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Penicillium verrucosum occurrence and Ochratoxin A contents in organically cultivated grain with special reference to ancient wheat types and drying practice

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Abstract

This study addresses the relationship between the ochratoxigenic strains of *Penicillium verrucosum* and ochratoxin A (OTA) contents in organically cultivated grain. It included 37 combined, non-dried grain samples from farmers with no drying facilities as well as 19 non-dried and 22 dried samples from six farms with on-farm drying facilities (Case studies 1-6). The study focused on the ancient wheat type spelt but also included samples of wheat, rye, barley, oats, triticale, emmer, and einkorn. All 78 samples were analysed for moisture content (MC) and occurrence of *P. verrucosum.* The latter was assessed by plating non-disinfected kernels on DYSG agar and counting those contaminated by the fungus. Fiftyfive samples were analysed for OTA. Most of the combine harvested samples (82%) were contaminated with *P. verrucosum* prior to drying. This was ascribed to difficult harvest conditions and many samples of spelt, which was significantly more contaminated by *P. verrucosum* than oats, wheat and barley. Though not statistically significant, the results also indicated that spelt was more contaminated than rye, which is usually regarded the most sensitive small grain cereal. No correlation was found between number of kernels contaminated by *P. verrucosum* and OTA content. Despite many non-dried samples being contaminated by *P. verrucosum*, only two exceeded the EU maximum limit for grain (5 ng OTA g^{-1}), both being spring spelt with 18 and 92 ng g^{-1}, respectively. The problems were most likely correlated to a late harvest and high MC of the grain. The case studies showed exceedings of the maximum limit in a batch of dried oats and spring wheat, respectively, probably to be explained by insufficient drying of late harvested grain with high MC. Furthermore, our results clearly indicate that OTA is not produced in significant amounts in samples with MCs below 17%. All dried samples with MCs above 18% exceeded the 5 ng OTA g^{-1} limit in grain. However, no correlation between MC and the amount of OTA produced was found.

Keywords: moisture content, natural air drying, Ochratoxin A, on-farm, rye, spelt

Introduction

The mycotoxin Ochratoxin A (OTA) has a number of toxicological effects and presents a well-known hazard to human health [1-3]. Denmark introduced maximum limits for OTA of 5 ng g^{-1} grain and 3 ng g^{-1} flour in 1995, and in 2001 these limits were introduced in the EU (Commission Regulation (EC) No. 466/2001 of 8 march 2001). Recently, the EU has set a maximum limit of 0.5 ng OTA g^{-1} baby food and processed cereal based food for infants and young children (Commission Regulation (EC) No. 683/2004 of 13 April 2004). OTA is resistant to high temperatures [4-5]. It is therefore essential to grain processors that the grain is not contaminated with OTA upon receipt. Danish producers and processors of organically grown cereals have been especially concerned about OTA contamination because a number of studies indicate OTA problems to be more prevalent in organic than conventional farming [6-8]. Quality criteria are crucial in organic production and one such criterion is the avoidance of toxic residues in cereal commodities. Partly therefore, pesticides are banned in organic farming and increased occurrence of other toxic substances like mycotoxins would jeopardize the credibility of organic farming in this respect.
In a review on mycotoxigenic fungi, Miller [9] points out that very little is known about the pre-harvest ecology of the OTA producing fungus, *Penicillium verrucosum* Dierkx, but that some kernels are commonly infested by the fungus at harvest. This was also found by Elmholt [8]. However, most problems seem related to insufficient drying facilities at small farms, which at least some years ago were more prevalent in organic farming [7-8]. A number of studies and surveys have shown differences in OTA susceptibility among the small grain cereals [6-7,10]. The Danish mill and bakery, Aurion ApS, uses only organically and biodynamically [11] grown grain, and they were the first to introduce ancient wheat types into commercial Danish bread production and flour

sale for home baking. These types include einkorn and emmer, which were the first wheats to be domesticated, and spelt which was developed from emmer. These ancient wheat types have not been exposed to modern breeding techniques, making them a more "natural" product and therefore attractive in the eyes of many organic growers. In addition, their chemical composition differs from modern wheat in ways that make them interesting for both bakers and consumers [12]. Nothing is known, however, of their susceptibility to *P. verrucosum* colonisation and OTA production.

This study was established in cooperation with Aurion ApS. The mill receives its grain from a range of farmers. Some of the farmers deliver their combine harvested grain directly by carrier. This grain is dried at the mill in a batch dryer and stored and processed according to demand. Other suppliers store their grain on-farm and deliver to Aurion upon request. Grain samples directly delivered to the mill as well as samples from farms with on-farm drying were analysed. The samples were tested for occurrence of *P. verrucosum* and moisture content, and selected samples were analysed for OTA. The relationship between occurrence of *Penicillium verrucosum*, moisture content and OTA was investigated with special attention to the ancient wheat type spelt and on-farm drying systems based on ambient air.

Materials and methods

Non-dried grain samples directly delivered to the mill

From Aug. 15th to Sept. 13th 2001, 37 combined (C), non-dried grain samples of approximately 300 g were forwarded by Aurion ApS for microbiological analysis. The samples originated from 22 farmers with no appropriate drying and storage facilities. The grain had been delivered on the day of harvest or the following day to be batch dried at Aurion. The miller sampled representatively from the delivered non-dried batch of grain. The following small grain species were represented: Spelt (19 samples, *Triticum aestivum* ssp. *spelta* (L.) Thell.); emmer wheat (two samples, *T.*

turgidum ssp. *dicoccon* (Schrank) Thell.); cultivated einkorn (one sample, *T. monococcum* ssp. *monococcum*); wheat (spring wheat one sample, winter wheat two samples, *T. aestivum* ssp. *aestivum* L.); triticale (one sample, x *Triticosecale* Wittm.); rye (six samples, *Secale cereale* L.); oats (two samples, *Avena sativa* L.); barley (three samples, *Hordeum vulgare* L. ssp. *vulgare*).

Non-dried and dried samples from the Case-study farms

To improve possibilities of relating *P. verrucosum* and OTA findings to management practice, samples of grain taken prior to drying (non-dried, combined grain, C) and after drying (dried grain, D) were obtained from six farms. These were farms with on-farm drying and storage facilities, and most of the farmers more or less regularly supply grain to Aurion ApS. Non-dried grain delivered by the farmers: The six farmers forwarded 19 combined grain samples of approximately 300 g from Aug. 20th to Sept. 24th 2001. They were asked to sample a handful of grain at ten different points in their heap, which typically consisted of 20-25 tonnes of grain, and forward it on the day of harvest or the following day. The grain was filled into a small cardboard box, which was sealed with tape. The box was sent by post, which in Denmark normally means delivery within one day. It was assumed that the combine harvester had mixed the grain well and that OTA and fungal conidia would be fairly homogenously distributed. The following number of species and samples were represented: Spelt (three samples), spring spelt (four samples), spring wheat (two samples), winter wheat (four samples), triticale (one sample), rye (one sample), oats (two samples) and barley (two samples). Dried grain sampled at the Case study farms: All six farms had ambient air drying systems, either unheated or with low heat (Table 1). Sampling of dried grain was performed on Nov. 15th and 28th with an open-throat hand probe with eight slots (2m, diameter 38mm, Rationel Kornservice A/S, Esbjerg, Denmark). With the slots closed, the probe was inserted at a slight angle to a depth of approximately 1.5 m. Then, with the slots facing upwards, the probe was opened and

moved slightly up and down to fill the compartment. Finally the probe was closed, withdrawn from the grain lot and the sample emptied into a plastic container. Where nothing else is mentioned, approximately 15 samples were combined to a composite sample.

Case 1: Combine harvested samples of oats, spelt and winter wheat were received. On Nov. 15th dried samples were collected. The dried spelt had been sold but a sample withheld for analysis. The wheat and oats lay in dryers to a height of approximately 3 m, oats in two driers (D1 and D2) and wheat in one (D3). The uppermost layer of D1 had been transferred to an airtight silo a few days before in an attempt to stop mould growth and further deterioration. From this airtight silo (AS), we obtained a sample, which had been rolled for cattle feed on the morning of sampling. Four replicate samples were taken in the now upper layer of D1 (0-1 m) to elucidate heterogeneity, while one, composite sample was taken in D2 and D3, respectively. Case 2: Combine harvested samples of barley, oats, spelt and winter wheat were received. On Nov. 15th dried spelt and wheat was sampled. Dried barley and oats was not sampled as these crops had been transferred to an airtight silo for cattle feed. Case 3: Combine harvested samples of barley, rye and spelt were received. On Nov. 28th dried samples were collected. Barley had been mixed with oats in a large silo for cattle feed, and one composite sample was taken in this mixture. Rye and spelt had been dried in closed, circular in-bin silos. One composite sample of rye and two of spelt (Da and Db) were taken at the outlet, placed at the bottom of the silos. Case 4: Combine harvested samples of triticale, winter wheat and spring wheat were received. On Nov. 28th dried samples were collected. One composite sample was taken in the spring wheat, placed in a natural air dryer. The winter wheat and triticale had been mixed and transferred to a loft to be used as cattle feed. A composite sample was taken from this lot. Case 5: Combine harvested samples of four different cultivars of spring spelt, grown in the same field were received. On Nov. 28th dried samples were collected. All spring spelt cultivars had been mixed during harvest and placed in the same low heat dryer. One composite sample was taken

from this lot. A composite sample was also taken of dried spring wheat, placed in the silo next to. At another location, the farmer dried and stored winter wheat and winter spelt to a height of approximately 4 m in large, low heat dryers. One composite sample was taken from each of these. Case 6: Combine harvested samples of spring wheat and winter wheat were received. On Nov. 28th dried composite samples were collected. The grain lay in natural air dryers, the winter wheat to a height of approximately 3 m and the spring wheat to approximately 1 m.

Enumeration of P. verrucosum contaminated kernels

Upon arrival, all grain samples were transferred to airtight plastic containers and stored at 2°C until analysis. The number of *P. verrucosum* contaminated kernels was assessed by direct plating of kernels on the selective and indicative nutrient agar, Dichloran Yeast Extract Sucrose Agar with 18% Glycerol (DYSG) [13-14]. Laboratory capacity did not allow all samples to be analysed upon receipt but with few exceptions all platings were performed within 1.5 month after sample receipt (Tables 2 and 3). Direct plating was performed in the following way: 300 kernels were drawn representatively from each sample and placed on DYSG with 10 kernels plate^{-1}. There were a few exceptions to this procedure: *a)* the four replicate samples from Case 1 (D1a-d) from which 75 kernels were tested (making up to a total of 300 for D1), *b)* the mixed sample from Case 3 from which 150 kernels of barley and oats, respectively, and *c)* the mixed sample from Case 4 from which 150 kernels of winter wheat and triticale, respectively, were tested. All plates were incubated for 7 days at 25°C. The kernels were not surface-disinfected prior to plating, because *P. verrucosum* is sensitive to this procedure [15]. Based on its terra-cotta coloured reverse, the number of kernels colonized by *P. verrucosum* were enumerated. These recordings were used to calculate the percentage of contamination (Cont. %). A number of *P verrucosum* strains were chemotaxonomically characterised in order to verify the identity of the strains [16]. The strains

were grown on two substrates, Czapek Yeast Autolysate agar (CYA) and Yeast Extract Sucrose agar (YES). Agar plugs with mycelial growth were analysed for extracellular and intracellular metabolites using thin layer chromatography (tlc), and production of OTA, citrinin and verrucolon verifies that the strain belongs to *P. verrucosum* chemotype II [16].

Spelt is difficult to thresh with a combine harvester as most kernels adhere firmly to the spikelets. The forwarded spelt samples consisted mostly of spikelets but had varying amounts of threshed out kernels. Normally, kernels are left within the hull during drying and storage and not threshed out until processing at the mill. Therefore we included assessments of both spikelets and kernels. It was decided to examine spikelets from samples with few threshed out kernels and kernels from samples with many threshed out kernels. From one spelt sample with particular high content of threshed out kernels (Sample ID 14-2, Table 2) both spikelets, kernels and damaged kernels were analysed. For the four spring spelt samples from Case 5, 100 threshed kernels per sample were plated in addition to the 300 spikelets.

Analysis of moisture content

The moisture content (MC) of the grain samples was determined according to the ISO 712:1998 standard (2 h at 130°C). No pre-conditioning was performed and all moisture contents are given at wet basis (w.b.).

Sample preparation and analysis of Ochratoxin A

OTA analyses were performed in the samples that had been stored at 2ºC. The samples were ground in a stone mill (hawo's Oktagon II). This mill has a grinding chamber with adjustable mill stones (finest setting 1 and coarsest setting 10). The stones are constructed of corundum bound in ceramic. At finest setting, the milling capacity is 220 g min^{-1}. Approximately 150 g were drawn

representatively from each sample. Wheat, barley, rye, and triticale were ground at setting 1. Spikelets of einkorn, spelt and emmer wheat were first ground at setting 10 followed by two grindings at setting 1. Oats was ground at setting 2.5. Between every two samples for OTA testing, a sample of bulk wheat (no contamination with *P. verrucosum*, OTA below limit of detection) was passed through the mill for cleaning purposes. When a sample had been ground, it was immediately stored at 2ºC until analysis for OTA.

Samples were analysed for OTA according to Jørgensen & Jacobsen [7] with minor modifications. Stock solutions of OTA (Sigma, St. Louis, USA) of approximately 75 µg ml^{-1} were made in toluene (99 v/v%)-acetic acid (1 v/v%). The exact concentration was measured by spectrophotometry at 330 nm by using ε330 = 5550 cm-1M-1. Stock solutions were stored at -20°C in portions of 1 ml for one year. Extraction and clean-up were carried out using Ochraprep immunoaffinity columns from Rhone Diagnostics Technologies (Glasgow, Scotland). Fifty grams of ground and homogenized sample were extracted with 200 ml aqueous 60 v/v% acetonitrile for two minutes in a Waring laboratory blender. The extract was filtered through a cellulose filter, and 4 ml of the extract was mixed with 44 ml phosphate (PBS) buffer (pH 7.4). The immunoaffinity column was precondioned with 10 ml PBS, and the diluted sample extract was sucked through the column at a flow rate of maximum 5 ml min^{-1}. The column was washed with 15 ml water at a maximum flow rate of 5 ml min^{-1} and subsequently dried by gentle vacuum. OTA was eluted with 3 ml 99 v/v% methanol in 1 v/v% acetic acid (flow rate <1 ml min^{-1}). After evaporation under nitrogen, the sample was dissolved in 200 µl HPLC mobile phase consisting of acetonitrile-water-acetic acid (50:49:1, v/v/v). Separation and detection of OTA were carried out using an RP-HPLC column (Hibar, LiChrosorb, 5 µm, 125 x 4 mm) at a flow rate of 1 ml min^{-1} and fluorescence detection using 385 nm as the excitation wavelength and 440 nm as the emission wavelength (Hewlett Packard Model HP1100). Post-column addition of 6% ammonia in water at 0.8 ml min^{-1}

was used. A standard solution (0–100 ng ml^{-1}) was used every day for calibration and prepared daily by dilution of the stock solution with the HPLC mobile phase. Sample volumes of 25 µl were injected. In each analytical series, a spiking experiment was performed at between 3.3 and 6.4 ng OTA g^{-1}. The mean recoveries and standard deviation (1 SD) for OTA was 94.1% ± 17.4% (n=17). The results were not corrected for recovery. The limit of detection (LOD) determined as the signal:noise ratio of 3:1 was approximately 0.1 ng OTA g^{-1} during the period of measuring.

Results

The occurrence of *Penicillium verrucosum* was assessed as percentage of contamination (Cont. %) in 56 non-dried, combine harvested samples (C). Results for each sample are shown in Tables 2 and 3 and summarized in Table 4. The Cont. % was measured only once for each sample but no correlation was obtained between Cont. % and storage for up to 102 days at 2ºC (results not shown). It is therefore assumed that storage prior to plating had no detectable effect on growth and proliferation of the fungus and thus no effect on the obtained Cont.%. *P. verrucosum* was found in 82% of the non-dried samples with a maximum value of 58.7%. There was no statistically significant difference in Cont. % between non-dried samples received from the mill and the case study farms (P=0.434). Furthermore, no clear relationship was obtained between MC at harvest and *P. verrucosum* contamination in the 56 non-dried samples.

The median contamination level for non-dried samples showed statistically significant differences among crop species (P=0.041, Table 4). The highest median values were obtained for spelt samples, both when spikelets were tested (5.0%) and when kernels were tested (median 4.4%). Spelt samples, from which kernels were assessed, showed significantly higher median contamination percentages than wheat, oats and barley samples but did not differ significantly from rye samples or spelt samples, from which spikelets were assessed.

Thirtythree non-dried and 22 dried samples were analysed for OTA (Tables 2 and 3). The OTA positive samples were divided into three groups (Table 4): 1) OTA below LOD, 2) OTA above LOD but below 5 ng g^{-1} and 3) OTA above 5 ng g^{-1}. The median Cont. % for the three groups with different levels of OTA was neither significantly different for non-dried (P=0.752) nor dried grain samples (P=0.177).

Figure 1 shows the relationship between MC and OTA in all samples containing OTA above the LOD. The figure clearly indicates that MCs above 17% are conducive to OTA production with two non-dried and five dried samples exceeding the EU limit of 5 ng OTA g^{-1} grain. Only two samples with moisture contents above 17% contained no detectable OTA, both being non-dried grain (111-95 and 38-72, Table 3). Nine samples with MC below 17% contained OTA (Figure 1). Three were non-dried samples with MCs below 15% and six were dried samples. A dried spring wheat (115-100) with 16.7% MC contained 3.2 ng OTA g^{-1} whereas the non-dried sample of this batch (48-38) had a similar MC and OTA <LOD. A dried spring wheat (126-97) with 12.2% MC contained 22 ng OTA g^{-1} grain. The drying history of this grain is unknown, as the farmer did not forward a sample prior to drying. From the present data, no significant correlation was found between MC and the amount of OTA produced.

All case studies showed examples of large increases in *P. verrucosum* Cont. % during drying. For example the Cont. % in spring wheat in Case 4 increased from 0.7% in non-dried to 37.3% in the dried grain. The highest OTA contents in dried samples were obtained in Case 1. Five samples ranged between 10 and 38 ng OTA g^{-1}. These samples originated from a batch of oats that had been harvested late and dried at low heat with no aeration (Table 1) and which had MCs above 18% (D1a-d). *P. verrucosum* contamination in these samples ranged between 11.3 and 16.3%. The farmer was aware that drying was too slow and had transferred an upper visibly mouldy layer to an airtight silo. A rolled sample of this grain contained less *P. verrucosum* contaminated kernels but

also exceeded the OTA limit for grain (D1-AS). An oats sample (D2) from another silo had a lower MC and its OTA content was only 0.5 ng g^{-1} (D2) despite a much higher *P. verrucosum* contamination (39.7 %). This sample even contained a few kernels with macroscopically visible, sporulated colonies of *Penicillium*, among which *P. verrucosum*. These findings exemplify that generally heavily contaminated samples did not contain similarly high amounts of OTA (Figure 2). When comparing drying efficiency in the two silos, it must be taken into account that the sampling procedure used in this study did not fulfil the guidelines for official testing of OTA contents in large batches of grain (Commission Directive 2002/26/EC of 13 March 2002). Therefore results may not be representative for the whole batch of grain. In Case 4, no MC decrease was detected following drying of the spring wheat, and the OTA content increased from a level below LOD to 3.2 ng g^{-1} after drying. In Case 5, four of the OTA positive non-dried samples originated from different cultivars of spring spelt grown in the same field. They had medium levels of *P. verrucosum* (2.0-8.7% for the spikelets and 2–11% for the threshed out kernels), while their contents of OTA ranged from 0.1 and 0.2 ng g^{-1} in two of the cultivars to rather high levels of 18 and 92 ng g^{-1} in the other two. The farmer mixed all four cultivars of spring spelt prior to drying, and the OTA content in a sample of this dried mixture (MC 11.4%) was 0.2 ng g^{-1} grain. Case 2, 3 and 6 all contained samples with high contamination by *P. verrucosum* but none exceeded 0.5 ng OTA g^{-1}.

Discussion

More than 80 % of the combine harvested samples contained *P. verrucosum* showing that much grain is contaminated prior to drying and storage. The major implication of early contamination is a latent risk of OTA production if the grain is not handled properly post-harvest. Considering the many contaminated kernels, this risk should be taken seriously especially if the grain is harvested at a high MC as discussed below. The origin of early contamination is not fully understood but some

soils contain *P. verrucosum* [8] and *P. verrucosum* conidia can survive in soil for many months [17]. Miller [9] pointed out that the combine may act as an efficient disseminator of fungal conidia within a batch of harvested grain and that infestation of some kernels by ochratoxigenic *P. verrucosum* is common at harvest. In 1998, Elmholt [8] analysed 35 combined samples from 15 farmers and found 51% of the samples to be contaminated by *P. verrucosum* (median 0.6%, maximum 5.8%). Thus, the combined samples from the present study had more contaminated samples and higher mean contamination levels (Table 4). There may be several reasons for this. The summer of 2001 was warm and sunny but accompanied by many showers in some parts of the country. Harvest conditions were especially difficult in September with about 80% more rain than average [18]. Quite exceptionally for Danish conditions, much grain could not be gathered in at all. This explains some of the problems in late harvested crops as oats, spring wheat and spring spelt. Probably a more important reason is that the present study included many samples of spelt. Spelt was significantly more contaminated than wheat, barley and oats. A higher contamination in spelt than rye was also indicated though not statistically significant (Table 4). Rye is normally considered the most sensitive of the small grain cereals regarding contamination with *P. verrucosum* [8] as well as formation of OTA [6-7].

According to a prediction model for OTA in cereal grain introduced by Lindblad et al. [19], there is only little risk of significant OTA formation at MC of 17% and below, even at high inoculum potentials. At MCs of 19-24% the risk of OTA formation increases and will further increase when the inoculum potential is high. Our results also show that MC is critical to OTA formation (Figure 1) and that MCs above 17% constitute a serious risk of OTA formation. Eleven of 13 such samples contained OTA and seven exceeded the EU limit of 5 ng OTA g^{-1} grain. Five of these originated from dried oats (Case 1) and the problems could be ascribed to insufficient on-farm drying of a late harvested crop with a MC of about 18%. As shown in Table 3, the grain did not

contain detectable amounts of OTA at the time of harvest. The other two samples with high MC and OTA contents originated from different cultivars of non-dried spring spelt. This grain had been harvested late in September and the two cultivars with MCs of 19.3 and 21.2%, respectively, contained 18 and 92 ng OTA g^{-1} grain. However, two other cultivars from the same field with similarly high MCs, contained only small amounts of OTA. This indicates cultivar differences in susceptibility to OTA accumulation, especially because all four cultivars had similar Cont.% at harvest, both for spikelets (2-8%) and threshed out kernels (2-11%). These findings are in accordance with other studies on barley and wheat, which also report cultivar differences in OTA susceptibility [20-21].

As predicted by Lindblad et al. [19], samples with MC below 17% contained no or little OTA (Figure 1), nine exceeding the LOD. Three of these were non-dried samples, in which the small amounts of OTA had seemingly been formed in the field. Field produced OTA is generally not regarded a problem but low levels have been found [20; 22]. The remaining six samples were dried grain, in which the OTA had probably been produced at some time prior to or during drying where the MC had exceeded a critical level. For example, the dried spring wheat (126-97) contained 22 ng OTA g^{-1} grain but only 12.2% moisture. Spring wheat is a late harvested crop in Denmark and many of the non-dried spring wheat samples contained more than 17% moisture. Although the drying history of this grain is unknown, the result illustrates that dried grain with low MC and a low number of contaminated kernels may contain OTA in significant amounts.

None of the winter spelt samples contained OTA above 1 ng g^{-1} despite some were heavily contaminated by *P. verrucosum*. The reason is probably that winter spelt was harvested before the rainy period started. In opposition to this, spring spelt is harvested late and these samples all had high MCs and two contained high levels of OTA. Most of the winter spelt samples had MCs of 13.5-15.5%, *i.e.* well below the critical level for OTA production. Furthermore the glumes of

spelt, which is a ‘covered wheat’, may help to protect the kernels inside from fungal infection and OTA contamination as demonstrated by Riesen *et al.* [23] for damping off caused by *Pythium.*

The presence of *P. verrucosum* is regarded an indicator of OTA formation [24] and in the present study, all OTA contaminated samples did contain *P. verrucosum.* However, no linear relationship was obtained between OTA and Cont. % (Figure 2). This is in accordance with Lund & Frisvad [15], who suggested that microbial interactions in the grain and microbial interactions with their environment are responsible for this lack of correlation. Some kernels and spikelets were most likely not infected by the fungus but merely surface contaminated. This may also account for the lack of correlation between OTA and Cont. %. Surface contamination is normally assessed by comparing fungi on disinfected and non-disinfected kernels. However, surface sterilisation seems detrimental to the growth of *P. verrucosum* [15] and was therefore not used in either this study or the study by Lund and Frisvad [15]. Lund and Frisvad [15] proposed that 7% or more *P. verrucosum* contaminated kernels in a sample indicates that the 5 ng g^{-1} limit of OTA is exceeded. Their study addressed wheat and barley but with no mention of whether samples originated from organically or conventionally cultivated grain. Our results did not support this hypothesis. Actually 52% of the OTA-negative samples exceeded the 7% limit (21 non-dried, 10 dried) with maximum values of 35.7% for non-dried and 44.7% for dried samples and actually. This shows that presence of *P. verrucosum* on a high number of kernels does not necessarily imply OTA formation, which is in accordance with the assumption that a certain MC is required for the onset of OTA production. Because microbiological analysis for *P. verrucosum* is cheaper than OTA analysis, Lund & Frisvad [15] propose the 7% limit as a criterion for further action in barley and wheat, either condemnation of the cereal batch or a subsequent determination of OTA. Based on the results of the present study, this limit warrants further investigation at least in rye and spelt, which constituted the main part of our samples and which were not included in the study by Lund & Frisvad [15]. If the 7% limit is

used to decide whether or not to condemn a batch of grain, much grain containing no OTA might be condemned.

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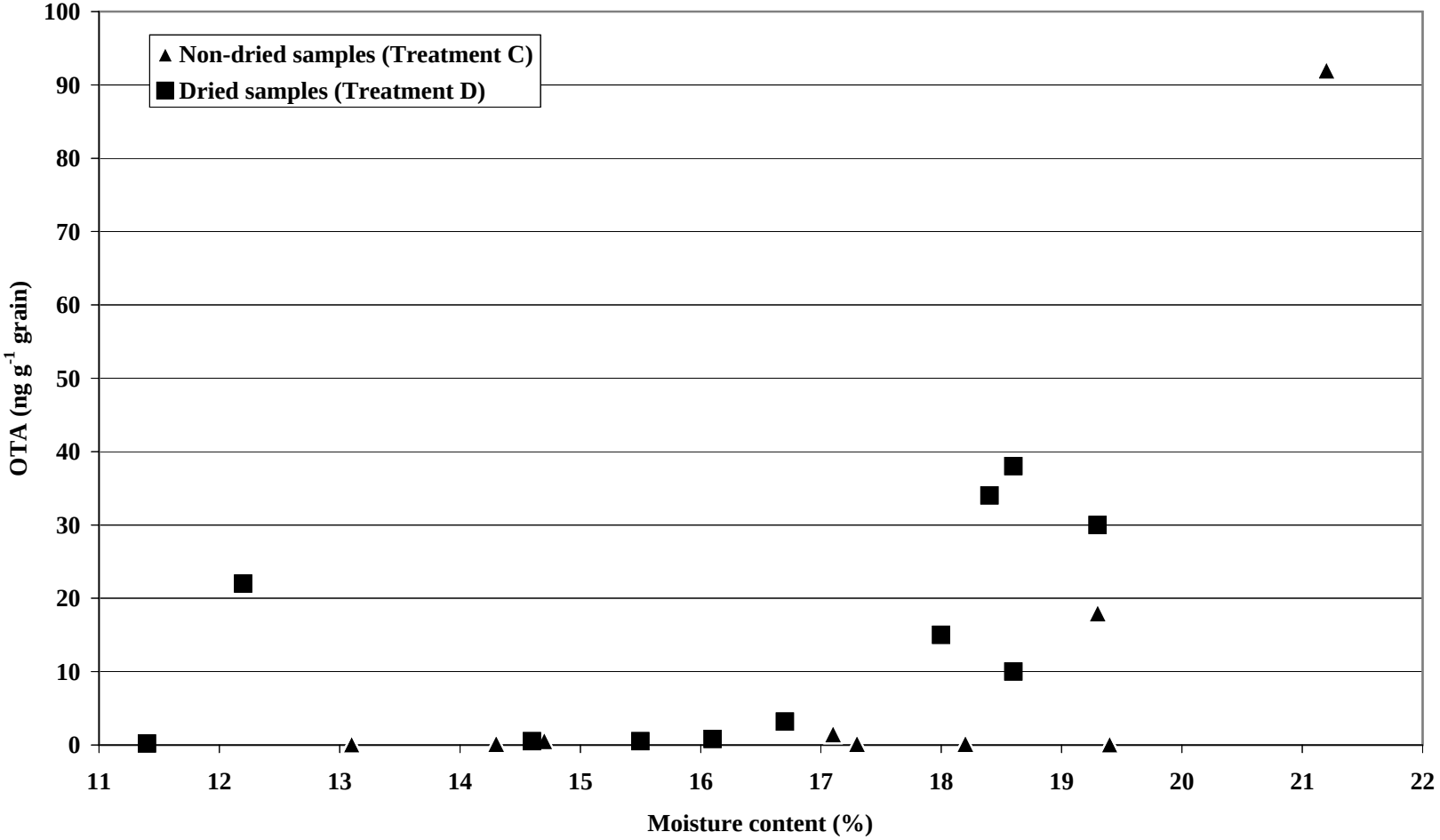
Figure captions

Figure 1. Relationship between MC of the grain samples (%) and Ochratoxin A contents (ng OTA g^{-1} grain) in non-dried (▲) and dried (■) samples. Data is drawn from OTA tested samples of Treatments C and D in Tables 2 and 3.

Figure 2. Relationship between kernels/spikelets with growth of *P. verrucosum* (Cont. %) and Ochratoxin A contents (ng OTA g^{-1} grain) in non-dried and dried samples. Data is drawn from OTA tested samples of Treatments C and D in Tables 2 and 3.


Non-dried samples (Treatment C)
Dried samples (Treatment D)
OTA (ng g-1 grain)
Moisture content (%)
100
90
80
70
60
50
40
30
20
10
0
11
12
13
14
15
16
17
18
19
20
21
22

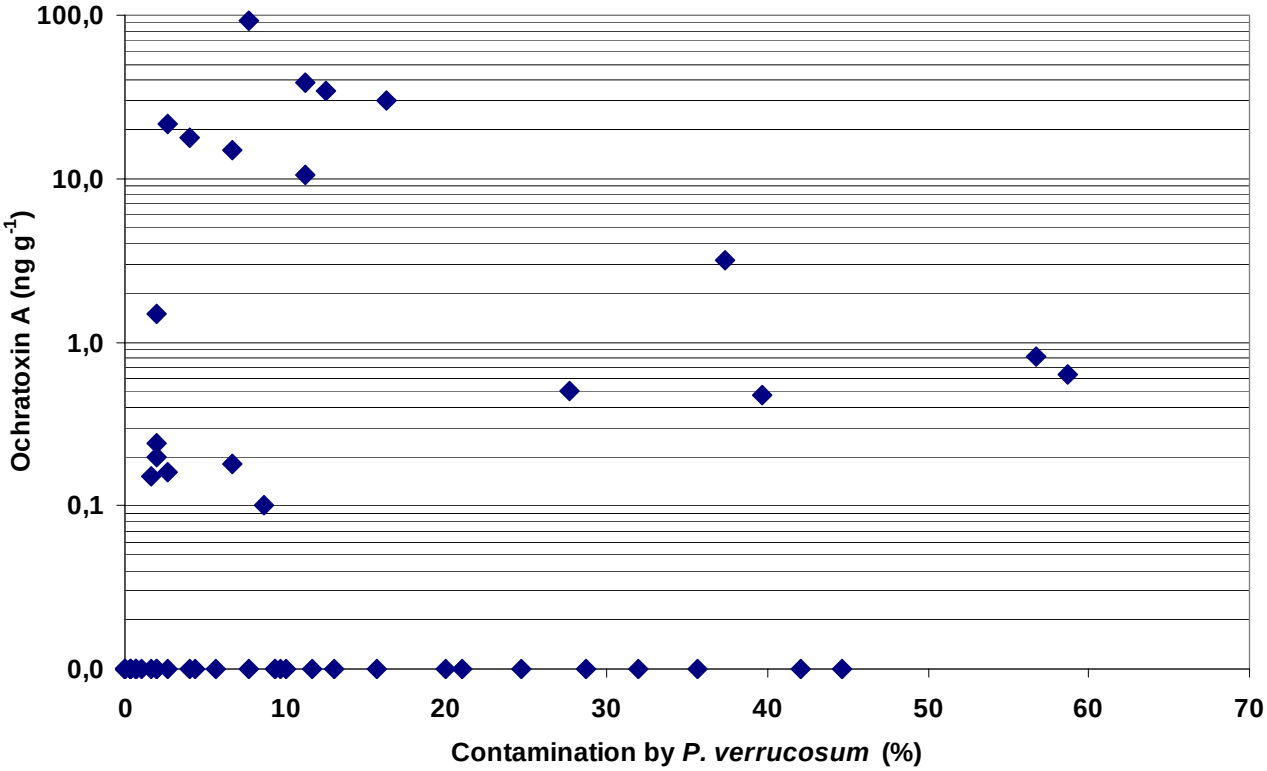

100,0
10,0
1,0
0,1
0,0
Ochratoxin A (ng g-1)
0
10
20
30
40
50
60
70
Contamination by P. verrucosum (%)

Table 1. Management data for case-study farms with on-farm drying

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Farm no.	Farm 23	Farm 24	Farm 25	Farm 26	Farm 27	Farm 28
Management	Dairy	Dairy	Dairy	Dairy	Plant production	Plant production, few suckler cows
Cereals in rotation	wheat, spelt, oats, barley	wheat, spelt, oats	wheat, rye, spelt, oats, barley	wheat, oats	wheat, spelt	wheat, oats
Home-grown seed	No	Occational	Occational	Occational	No	No
On-farm combine harvester	Yes	Yes	Yes	Yes	Yes	No
Drying system	Low heat	Natural air	Low heat	Natural air	Low heat	Natural air
Motor power of fan (KW)	7.5	7.5	11	11	11	7.5
Main duct	Metal	Wood	Wood	Concrete and chipboard	Chipboard	Wood
Side ducts	Metal	Wire mesh with hessian covering	Wire mesh with hessian covering	Galvanised steel	Galvanised steel	Metal
Heating	Oil	No	Oil	No	Oil	No
Aeration	No	Yes	Yes	Yes	Yes	Yes
Are cereals moved after drying	No	No	No	Yes	No	No
Grain thermometer	Yes	No	Yes	Yes	Yes	Yes
Moisture measurer	Yes	No	Yes	Yes	Yes	Yes

Table 2. Combined, non-dried (Treatment C) grain samples directly delivered to the mill

Farm ID	Sample ID	Sample receival	Storage at 2ºC (days)	Crop	Objects for determining Cont. %	Moisture (%)	*P. verrucosum* Cont. %	OTA (ng g^{-1}) [a]
1	1-46	31-aug	13	Rye	Kernels	16,7	9,7	<LOD
	2-9	20-aug	16	W-spelt	Kernels	15,0	37,7	n.d.
	3-27	24-aug	28	W-spelt	Kernels	16,2	4,0	n.d.
	4-28	24-aug	28	W-spelt	Kernels	16,7	3,7	n.d.
2	5-32	24-aug	0	Barley	Kernels	15,2	1,0	n.d.
	6-34	24-aug	119	Rye	Kernels	14,8	0	n.d.
3	7-49	31-aug	75	Rye	Kernels	15,5	0	<LOD
	8-18	22-aug	14	W-spelt	Spikelets	15,7	0,3	<LOD
4	9-69	13-sep	0	Oats	Kernels	17,9	0	n.d.
	10-39	28-aug	8	Rye	Kernels	14,6	1,3	n.d.
	11-67	10-sep	10	S-wheat	Kernels	16,0	1,0	n.d.
5	12-30	24-aug	20	W-spelt	Spikelets	14,4	0	n.d.
6	13-20	22-aug	15	W-spelt	Spikelets	14,1	0,7	<LOD
7	14-2	15-aug	8	W-spelt	Spikelets	12,3	1,7	n.d.
			8		Kernels (U) [b]		1,3	n.d.
			8		Kernels (D) [b]		0	n.d.
8	15-5	20-aug	24	W-spelt	Spikelets	13,7	0	n.d.
9	16-65	10-sep	102	Rye	Kernels	17,1	2,0	1,5
	17-66	10-sep	102	W-spelt	Spikelets	15,5	7,7	<LOD
10	18-43	28-aug	13	W-wheat	Kernels	15,9	1,7	n.d.
	19-44	29-aug	13	W-spelt	Spikelets	13,7	0	n.d.
	20-45	30-aug	13	Barley	Kernels	14,4	0	n.d.
11	21-15	21-aug	17	W-spelt	Spikelets	15,0	21,0	<LOD
12	22-47	31-aug	14	W-spelt	Kernels	15,0	4,1	n.d.
13	23-50	31-aug	13	Emmer wheat	Spikelets	13,1	0	<LOD
14	24-13	21-aug	0	Barley	Kernels	13,3	2,0	<LOD
	25-4	20-aug	16	Einkorn	Spikelets	12,1	4,3	<LOD
	26-33	24-aug	0	Oats	Kernels	13,7	0,7	<LOD
	27-3	20-aug	2	Triticale	Kernels	14,6	9,3	<LOD
15	28-62	12-sep	49	W-spelt	Spikelets	14,7	58,7	0,6
16	29-17	22-aug	0	W-wheat	Kernels	14,3	2,7	0,2
17	30-29	24-aug	21	W-spelt	Kernels	15,1	6,3	n.d.
18	31-21	22-aug	23	W-spelt	Kernels	14,8	1,7	n.d.
19	32-1	15-aug	21	W-spelt	Kernels	14,1	7,3	n.d.
	33-8	20-aug	32	W-spelt	Kernels	13,3	4,7	n.d.
	34-19	22-aug	30	W-spelt	Kernels	13,8	43,7	n.d.
20	35-64	10-sep	51	Emmer wheat	Spikelets	14,5	9,3	<LOD
21	36-10	20-aug	1	Rye	Kernels	15,6	0	<LOD
22	37-52	31-aug	7	W-spelt	Kernels	15,7	0,3	n.d.

[a] <LOD=lower than the limit of detection (0.1 ng g^{-1}); n.d.=not determined

[b] Threshed out kernels (U=Undamaged; D=damaged) of Sample 14-2

Table 3. Combined, non-dried (Treatment C) and dried (Treatment D) grain samples from the Case study farms

Farm ID	Sample ID	Sample receival	Storage at 2ºC (days)	Crop	Treatment	Objects for determining Cont. %	Moisture (%)	*P. verrucosum* Cont. %	OTA (ng g^{-1}) [a]
Case 1 ('23)	38-72	19-sep	1	Oats	C	Kernels	17,7	0,7	<LOD
	101-86	15-nov	31	Oats	D (D2)	Kernels	14,6	39,7	0,5
	102-81	15-nov	36	Oats	D (D1a)	Kernels	19,3	16,3	30
	103-83	15-nov	36	Oats	D (D1b)	Kernels	18,4	12,5	34
	104-84	15-nov	36	Oats	D (D1c)	Kernels	18,6	11,3	38
	105-85	15-nov	36	Oats	D (D1d)	Kernels	18,6	11,3	10
	106-82	15-nov	41	Oats	D (D1-AS)	Rolled kernels	18,0	6,7	15
	39-54	03-sep	7	W-wheat	C	Kernels	14,5	4,0	<LOD
	107-87	15-nov	30	W-wheat	D	Kernels	15,3	24,7	<LOD
	40-51	12-sep	10	W-spelt	C	Spikelets	14,9	15,7	<LOD
	108-88	15-nov	8	W-spelt	D	Spikelets	14,5	10,0	<LOD
Case 2 (24)	41-40	28-aug	9	Barley	C	Kernels	15,5	0	n.d.
	42-41	28-aug	8	Oats	C	Kernels	13,7	0,3	n.d.
	43-42	28-aug	9	W-wheat	C	Kernels	14,5	0,3	<LOD
	109-78	15-nov	30	W-wheat	D	Kernels	16,3	2,0	<LOD
	44-16	22-aug	15	W-spelt	C	Spikelets	14,1	5,7	<LOD
	110-80	15-nov	1	W-spelt	D	Spikelets	15,5	27,7	0,5
Case 3 (25)	45-53	03-sep	2	Barley	C	Kernels	13,1	1,7	0,1
	111-95	28-nov	23	Barley/oats	D	Kernels	17,0	0	<LOD
	46-12	21-aug	0	Rye	C	Kernels	14,0	35,7	<LOD
	112-92	28-nov	5	Rye	D	Kernels	14,7	11,7	<LOD
	47-14	21-aug	15	W-spelt	C	Spikelets	12,6	20,0	<LOD
	113-93	28-nov	23	W-spelt	D - a	Spikelets	14,1	42,1	<LOD
	114-94	28-nov	5	W-spelt	D - b	Spikelets	14,5	44,7	<LOD
Case 4 (26)	48-38	28-aug	23	S-wheat	C	Kernels	16,5	0,7	<LOD
	115-100	28-nov	5	S-wheat	D	Kernels	16,7	37,3	3,2
	49-6	20-aug	31	Triticale	C	Kernels	12,9	13,0	<LOD
	50-37	28-aug	23	W-wheat	C	Kernels	15,7	0,3	<LOD
	116-101	28-nov	23	Triticale	D	Kernels	16,1	50,0	0,8
			23	W-wheat		Kernels		63,3	
Case 5 (27)	51-73	24-sep	44	S-spelt	C	Spikelets	18,2	2,0	0,2
			44			Kernels		3,0	
	52-74	24-sep	44	S-spelt	C	Spikelets	19,3	4,0	18
			44			Kernels		2,0	
	53-75	24-sep	46	S-spelt	C	Spikelets	19,4	8,7	0,1
			46			Kernels		6,0	
	54-76	24-sep	46	S-spelt	C	Spikelets	21,2	7,7	92
			46			Kernels		11,0	
	125-96	28-nov	5	S-spelt	D	Spikelets	11,4	6,7	0,2
	126-97	28-nov	23	S-wheat	D	Kernels	12,2	2,7	22
	127-98	28-nov	23	W-wheat	D	Kernels	12,9	2,7	<LOD
	128-99	28-nov	23	W-spelt	D	Spikelets	14,7	32,0	<LOD
Case 6 (28)	55-11	21-aug	0	W-wheat	C	Kernels	14,4	1,0	<LOD
	129-90	28-nov	5	W-wheat	D	Kernels	14,5	1,7	<LOD
	56-59	07-sep	68	S-wheat	C	Kernels	17,3	2,0	0,20
	130-91	28-nov	5	S-wheat	D	Kernels	16,4	28,7	<LOD

[a] <LOD=lower than the limit of detection (0.1 ng g^{-1}); n.d.=not determined

Table 4. ***Penicillium verrucosum*** **contamination in combined, non-dried (C) and dried (D) samples as related to sample origin (mill vs. case study farms), crop species and ochratoxin A contents**

			Penicillium verrucosum				
	Treatment	Number of samples	Positive samples	Cont. % (Mean)	Cont. % (Median)	P-value [a)]	Cont. % (Maximum)
Non-dried samples from the mill	C	37	28	6,7	1,7	0.434	58,7
Non-dried samples from case farms	C	19	18	6,5	2,0		35,7
Wheat	C	9	9	1,5	1.0 [a]	0.041	4,0
Spelt (spikelets investigated) [b)]	C	16	13	9,6	5.0 [ab]		58,7
Spelt (kernels investigated)	C	10	10	11,4	4.4 [b]		43,7
Rye	C	7	4	7,0	1.3 [ab]		35,7
Oats	C	4	3	0,4	0.5 [a]		0,7
Barley	C	5	3	0,9	1.1 [a]		2,0
OTA <LOD (0.1 ng g^{-1})	C	24	21	6,8	3,0	0.752	35,7
LOD < OTA < 5 ng g^{-1}	C	7	7	11,1	2,0		58,7
OTA > 5 ng g^{-1}	C	2	2	5,8	5,8		7,7
OTA <LOD (0.1 ng g^{-1})	D	11	10	18,2	11,7	0.177	44,7
LOD < OTA < 5 ng g^{-1}	D	5	5	33,6	37,3		56.7 [c)]
OTA > 5 ng g^{-1}	D	6	6	10,1	11,3		16,3

[a)] Median values were compared by Kruskal Wallis test as the conditions did not allow the use of binominal distribution based methods. Though the median value of spelt samples from which spikelets were examined was the higest, it did not differ from any of the other groups. This might be due to very high variation within this group ranging from three samples with no contaminated spikelets to a sample with 58.7% contaminated spikelets.

[b)] Spelt samples where both spikelets and kernels were analysed (Table 2: 14-2. Table 3: 51-73, 52-74, 53-75 and 54-76) have been included in Table 4 as spikelet samples.

[c)] *P. verrucosum* contamination in sample 116-101 was calculated to be 56.7% (average of data from triticale and winter wheat)