved to be producers of aflatoxins at medium to high level [16]. Additionally it can be noted, that nine samples out of ten randomly collected from public market places in Bhagalpur, India (produced in North Bihar province around 1990) were shown to be contaminated with from one to three *Alternaria* mycotoxins [17]. Furthermore, that a clear correlation between the formation of smelling volatiles such as 2-methylbutanal and infection of sorghum with mold fungi has been demonstrated [18].

Pearl millet: Freshly harvested grain samples of pearl millet (*Pennisetum glaucum*) grown at different locations in West Africa 2005 showed an average content of total aflatoxins of 4.6 µg kg^{-1} [7]. One isolate of *Aspergillus flavus* isolated from grains of "Dukhn" (*Pennisetum* sp.) collected in Sudan around 1997 did not produce aflatoxins [16]. Growing two cultivars each of corn and pearl millet in the years 2000 and 2001 in Georgia (USA) the content of total aflatoxins as well as of fumonisins was demonstrated to be much lower in pearl millet at compared to corn (maize). The average content of aflatoxins in millet was around 6 4.6 µg kg^{-1}, fumonisins differing according to cultivar between 11 and 121 µg kg^{-1} [22].

Finger millet: Three out of eight samples *of Eleusine coracana* (Ragi) randomly collected from public market places in Bhagalpur, India (produced in North Bihar province) around 1990 were shown to be contaminated with from one to three *Alternaria* mycotoxins [17]

Kodo millet: Around 50% of grain samples from *Paspalum scrobiculatum* grown and collected in India was found to contain aflatoxin B_1 in concentrations between 12 and 44 ppb (µg kg^{-1}) [19].

Unspecified millet: In an investigation of 219 samples of foodstuff of plant origin, consisting of grain-based food, psedocereals and gluten-free food in Germany (all randomly purchased 2003 from commercial food stores/health food stores), five samples of "millet" were all found free of any of 13 trichothecenes analysed for [8].

Tef(f): samples of teff (*Eragrostis tef*) collected 1999 from traditional storage facilities (bags) in Ethiopia showed an average content of aflatoxin B_1 of 5.1 µg kg^{-1} (maximum level found 15.6), OTA 32.7 µg kg^{-1} (max. 80) but were shown to not contain any of the toxins DON, NIV, ZEA and FUM [15].

Amaranth: Two moldy (from not dry storage) samples of amaranth (*Amaranthus cruentus*) produced in Argentina were analysed for the occurrence of aflatoxins, ZEA and OTA. ZEA was found at levels of 1980 and 420 µg kg^{-1}, respectively [20]. In an investigation of 219 samples of foodstuff of plant origin, consisting of grain-based food, psedocereals and gluten-free food in Germany (all randomly purchased 2003 from commercial food stores/health food stores), five samples of amaranth were all found free of any of 13 trichothecenes analysed for [8].

Quinoa: In an investigation of 219 samples of foodstuff of plant origin, consisting of grain-based food, psedocereals and gluten-free food in Germany (all randomly purchased 2003 from commercial food stores/health food stores), five samples of quinoa (*Chenpodium quinoa*) were all found free of any of 13 trichothecenes analysed for [8].

White fonio: Moldy grain samples of *Digitaria exilis* were collected in Nigeria at three different periods of the year. Aflatoxin B_1 and B_2, together with ZEA and DON (Vomitoxin) were detected. Concentrations in general were highest for samples collected March to May, where aflatoxin B_1 ranged from 2.4 to 20 μg kg^{-1} , ZEA from 200 to 602 μg kg^{-1}, and DON from 7 to 58 μg kg^{-1} [21].

Conclusion

The above cited findings from databases and alerts tell us, that there in general is no reason to expect higher levels of mycotoxins from most of the so-called traditional grains, when grown in their natural habits (typically tropical developing countries), than from traditional grains. Indeed especially the storage/drying conditions after harvest do influence the final level found for many of the mycotoxins assayed for; as is seen for traditional grains too. However, the findings also clearly indicate that the control must be just as strict in order to ensure healthy food products free of indeed very toxic compounds such as e.g. aflatoxins.

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