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Susceptibility of spelt to Ochratoxin A producing fungi

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Ochratoxin A (OTA) is a hazard to man and animals. It is the cause of porcine nephropathy and it is considered carcinogenic **(see pdf document)**. Maximum limits of 5 ng OTA per g grain and 3 ng OTA per g flour were introduced in Denmark in 1995 and the EU in 2001 **(see pdf document)**. Special limits of 0.5 ng OTA per g were recently introduced for processed cereal-based foods for infants and young children **(see pdf document)**.

OTA is rather resistant to high temperatures. It is therefore essential to grain processors that the grain is not contaminated with OTA upon receipt. Producers and processors of organically grown cereals have been especially concerned because quality criteria are crucial in organic production and because a number of Danish studies indicate OTA problems to be more prevalent in organic than conventional farming (Jørgensen et al., 1996; Jørgensen & Jacobsen, 2002; Elmholt, 2003).

Differences in sensitivity among small grain cereals

A number of studies have shown differences in OTA susceptibility among small grain cereals. For example, rye is more sensitive than wheat regarding contamination with *Penicillium verrucosum* (Elmholt, 2003) and formation of OTA (Jørgensen & Jacobsen, 2002). *P. verrucosum* is the fungus that produces OTA under Danish conditions (figure 1).

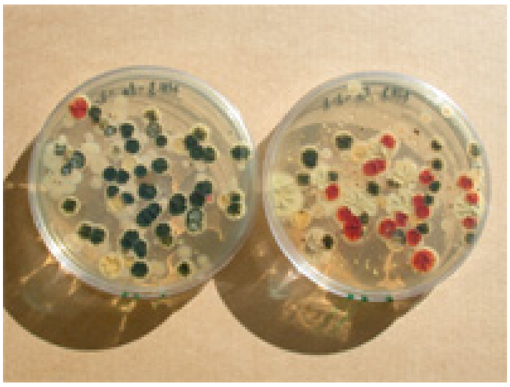

Figure 1.

Penicillium verrucosum growing on DYSG agar. The terra-cotta coloured reverse of the colony is indicative of this particular species and used in species identification.

Reintroduction of ancient wheat types

The biodynamical mill and bakery, **Aurion ApS**, has introduced ancient wheat types into commercial Danish bread production and flour sale for home baking. These types include einkorn, emmer and spelt. The ancient wheat types are considered a more 'natural' product and therefore attractive to many organic growers. In addition, their chemical composition makes them interesting for both bakers and consumers. Nothing is known about the susceptibility of the ancient wheat types to *P. verrucosum* colonisation and

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Sensitivity of ancient wheat types to ochratoxin A

Aurion receives its grain from a range of farmers. Some deliver their combine harvested grain directly to the mill by carrier and some dry and store on-farm and deliver to the mill upon request. With focus on spelt, a number of grain samples were analysed for occurrence of *P. verrucosum* and contents of OTA.

Sampling was performed in 2001. The study included 37 combined, not dried grain samples from farmers with no drying facilities. And, additionally, the study included 19 combined and 22 dried samples from six farms with on-farm drying facilities. Thus, in total, 56 combined and 22 dried samples were analyzed. The cereal species were spelt, wheat, rye, barley, oats, triticale, emmer, and einkorn. All 78 samples were analysed for moisture contents and abundance of *P. verrucosum*. 55 of the samples were analysed for OTA **(table 1)**.

Spelt is a 'covered wheat' meaning that the kernels do not readily thresh free of glumes, lemma and palea. Most spikelets contain two kernels. According to the setting of the combine harvester, harvested spelt typically contains about 60% threshed out kernels.

Figure 2.

Sample of spelt showing spikelets and threshed out kernels, some of which are damaged.

The forwarded samples had varying amounts of threshed out kernels (figure 2). Normally, kernels are left within the hull during drying and storage and not threshed out until processing at the mill. Therefore, we made assessments of both spikelets and kernels. *P. verrucosum* was isolated by plating 300 kernels or spikelets (figure 3) on a specific nutrient agar (DYSG) and incubating for 7 days at 25°C.

Based on its terra-cotta coloured reverse (figure 1), the number of kernels/ spikelets colonized by *P. verrucosum* were enumerated. These recordings were used to calculate the percentage of contamination (Cont. %). Samples for OTA analysis were ground in a stone mill. Extraction and clean-up were carried out using Ochraprep immunoaffinity columns and analysis for OTA was performed by HPLC according to standard procedures (Jørgensen & Jacobsen, 2002).

Figure 3.

Plating of kernels or spikelets on a specific nutrient agar (DYSG).

Grain contamination is a latent risk

P. verrucosum was present in 82% of the 56 combined samples showing that much grain is contaminated prior to drying and storage. This has been found in other studies too (Elmholt, 2003). Regarding the amount of *P. verrucosum*, the median contamination of the combined samples was 2.0% **(table 1)**. No clear relationship was obtained between moisture content at harvest and *P. verrucosum* contamination. 33 combined samples were tested for OTA **(table 1)**. Twenty-four of these contained no detectable OTA though most of them contained *P. verrucosum* (median contamination 3.0%). Even a rye sample with 35.7% contaminated kernels contained no OTA. This indicates that the major implication of early contamination with *P. verrucosum* is a latent risk of OTA production if the grain is not properly dried and stored. However, 27% of the 33 tested combined samples did actually contain detectable amounts of OTA prior to drying (Table 4), and two spring spelt samples even exceeded the maximum EU limit of 5 ng OTA (see below).

Highly contaminated spelt without OTA

Spelt was more contaminated by *P. verrucosum* than the other grain species, including rye **(table 1)**. The combined, not-dried spelt samples had a median contamination of 4.4% and a maximum contamination of 58.7% for the 26 investigated samples. In comparison, the median contamination levels for the other species were below 1.5%.

We expected more *P. verrucosum* contaminated spikelets than kernels, because a spikelet has a larger surface area than a kernel. Yet, only minor differences in Cont. % were observed between samples from which spikelets were analysed and samples from which kernels were analysed.

Despite the heavy contamination by *P. verrucosum*, only one of the combined winter spelt samples contained OTA (0.6 ng per g). Spring spelt, which is harvested later than winter spelt, seems to be more sensitive to OTA formation. Two combined spring spelt samples exceeded the maximum EU limit of 5 ng OTA per g containing 18 and 92 ng OTA per g, respectively. Although the dried winter spelt samples were heavily contaminated containing 10 to 45% spikelets with *P. verrucosum*, none of these samples contained OTA above 0.5 ng per g.

It is likely that the glumes of the spikelet protect the kernels inside from fungal infection and toxin formation. However, the high contents of OTA of the two spring spelt samples indicate that this protection is not always sufficient and should be investigated more closely.

Indications of cultivar differences

The two combined samples containing 18 and 92 ng OTA per g, respectively, originate from two different cultivars of spring spelt. They had been grown in the same field together with two other cultivars, but these contained less than 0.5 ng OTA per g. The spelt cultivars had not been harvested until late September and contained more than 18% moisture. The results indicate cultivar differences in the susceptibility of spelt to OTA formation. It should be noted that all four cultivars had similar levels of *P. verrucosum* contamination at harvest, both for spikelets (2-8%) and threshed out kernels (2-11%).

No linear relationship between contamination and OTA

21 of the 22 OTA-tested dried samples contained *P. verrucosum*, and OTA was found in half of these samples **(table 1)**. OTA development could generally be ascribed to poor on-farm drying and storage facilities, especially in cases where poor facilities were combined with late harvest and high moisture contents of the harvested grain.

All OTA contaminated samples contained *P. verrucosum* supporting the hypothesis that *P. verrucosum* is the sole producer of OTA in Danish grain. As expected, dried samples were generally more contaminated by *P. verrucosum* than combined samples. Dried samples below the limit of detection (0.1 ng OTA per g) had a median *P. verrucosum* contamination of 11.7%. Dried samples with OTA contents between 0.1 and 5 ng per g had a median contamination of 37.3% while dried samples with more than 5 ng OTA per g had a median contamination of 11.3% **(table 1)**. This illustrates that there is no linear relationship between OTA contents and the percentage of kernels contaminated with *P. verrucosum*. Heavily contaminated samples do not necessarily contain similarly high amounts of OTA **(figure 4)**. A high production of conidia, however, may also be detrimental because conidia may be dispersed to large parts of the grain during drying and aeration (Haase, 2003).

Further reading

Elmholt, S. (2003). Ecology of the ochratoxin A producing Penicillium verrucosum: Occurrence in field soil and grain with special attention to farming system and on-farm drying practices. Biological Agriculture and Horticulture 20, 311-337.

Haase, M.S. (2003). Prevention of mycotoxin formation in organically cultivated bread grain - focusing on Penicillium verrucosum and ochratoxin A formation (In Danish). M.Sc. Thesis, Biological Institute, University of Aarhus.

Jørgensen, K., Rasmussen, G., Thorup, I. (1996). Ochratoxin A in Danish cereals 1986-1992 and daily intake by the Danish population. Food Additives and Contaminants 13, 95-104.

Jørgensen, K., Jacobsen, J.S. (2002). Occurrence of ochratoxin A in Danish wheat and rye, 1992-99. Food Additives and Contaminants 19, 1184-1189.