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Mycotoxins in Brewing Grain Raw Material (Barley, Malt) in Russia

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Abstract: The levels of five mycotoxins (MT): deoxynivalenol (DON), T-2-toxin (T-2), zearalenone (ZEA), aflatoxin (Afl), ochratoxin A (OTA) were measured in malting barley and malt samples by enzyme immunoassay (ELISA), using test systems RIDASCREEN FAST (R-Biopharm, Germany). 40 samples of malting barley, mainly from the Central part of European Russia and fewer from the Southern part of, and also some samples from Altai (Asian Russia) were analyzed during 2007-2011 years as well as 120 samples of malt from Russian malting companies. It was found that 17% of barley samples were contaminated with MT; in two cases (5%), the MT concentration exceeded maximum allowable levels (MAL). Among malt samples in more than half (in 56%) MT were detected, in 9% of samples, the MT concentration exceeded MAL (Afl-3 incidents, T-2-3 incidents, OTA-2 incidents, ZEA-1 incident). Maximum levels of mycotoxins in malt were found to be higher than those in barley. These facts support the idea about risky conditions during malting processing.

Key words: Barley, malt, malting, field fungi *Fusarium*, storage fungi *Penicillium* and *Aspergillus*, mycotoxins.

1. Introduction

According to "The Law on Purity" established in Thuringia and Bavaria in 1516, and in Germany in 1906, only barley malt, hops and water can be used in brewing [1]. Also, wheat malt was used for preparing wheat beer (top fermentation beer). In 60-70 years of the 19th century brewers, especially in the USA, acknowledged economic benefits of adding unmalted cereals—corn, rice and wheat as adjuncts, at the rate of 15%-20% by weight.

Malt is considered as a main brewing grain material prepared from barley by germination and modification processes, as well as so-called unmalted (non-modified) materials—barley, wheat, rice, corn and sorghum.

Mycotoxins can be produced on a wide range of cereals, particularly on barley, wheat, oat, corn, rice et cet. Grain contamination and damage may occur in the field during kernel maturation, in harvest, transportation and storage processes, if conditions of high moisture are present. Mycotoxins produced by field fungi *Fusarium*: trichothecenes-nivalenol (NIV), deoxynivalenol (DON) and its derivatives, T-2 and HT-2 toxins (T-2, HT-2) and also zearalenone (ZEA), moniliformin, fumonisins were detected in some samples of wheat, barley, rice, corn and oat from different countries [2-15]. Mycotoxins of storage fungi *Penicillium* and *Aspergillus*: aflatoxins (Afl), ochratoxin A (OTA), citrinin and other were detected in part of samples of all cereals [3, 12, 16-19] and there would appear to be a significant risk of their formation in grain which is stored improperly or for extended period of time.

The malting, the process of barley germination and one of the key steps in the brewing process, is also a very risky stage, regarding MT problem because of specific moisture and temperature conditions which are very favorable for yeast and mould growth.

The production and accumulation of extra amounts of mycotoxins, mostly, *Fusarium* toxins, may occur

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during this process. In some samples of malt trichothecenes of type A (T-2, HT-2, 3-Ac-DON, 15-Ac-DON, diacetoxyscirpenol (DAS)), and type B (DON, NIV, fusarenone X), ZEA, fumonisins and also aflatoxins and OTA have been found [19-24].

In several works, the fate of naturally occurring and artificially added mycotoxins has been investigated at various stages of the brewing process [9, 19, 25-30]. *Fusarium* MT trichothecenes (DON, NIV, fusarenone X, DAS, neosolaniol, T-2) were estimated as very thermo stable and they do not disturb after heating near 120 °C during 45 min [24, 29, 30]. These MT survived after kilning of malt and boiling of wort. On the other hand, DON is good water-soluble and OTA is partly water-soluble and its concentration declines during steeping [23]. In spite of that, some quantities of water soluble MT may be transferred in mashing stage from contaminated grain into wort and then into beer [23, 24, 29, 30]. In Nigeria, about 50% of ZEA transferred from naturally contaminated corn (maize) into beer have been observed [29].

The fate of storage fungi mycotoxins-OTA, citrinin, Afl B_1 have been investigated by Gjersten et al. [26, 28] and Chu et al. [31]. The latter authors have demonstrated transmission of 14%-18% of Afl B_1 and 27%-28% OTA from initial barley into finished beer. Afl and OTA resist heating to 200 °C and soluble in ethanol [16]. More recent brewing studies have confirmed that at least one third of OTA in malt can survive into the beer [25].

Specificity of brewing: the technology of grain processing uses all grind fractions, including husks (lemmas and paleas) as filtration layer for wort. It is known that in the husks there is a maximum part of total mycotoxins content [20, 29, 32-34]. At the stages of mashing and filtration, the extraction of water-soluble MT occurs with sequent transmitting into the wort and final beer.

The potential for mould growth and toxin formation during malting was investigated using artificially infected barley [25]. Only limited loss of DON or NIV was observed during malting. Results suggest that if viable mould capable of producing these mycotoxins were present on barley used for malting, then there would be some potential for concentrations to rise during malting. DON metabolites such as 3-Acetil DON may also form during malting. In contrast, significant loss of fusarenone-X, neosolaniol and T-2 toxin were observed during malting. Diacetoxyscirpenol (DAS) and citrinin are very largely destroyed during malting.

Malts containing high levels of mycotoxins were brewed on the pilot scale [25]. Brewing performance and beer quality were noticeably affected. Both worts and beers were cloudy, and the beers displayed a strong tendency to gush. Substantial proportions of DON, NIV, fusarenone-X and neosolaniol persisted into the final beer. All the NIV and fusarenone-X, and on average around 80% of the DON present in malt, could be recovered in the beer. In contrast, about 30% of neosolaniol was lost during brewing. Significant losses of T-2, HT-2 and DAS were observed, and less than half of that present in the malt persisted into the beer.

Numerous surveys have reported the natural occurrence of *Fusarium* MT (DON, NIV, T-2, DAS, ZEA and fumonisins) and storage fungi MT (OTA, Afl and B_1) in some beer samples [29, 32, 35-42]. Small amounts of ZEA, DON, NIV, T-2, DAS and OTA were detected in commercial samples of light lager beer from different countries, including Canada, European regions, Balkan, Africa and South America.

All these mycotoxins represent health hazard and have been associated with human and animal diseases [34, 43, 44]. But mycotoxins not only pose some risk to human health, but they also may affect the brewing processing technology because of their phytotoxic and antibacterial activity. For example, T-2, DAS and ZEA may cause reduction in cell number, dry mass and fermentation velocity in brewing yeast, may affect the development of rootlets and enzyme (particularly, α-amylase) synthesis during malting of barley (germination stage) [45-50]. In our practice, we

observed the stop of fermentation in brewery because of strong infestation of grain with *Aspergillus clavatus* (producer of patulin, citochalasin E and some other MT).

The connection is known between *Fusarium* infestation of grain and the gushing of finished beer [20, 23]. The positive correlation was observed between gushing potential of malt and concentration of DON in barley and in malt (r = 0.74 and r = 0.77, respectively) [23].

All these investigations clearly show the importance of monitoring the occurrence of these mycotoxins in barley and malt grain.

The objective of this study was to evaluate the incidence and contamination levels of five mycotoxins (DON, T-2, ZEA, Afl and OTA), in malting barley and malt in Russia.

2. Materials and Methods

A total of 40 samples of malting barley mainly from central and fewer from the Southern parts of European Russia (regions of Belgorod, Voronezh, Kursk, Rostov-on-Don and Stavropol) and some samples from region of Altai (Asian Russia) and of 120 samples of malt from different Russian malting companies were analyzed in the years 2007-2011. Samples were randomly collected from brewing plants, maltings and farms where they had different storage periods, although most of barley samples were freshly harvested or had storage periods of 3-5 months, and most of malt samples were freshly kilned.

The levels of five mycotoxins, for which the maximum allowable levels (MAL) in cereals have been regulated in Russia [51, 52] and in EU [53]: DON, T-2, ZEA, Afl and OTA were determined with commercial direct competitive ELISA test kits RIDASCREEN FAST (R-Biopharm AG, Germany) (Table 1). (Test kit RIDASCREEN FAST Aflatoxin for the quantitative analysis of sum of aflatoxins B_1, B_2, G_1 and G_2). Sample processing (extraction, dilution, etc.) was carried out according to the producer's recommendations.

3. Results and Discussion

Table 2 shows the occurrence of three *Fusarium* mycotoxins and two storage fungi mycotoxins in malting barley and malt samples from 2007-2010 harvests.

Of 40 malting barley samples analyzed during 2007-2011, seven (17.5%) were contaminated with MT and in two (5%) incidents the MT levels were higher than maximum allowable levels. It was T-2 (150 μg/kg) and OTA (10 μg/kg). The last case was with barley sample, which had been stored on farm for 2 years in unfavorable conditions and its germination index was below 50%. The 33 barley samples (82.5%) which arrived on malt houses for processing were free from MT.

Of 120 malt samples examined, 67 (56%) were contaminated with MT: T-2 was detected in 36 incidents (30% of total); Afl in 28 incidents (23.3%); ZEA in 22 incidents (18.3%); DON in 10 (8.3%); OTA in three cases (2.5%). In 11 (9.2%) samples, the MT concentrations were found to be higher than maximum

Table 1 Maximum allowable levels of MT in cereals for food in EU and in Russian Federation (RF) and characteristics of RIDASCREEN FAST method.

MT	Maximum allowable levels, (μg/kg) (ppb) (grain for food)		RIDASCREEN FAST (R-Biopharm)		
	RF, 2002, 2008 [51, 52]	EU 2006 [53]	Limit of detection (LOD), ppb	Limit of quantification (LOQ), ppb	Measuring range, ppb
DON	1,000 barley 700 wheat	1,250 (unprocessed cereals other than durum wheat, oats and maize)	200	200	200-6,000
T-2	100	100	< 20	50	50-400
ZEA	1,000	100 (unprocessed cereals)	17-41	50	50-400
Afl	5.0 (Afl B_1)	2.0 (Afl$_1$) 4.0 (Afl sum)	< 1.7 (Afl sum)	5 (Afl sum)	1.7-45 (Afl sum)
OTA	5.0	5.0 barley, 3.0 malt	5	5	5-40

Table 2 Occurrence of MT in malting barley and malt samples from 2007-2010 harvests.

MT	Barley		Malt	
	Occurrence of MT, incidents (% of total)	MT > permitted level, incidents (% of total)	Occurrence of MT, incidents (% of total)	MT > permitted level, incidents (% of total)
Samples total	40 (100%)		120 (100%)	
DON	2 (5%)	0	10 (8.3%)	0
T-2	2 (5%)	1 (2.5%)	36 (30%)	3 (2.5%)
ZEA	2 (5%)	0	22 (18.3%)	1 (0.8%)
Afl	2 (5%)	0	28 (23.3%)	4 (3.3%)
OTA	1 (2.5%)	1 (2.5%)	3 (2.5%)	3 (2.5%)
Samples contaminated total*	7 (17.5%)	2 (5%)	67 (56%)	11 (9.2%)
Incidents total*	9	2	99	11

*Co-existence of two or three different MT were detected in some samples, therefore, "samples contaminated total" and "incidents total" were unequal in some cases. There was one case of MT co-existence in barley: OTA + ZEA; and there were three incidents in malt: (1) T-2 + ZEA; (2) DON + T-2 + ZEA; (3) ZEA + OTA.

allowable levels: Afl in four cases (3.3%) with concentrations ranging from 5 μg/kg to 15 μg/kg; OTA in three incidents (2.5%), samples consisted respectively 25, 30 and 40 μg/kg; T-2-3 incidents (2.5%), ranging from 200 μg/kg to 400 μg/kg; ZEA-1 incident, 150 μg/kg. DON concentrations were lower MAL in all incidents.

The incidence of MT contamination in malt tended to be higher than in barley nearly 3 times. MT exceeds of maximum allowable levels were detected in malt 1.5 times more frequently than in barley. Maximum exceeds were 1.5-2 units of MAL in barley and about 6-8 units of MAL in malt. So, the results indicated that mycotoxins contamination was more frequent and more sever in malt than in barley.

The increase of mycotoxins levels in grain mass after malting completion was detected in two cases on industrial malting, when we could analyze one batch of grain before and after malting. In both cases, there was T-2 toxin and in one incident there was ZEA (Table 3). Although, there must be a note that T-2 toxin levels in the first case were lower than limit of quantification equal 50 μg/kg.

Three of those surveyed mycotoxins—DON, T-2 and ZEA—are produced by *Fusarium* species. Ochratoxin A is a metabolite of *Penicillium verrucosum* in temperate zone of North and Central European Russia and *Aspergillus flavus* is a producer of aflatoxins.

Table 3 The increase of MT content in grain mass during malting processing (μg/kg).

Samples	MT	Mycotoxins concentration (μg/kg)	
		Initial barley	Freshly kilned malt
1	T-2	24	32
	ZEA	100	415
2	T-2	traces	650

The main part of studied samples was collected from Central and Central-Chernozem regions of European Russia. More than 20 years monitoring of grain microbiota of malting barley has shown that freshly harvested grain with high germination index (95% and higher) did not contain storage fungi, and *Fusarium* contamination ranged from 0% to 68% of kernels with mean index of 17% [54].

Malting barley grain in the Central part of European Russia in most cases has been contaminated with *Fusarium* species of *Sporotrichiella* group: *Fusarium sporotrichioides*, *F. poae*, *F. tricinctum* and *F. langsethiae* [54-56], with grate prevalence of *F. sporotrichioides* [54]. *Fusarium sporotrichioides* and *F. langsethiae* are known as the most important producers of T-2, to a lesser extent this is *F. poae* [34, 43, 55].

Fusarium verticillioides (formerly named *F. moniliforme)*, the main producer of high yields of fumonisins (FUM) [34, 43], was also frequently isolated from barley in our samples. *F. graminearum* and *F. culmorum*, producers of great amount of DON

and a lesser amount of zearalenone, were isolated most frequently in the South of European Russia. The frequency of contamination by mycotoxins obtained in these studies seems to correspond well with proportion of *Fusarium* species and their associated toxins in Central part of European Russia.

The species-producers of Afl and OTA have been found in batches of grain in the cases of disturbed storage and transportation conditions. In our analyses, the species of genera *Penicillium* have been found most of the time, but not all of them were *P. verrucosum* and not all isolates of *P. verrucosum* were toxigenic. Therefore, the occurrence of OTA was not frequent, and seems to be located in the grain mass in the form of "nests". *Aspergillus flavus* was found on the second place among storage fungi by the frequency of occurrence and on the first place among *Aspergillus* species.

MT contamination of malt may occur in malting processing (barley germination stage) (*Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp.), in storage period and also during long-term transportation when ill-conditioned control (*Penicillium* spp. and *Aspergillus* spp.).

4. Conclusions

Of 40 malting barley samples harvested during 4 years (2007-2010), seven (17.5%) have been contaminated with MT and in two (5%) incidents the MT levels were higher than maximum allowable levels. 33 (82.5%) barley samples which have arrived on malt houses for processing were free from MT.

Of 120 malt samples from Russian malting companies examined during 2007-2011, 67 (56%) have been contaminated with MT and in 11 (9.2%) samples the MT concentrations were found to be higher than maximum allowable levels. Thus, the malting process tended to increase nearly 3 times the incidence of MT contamination. The results indicated that mycotoxins contamination was more frequent and more sever in malt than in barley. The risky conditions during malting process require monitoring of sanitary in malt-houses and mycological control of arrived grain raw material.

T-2 toxin is the most common between *Fusarium* toxins in barley and malt samples from Central parts of European Russia. This corresponds well with proportion of *Fusarium* species producers of T-2 toxin (*F. sporotrichioides*, *F. poae* and *F. langsethiae*) living in this area.

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