Review

Mycotoxins and beer. Impact of beer production process on mycotoxin contamination. A review

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Abstract

Beer is the most consumed alcoholic beverage in the world. Its contamination with mycotoxins is of public health concern, especially for heavy drinkers. Beer production implies a variety of operations which might impact the initial level of mycotoxins in a positive or negative way. The complexity of these operations do not give to the brewer a complete control on chemical and biochemical reactions that take place in the batch, but the knowledge about mycotoxin properties can help in identifying the operations decreasing their level in foodstuffs and in the development of mitigation strategies. This review discusses available data about mycotoxin evolution during malting and brewing process. The operations that may lead to a decrease in mycotoxin load are found to be steeping, kilning, roasting, fermentation and stabilization operations applied over the process (e.g. clarification). Also, other general decontamination strategies usually employed in food industry, such as hot water treatment of barley, ozonation or even the use of lactic acid bacteria starter cultures during malting or fermentation are considered.

Keywords: ~~b~~Barley; ~~b~~Beer; ~~b~~Brewing; ~~m~~Mycotoxin; ~~d~~Deoxynivalenol; ~~f~~Fumonisins; ~~a~~Aflatoxin; ~~d~~Decontamination

~~1.~~1 Introduction

Mycotoxins are natural compounds with a low molecular weight produced by filamentous fungi as secondary metabolites with no biochemical significance for fungal development. When exposed to optimal mycotoxin synthesis conditions, they create a toxic environment being able to cause diseases in animals and human beings (Benett & Klich, 2003).

The mycotoxins with the greatest agro-economic and public health impact are aflatoxins (AFs), ochratoxin A (OTA), patulin (PAT), trichothecenes (deoxynivalenol DON, nivalenol NIV, HT-2 toxin, T-2 toxin), zearalenone (ZEN), fumonisins (FUM), tremorgenic toxins and ergot alkaloids (Hussein & Brassel, 2001) mainly produced by *Aspergillus* (AFs, OTA, PAT), *Penicillium* (OTA and PAT) and *Fusarium* (DON, NIV, HT-2, T-2, ZEN) genera. Many commodities and products used in food and feed industry may be contaminated by mycotoxinogenic fungi which lead to mycotoxin synthesis. Frequently contaminated commodities are cereals, peanuts, milk and dairy products, coffee, wine, beer, cottonseeds, fresh and dried fruits, vegetables and nuts.

Barley represents one of the main ingredients in beer production together with water, hops and yeast. Its quality is decisive for the quality and acceptance of the beer on the market. Beer also can be subjected to mycotoxin contamination coming from infected raw materials: barley, malt, hops or adjuncts.

Many studies have been published concerning the fate of mycotoxins in beer production, analysing the overall production process or only a part of it and highlighting the physical parameters leading to the variation in mycotoxins' concentration (Inoue, Nagatomi, Uyama, & Mochizuki, 2013; Malachova et al., 2010; Pietri, Bertuzzi, Agosti, & Donadini, 2010; Vaclavikova et al., 2013). A review on the evolution of mycotoxin during brewing is available, considering also the existing physical, chemical and biological decontamination methods that could be applied (Wolf-Hall, 2007). The present review compiles the available updated information on the incidence of mycotoxins in beer, the impact of beer processing operations on mycotoxin levels and several mycotoxin decontamination strategies that could be applied in brewing industry.

~~2.~~2 Incidence of mycotoxins in beer

Many studies in beer have focused their investigation on DON, which is the most abundant mycotoxin and which represents the highest public health concern related to the consumption of beer (Yoshizawa & Morooka, 1973; Tanaka et al., 1988; Lancova et al., 2008; Kuzdraliński, Solarska, & Muszyńska, 2013; Piacentini, Savi, Olivo, & Scussel, 2015).There are thousands of beer brands in the world and, in order to find a place on the market, each producer aims to obtain its own one according to the demand of the consumer. However, two beer styles are defined worldwide with respect to the fermentation style: top-fermented beer or ale and bottom-fermented beer, known as lager. Apart from different yeast strains used in the two aforementioned beer styles, other fermentative characteristics, such as secondary products formed and fermented sugars, define the particularities of these beers (Kunze, 2006).

Current European regulations on mycotoxin ~~sets~~ set maximum levels in foodstuff for 13 compounds (EC 1881/2006; Commission Recommendation, 2013/165/EU). The limits for cereal based products (e.g. beer) are set as following: 2 µg/kg for AFB1 and 4 µg/kg for total AFs, 750 µg/kg for DON, 75 µg/kg for ZEN, 400 µg/kg for the sum of FUMB1 and FUMB2, 5 µg/kg for OTA. Due to its high worldwide acceptance, beer may contribute to mycotoxins intake, particularly in the case of heavy consumers. Mycotoxin contamination may occur at different stages of brewing. Some of them can be transferred from cereals to malt and then to beer due to high thermal stability (AFs, ZEN and DON) and water solubility of mycotoxins (DON and FUM) (Rodríguez-Carrasco, Fattore, Albrizio, Berrada, & Mañes, 2015). Whatever the origin, numerous surveys on the occurrence of mycotoxins in beer were conducted worldwide up to nowadays analysing different styles of beer making (Table 1). Many surveys performed on beer are mycotoxin specific, searching for the occurrence and people's exposure to different *Fusarium* mycotoxins found in beer (Shim et al., 1997; Torres et al.,1998; Molto, Samar, Resnik, Martínez, & Pacin, 2000; Papadopoulou-Bouraoui, Vrabcheva, Valzacchi, Stroka, & Anklam, 2004; Bertuzzi, Rastelli, Mulazzi, Donadini, & Pietri, 2011; Rubert et al., 2013; Piacentini, Savi, Pereira, and Scussel, 2015; Rodríguez-Carrasco et al., 2015; Piacentini et al., 2017). Others are beer style specific, regrouping the beer samples according to the production style applied to malting barley that they are made from.

Table 1 Occurrence of mycotoxins in commercial beer of different production styles.

alt-text: Table 1

Beer type	Toxin	Positive samples, % (n)	Mycotoxin concentration, µg/L		References
			Range	Mean*	
Non-alcoholic (< 1% vol. alc.)	DON-3-Glc	NA	0–3.1[f]	2.3	[a] Niessen, Vogel, & S. D., 1993 [b] Shim, Seo, Lee, & K. J. C., 1997 [c] Mbugua & Gathumbi, 2004 [d] Zachariasova et al., 2008 [e] Roger, 2011 [f] Malachova, Varga, Schwartz, Krska, & Berthiller, 2012 [g] Kuzdraliński et al., 2013 [h] Rubert, Soler, Marín, James, & Mañes, 2013 [i] Varga, Malachova, Schwartz, Krska, & Berthiller, 2013 [j] Piacentini, Savi, Pereira, & Scussel, 2015 [k] Rodríguez-Carrasco et al., 2015 [l] Habler & Rychlik, 2016 [m] Piacentini et al., 2017
		47.4 (19)	2.0–6.6[i]	3.0	
	DON	66.7 (3)	18.0–23.0[b]	20.5	
		NA	0.0–3.7[f]	3.7	
		26.3 (19)	3.2–26.1[i]	8.7	
		NA	NA[k]	19.1	
Shandy beer	DON-3-Glc	NA	0.0–5.5[f]	3.5	
		80 (25)	1.8–7.9[i]	3.8	
	DON	NA	0.0–6.4[f]	6.4	
		52 (25)	4.2–12.7[i]	6.9	
		NA	NA[k]	9.4	
Light beer (1.0 to 3.5% vol.)	NIV	100 (6)	3.3–38.0[b]	17.97	
	DON	NA	NA[k]	20.6	
Lager beer (bottom-fermented)	ADONs	85.7 (7)	< 5–27.6[d]	11.65	
	FUMB1	72 (75)	0.0–0.78[c]	0.3	

		50 (14)	201.7–1568.62[m]	367.47
	ZEN	100 (75)	4.3–107.0[c]	8.16
	NIV	97 (36)	1.0–20.0[b]	4.05
	DON-3-Glc	85.7 (7)	< 2.5–25.8[d]	9.7
	DON	28.5 (123)	0.0–478[a]	148
		55.5 (18)	1.0–10.0[b]	3.1
		100 (75)	1.56–6.4[c]	3.42
		71.4 (7)	< 5–35.9[d]	21.3
		100 (46)	6.0–70.2[g]	20.01
		72.4 (58)	< LOQ-42.0[k]	22.9
		28.6 (14)	4.3–10.1[l]	7.1
Ale beer (top-fermented)	OTA	10 (10)	3.2[h]	NA
	DON	100 (17)	8.6–43.3[g]	25.21
Craft beer	FUMB1	100 (8)	29.0–285.0[j]	105.0
	DON	100 (17)	127–501[j]	221.0
Sorghum beer	FUMB1	87.5 (120)	0.0–340.0[e]	180.0
	DON	89.2 (120)	0.0–730.0[e]	485.0
Pale beer	T-2	15.8 (19)	4.0–12.1[h]	NA
	HT-2	15.8 (19)	15.1–20.0[h]	NA
	FUMB2	36.8 (19)	71.0–87.0[h]	NA
	FUMB1	36.8 (19)	71.2–118[h]	NA
	OTA	21 (19)	2.7–6.9[h]	NA
	DON-3-Glc	NA	0.0–19.0[f]	8.3
		65.4 (217)	3.6–81.3[i]	9.3
	DON	NA	0.0-30[f]	13
		100 (55)	6.0–70.2[g]	18.3
		54.4 (217)	5.4–89.3[i]	12.0
Dark beer	DON-3-Glc	NA	0.0–16.0[f]	9.6
		59.6 (47)	4.2–26.2[i]	10.7
	DON	NA	0.0–11.0[f]	11

		100 (12)	14.3–52.9[g]	28.3
		29.8 (47)	11.1–45.0[i]	22.4
		NA	< LOQ-32.8[k]	23.6
Wheat beer	ENNB	60 (5)	0.01–0.24[l]	0.16
	HT-2	56 (25)	< LOQ-38.2[k]	30.9
	ADONs	50 (6)	5.1–22.8[d]	11.7
	DON-3-Glc	100 (6)	6.3–21.0[d]	13.1
		NA	0.0–15.0[f]	8.6
		69.6 (46)	13.5–28.4[i]	11.5
	DON	74.6 (67)	0.0–569.0[a]	245
		100 (6)	9.0–31.4[d]	24.8
		NA	0.0-27[f]	14
		78.3 (46)	5.2–49.6[i]	18.4
		76 (25)	< LOQ-47.7[k]	34.0
		40 (5)	3.6–5.8[l]	4.7

Notes: ENN B: enniantin B; ADONs: 3- and 15-acetyl-deoxynivalenol; DON-3-G: deoxynivalenol-3-glycoside; NA = not available; LOQ = limit of quantification; n = number of samples.

* Mean from contaminated samples

Niessen et al. (1993) have found wheat beer containing higher levels of DON and its derivatives compared to barley beer. This can be explained by existing matrix differences between wheat and barley which determine crops mycobiota. Taking into account that different beer styles imply a slightly different physical treatment and substrate composition, Malachova et al. (2012) developed a matrix specific LC-MS/MS method (mycotoxin extraction ~~protocole~~ protocol adjusted to the type of beer) to evaluate the levels of DON and its conjugates in beer samples purchased in Austria, reporting an average concentration of 6.6 µg/L for DON and DON-3-Glc, which does not overcome regulated limits. Varga et al. (2013), focusing their research on different beer styles (374 samples) from 38 countries, have identified that the lowest contamination level of DON and DON-3-Glc was observed in non-alcoholic (2.7 and 1.5 µg/L respectively) and in shandy beers (4.4 and 3.2 µg/L respectively), reaching the same conclusion as Kostelanska et al. (2009), but could not prove it as the information concerning raw materials was not available. From the data presented in ~~t~~Table 1, it can be seen that T-2 and HT-2 toxins concentration in pale and wheat beers are near or overcoming the limits recommended by the European Commission (Commission Recommendation, 2013/165/EU) (Rodríguez-Carrasco et al., 2015; Rubert et al., 2013). One of the recent studies performed by Piacentini et al. (2017) have identified very high levels of FUMB1 in lager beer (four times overpassing the maximum allowed concentration).

DON was firstly isolated in Japan (1972) from mouldy barley. In 1973, Yoshizawa and Morooka published an article about the finding of a new mycotoxin, Deoxynivalenol monoacetate, found in barley contaminated with *Fusarium roseum*. Consequently, considering a possible carry-over of the toxin and its conjugate, many surveys on the occurrence of DON and its derivatives in beer were performed in different countries such as Germany, with detected levels between 172 and 569 µg/L (Niessen et al., 1993); Canada, where ~~more than~~ ≥ 50% of beer samples contained up to 50 µg/L of DON (Scott, 1996); Argentina, with a range of 5 to 221 µg/L (Molto et al., 2000); Czech Republic, with 10.9 µg/L and 9.2 µg/L of DON and DON-3-Glc, respectively found (Benešová, Běláková, Mikulíková, & Svoboda, 2012; Kostelanska et al., 2009); Poland where DON and ZEN concentrations were about 7.5–70.2 µg/L and < 0.26–0.36 µg/L, respectively (Kuzdraliński et al., 2013); Brazil, with levels from 127 to 501 µg/L of DON and from 29 to 285 µg/L of ZEN were found (Piacentini et al., 2015). On a larger regional scale, regrouping several European countries where a contamination range between 4 and 56.7 µg/L of DON was found (Papadopoulou-Bouraoui et al., 2004).

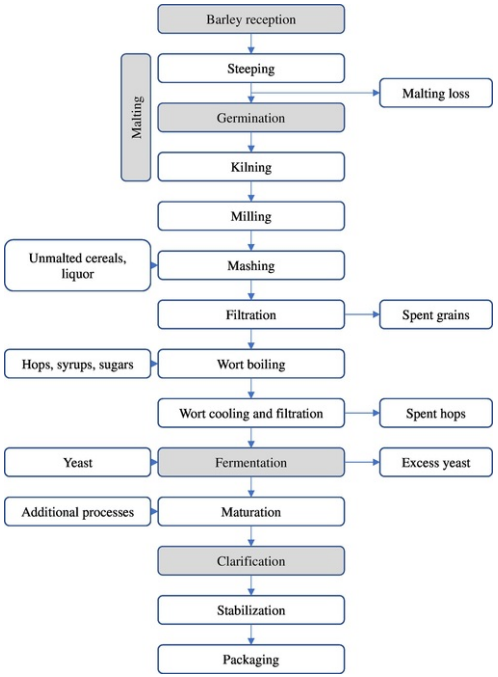
Bertuzzi et al. (2011) have studied the occurrence of OTA, trichothecenes, FUMs and AFs in beer produced in several European countries. In this study aflatoxins were not found in any of the analysed samples which was

confirmed by another study ~~analyzing~~analysing 117 beer samples (no information was given concerning beer production style)~~.~~, one year later, performed by Benešová et al. (2012). However, detectable amounts of other mycotoxins were identified in the majority of the samples (mean levels of 2.1 µg/L for DON, 5.8 µg/L and 0.6 µg/L for fumonisins B1 and B2 respectively and 0.019 µg/L for ochratoxin A) with small differences observed between the countries concerned with the study.

Considering the aforementioned information, researchers are continuously working on the elaboration of fast and reliable methods for mycotoxin identification in both raw materials (such as cereals) and final products as well as preventive and corrective measures in the food and feed chain, but the best measure to avoid mycotoxin ~~acumulation~~ accumulation is still prevention of moulds' growth in raw materials.

~~3.~~3 Mycotoxins during beer production process

Beer production process implies three main biochemical reactions: enzyme activation in barley grain during germination, starch degradation into fermentable sugars thanks to grain's enzymatic equipment and alcoholic fermentation realized by *Saccharomyces* yeasts with ethanol and CO_2 formation. In terms of raw materials, five commodities are involved in beer production, namely barley, hops, water, yeasts and adjuncts. The quality of these commodities plays a decisive role in the creation of organoleptic characteristics of the final product, beer. The production process includes the following main steps: malting, milling, mashing, fermentation, maturation, filtration, stabilization (e.g. clarification or pasteurization) and packaging (Fig~~ure.~~ 1).



~~Figure 1~~**Fig. 1** Beer production scheme and steps where possible decontamination strategies could be applied (blocks in grey).

Modified from Lewis & Young, 1995.

alt-text: Fig. 1

As it was previously said, mycotoxins are highly stable compounds (resistant to high temperatures and extreme pH levels) (Wolf & Bullerman, 1998). Although, beer processing operations have maximum operation temperatures below the ones able to destroy the mycotoxins, it may influence mycotoxin concentration due to physical, chemical and biochemical changes that are taking place.

~~3.1.~~3.1 Barley reception

The aim of obtaining a homogeneous quality of the final product within different batches and different harvesting years are making the production process quite challenging. Barley reception and malting are the first decisive

steps in beer fabrication. The use of barley in beer production is explained by its high starch content and the good adherence of the husks to the grain body even after malting and milling. Various parameters of barley and malt are to be considered. At the arrival of barley to the brewery, it is first of all submitted to a process of cleaning (to eliminate the present physical contaminants) and classification (to ensure a maximum of grains size and shape homogeneity) (Kunze, 2006).

~~3.1.1.~~3.1.1 Incidence of mycotoxins in malting barley

The main mycotoxins present in malting barley are the ones produced by *Fusarium* species. The plant disease *Fusarium* head blight (FHB) or scab is of a high concern in the production of malting barley (Wolf-Hall, 2007). The major species involved in FHB disease in Europe are *F. graminearum, F. avenaceum* and *F. culmorum* and others of the same genera but in a smaller rate (Nielsen, Cook, Edwards, & Ray, 2014). The most important damage for brewing industry caused by this disease is the negative impact on germination rates which results in worse malting quality and yield reduction (Piacentini et al., 2015). There is a clear relationship between FHB and mycotoxin contamination as the main fungal species responsible for the disease are mycotoxinogenic. The predominant mycotoxin present in malting barley is DON, besides ZEN, NIV, T2 and HT-2 toxins, whose accumulation in human bodies results in neurotoxic, immunosuppressive, teratogenic and carcinogenic effects (Pestka, 2007). Various studies have proved the existing positive correlation between FHB intensity and DON accumulation level (Paul et al., 2005; Urrea et al., 2002). However, a meta – analysis performed by Paul et al., 2005 evaluating Pearson's correlation between FHB disease parameters and DON accumulation performed on wheat, ranged from 0.43 to 0.94 for the correlation between disease severity and DON concentration and from 0.47 to 0.98 for the correlation between *Fusarium* damaged kernels rate and DON concentration. This result shows that apart from meteorological conditions, defining mycotoxin accumulation, barley variety and its resistance to FHB is an important barrier to mycotoxin synthesis and a factor to consider while evaluating the correlation coefficient (Urrea et al., 2002). Table 2 regroups several surveys concerning the main mycotoxins encountered in malting barley. The natural occurrence of DON is the most studied over years in different regions of the world, as being the mycotoxin reaching the highest concentration levels on barley matrix as compared to AF, ZEN, OTA etc. (Malachova et al., 2010).

Table 2 Mycotoxins in malting barley.

alt-text: Table 2

Mycotoxin	Positive samples, % (n)	Concentration, µg/kg		References
		Range	Mean	
DON	NA	300–50–800	NA	Ruan, Li, Lin, & Chen, 2002
	50 (10)	5.0–80.0	31.0	Olsson, Börjesson, Lundstedt, & Schnürer, 2002
	100 (37)	500–10–000	4098	Pan, Bonsignore, Rivas, Perera, & Bettucci, 2007
	NA	NA	12.0	Lancova et al., 2008
	61.9 (21)	0.0–4000.0	3923.8	Tabuc, Marin, Guerre, Sesan, & Bailly, 2009
	53 (36)	NA	17.0	Malachova et al., 2010
	72 (29)	3.9–112.3	34.4	Gil-Serna et al., 2013
	77.5 (80)	0.0–985.9	48.4	Běláková, Benešová, Čáslavský, Svoboda, & Mikulíková, 2014
	18 (15)	200.0–15–100.0	3400	Piacentini et al., 2015
DON-3-Glc	20 (36)	NA	2.0	Malachova et al., 2010
ADONs	2.8 (36)	NA	1.0	Malachova et al., 2010
NIV	20 (36)	NA	4.0	Malachova et al., 2010
	17.2 (29)	3.5–5.8	4.6	Gil-Serna et al., 2013
ZEN	8 (25)	0.7–21.5	15	Ghali, Hmaissia-khlifa, Ghorbel, Maaroufi, & Hedili, 2008
	11.1 (18)	0.0–36.3	29.0	Manova & Mladenova, 2009
	71.4 (21)	86.0–202.0	132.7	Tabuc et al., 2009

	39 (123)	0.0–18.5	1.89	Ibáñez-Vea, González-Peñas, Lizarraga, & de Cerain, 2012
	37.9 (29)	10.4–34.1	18.5	Gil-Serna et al., 2013
	33.8 (80)	0.0–47.9	3.7	Běláková et al., 2014
OTA	79 (40)	6.7–57.0	25.7	Czerwiecki, Czajkowska, & Witkowska-Gwiazdowska, 2002
	89.6 (295)	0.53–12.0	4.93	Gumus, Arici, & Demirci, 2004
	52 (25)	0.6–3.4	1.9	Ghali et al., 2008
	58 (123)	0.0–3.53	0.1	Ibáñez-Vea et al., 2012
AFB1	4.8 (21)	0.0–7.2	2.0	Tabuc et al., 2009
AFs	44 (25)	3.5–11.7	7.5	Ghali et al., 2008
	100 (123)	0.0–0.75	0.14	Ibáñez-Vea et al., 2012
FUMs	10 (15)	10.0–13.0	6.0	Piacentini et al., 2015
	34.5 (29)	186.5–347.5	249.1	Gil-Serna et al., 2013
T-2 + HT-2	10.3 (29)	14.4–22.7	17.8	Gil-Serna et al., 2013
	40 (80)	0.0–53.4	5.2	Běláková et al., 2014
T-2	86 (36)	NA	34.0	Malachova et al., 2010
HT-2	100 (36)	NA	262.0	Malachova et al., 2010

Note: NA = not available; n = number of samples.

Considering that the most important part of the contamination takes place on the field, the level of mycotoxins looks to be highly dependent on weather conditions (humidity and temperature) (Ghali et al., 2008; Manova & Mladenova, 2009; Pietri et al., 2010). Particularly important are climate conditions during critical phases of barley plant growth: more fungal and mycotoxin diversity was observed in warm and humid regions and less in countries with a noticeable difference between seasons (Manova & Mladenova, 2009; Piacentini et al., 2015; Tabuc et al., 2009). In cold regions like Romania, the longer period of cold weather barrier the accumulation of OTA and FUM but not of ZEN and AFs, although on comparatively low concentrations (Tabuc et al., 2009). This, together with applied agricultural practices, explains the fungal populations invading the crops on the field (Piacentini et al., 2015). Mbugua and Gathumbi (2004) calculated Pearson's correlation coefficient for the co-occurrence of DON and other mycotoxins and have found a positive association between DON and FB1 and DON and ZEN (if DON is present it is more likely that other mycotoxins are also present in the product), which is explained by the fact that these are all *Fusarium* toxins. Taking into account that different strains are responsible for FB1 and ZEN production, their co-occurrence would mean the presence of more than one fungal strain within the same batch or sample.

Legal limits are also installed for cereals or barley specifically, namely: 2 µg/kg for AFB1 and 4 µg/kg for total AFs; 100 µg/kg for ZEN; 1250 µg/kg for DON and 2000 µg/kg for the sum of FUMB1 and FUMB2. It is important to notice that a high ratio of malting barley samples from the presented studies were contaminated with mycotoxins at levels that overcome maximum allowed concentrations (especially researches between 2002 and 2010). However, the more recent studies do show a tendency of decreasing contamination levels.

Co-occurrence of DON and its derivatives with other *Fusarium* mycotoxins, such as FUM, NIV, ZEN, etc., is very frequent in cereals (Ruprich & Ostrý, 2008) and can result in the contamination of the processed cereal based products such as beer (Cole, Dorner, Cox, & Raymond, 1983; Kostelanska et al., 2011; Medina, Jiménez, Gimeno-Adelantado, Valle-Algarra, & Mateo, 2005). Although mycotoxins produced by *Fusarium* are quite similar, their derivatives differ in both physico-chemical properties and incorporation and distribution into the grain body. This explains the different and sometimes contradictory results obtained concerning mycotoxin concentration in barley samples.

~~3.2.~~3.2 Malting

Malting is a controlled germination process to produce the malt. It consists of three stages: steeping, germination and kilning. Steeping is a process initiated under specific conditions of temperature and humidity (controlled cycles of water spraying or immersion and aeration until grain water content reaches 42–48%). The humidity of the barley after steeping is determined by the type of malt that is aimed to be obtained (42–44% for Pilsner and 47–48% for dark beers). Steeping purpose is to create favourable humidity conditions inside the grain and activate the enzymes involved in germination. It generally takes place at 10–15 °C where, after approximately 30–50 ~~hours~~h, the water

enters the kernel and first signs of germination appear (Kunze, 2006). Water flow during respective treatment cycle may lead to a spread of fungal contamination into the batch by 15–90% (Vegi, Schwarz, & Wolf-Hall, 2011). Also, as barley steeping implies a treatment of the grain with a quite high amount of water, this process may have an impact on the level of water soluble mycotoxins, such as DON and FUM, by eluting them from the matrix (Schwarz et al., 1995). Lancova et al. (2008) proved a decrease of DON concentration up to 10% compare to the initial content. In addition to that, Maul et al. (2012) found that thanks to the plant cell structure, first 17 h of steeping and germination later induce the glycosylation of DON to DON-3-Glc, which explain the decrease in DON concentration (in the favour of its glycosylated metabolite). Oliveira, Mauch, Jacob, Waters, and Arendt (2012) proved an augmentation in fungal infestation after 48 h of steeping (76%) and a 75% increase in DON concentration, which was not detectable after kilning. Vaclavikova et al. (2013), on the contrary, found that after two days of steeping the decrease in DON concentration has begun showing up to 30% decline in the final malt.

Germination implies the activation of all enzymatic equipment for the break of reserves of starch and proteins. It starts few hours after water penetration into the grain during steeping and begins with the transport of gibberellic acid (growth promoter) to the aleurone layer where enzyme production and activation take place (Oliveira et al., 2012). The following enzymes are synthetized: amylases and dextrinases, cytolytic enzymes, proteolytic enzymes, lipases, lipoxygenases and phosphatases (Kunze, 2006). The germination temperature, likewise steeping final humidity, is determined by the type of malt that the brewer wants to obtain (17–18 °C for Pilsner malt and 23–25 °C in the case of dark malt). The main role of malting is expressed in enriching the malt with enzymes and the formation of flavour and aroma compounds. The germination is stopped by kilning (drying) and/or roasting in order to prevent future structural changes of the barley. Fungal biomass may be growing during barley germination (increase of fungal infection up to 39.3% at the end of germination) probably as a result of cross contamination from the residual steeping water or because of a latent barley grain infection, which may be activated with the increase of humidity during this production step (Vegi et al., 2011). Fungal infection of barley drastically influences its germinative energy (decrease of germinative energy up to 45%), increase grain water sensitivity and promote DON accumulation up to 199 µg/kg (Oliveira et al., 2012). According to Maul et al. (2012), the possible reason to DON glycosylation during germination is related to the increase in glucose content which might activate the enzyme responsible for the respective reaction and DON transformation into DON-3-Glc (approximately 50% of DON is converted after 5 days of germination).

The kilning prepares the malt for storage and transportation, if needed. It usually takes place at several temperature scales: < 50 °C until the water humidity of grains reaches 10–12% and then the temperature is gradually increased until 80–90 °C. The temperatures chosen are aiming to reduce at a minimum level the degradation of the enzymes (Kunze, 2006). The intensity of kilning and roasting (if applied) is crucial in malt flavour and colour formation (Pires & Branyik, 2015). However, the early stages of kilning may promote fungal growth and mycotoxin accumulation by some *Fusarium* strains (Wolf-Hall, 2007). Kostelanska et al. (2011) found that DON may be degraded while malt roasting at 150 °C leading to the formation of de-epoxidized compounds. Also, the enzymatic hydrolitical activity is leading to the increase of DON concentration in the product due to its ~~relese~~ release from the matrix (Vegi et al., 2011).

An increased attention is paid to the transfer of trichothecenes (HT-2 toxin, T-2 toxin, DON and its derivatives) from barley to malt but quite little information is available concerning the fate of AFs and ZEN during malting process which is probably due to their lower occurrence in barley and probably a higher weather dependence (Rodríguez-Carrasco et al., 2015). Weather, fungicide treatment and barley variety look to be the main factors influencing fungal invasion and mycotoxin synthesis in malting barley. Fungicide treated barley showed a 80% decrease in DON concentration after malting (Malachova et al., 2012). Barley rootlets removed at the end of malting were found to contain from 564 to 1383 µg/kg of FUM B1 (highest rate compare to other brewing intermediates) (Cavaglieri et al., 2009).

~~3.3.~~3.3 Milling

Milling of the malt and other grain aims to increase the contact surface between the brewing liquor and malt. Usually, roller and hammer mills are used to obtain the best results because this way the husks are almost intact, which barrier the extraction of tannins and other undesirable compounds (Lewis & Young, 1995). Finer the particles, better the breakdown of malt into fermentable materials such as sugars and assimilable nitrogen compounds while mashing. However, too small particle size may have a negative impact by decreasing filtration yields and increasing wort turbidity (Kunze, 2006). Some researchers have found that the efficiency of milling is not only expressed in the size of final granules, but it should be evaluated together with mashing temperature levels, because starch α-amylase activity depends on both granules size and treatment temperature (Mousia, Balkin, & Pandiella, 2004). No direct impact on mycotoxin levels at this stage occurs but probably milling would promote mycotoxin homogeneous spread into all malt batch and its later solubilisation into mashing water.

~~3.4.~~3.4 Mashing

Mashing is the mix of milled malt and a large amount of water (approximately 17 kg of malt are needed for 1 hL of beer) under specific temperatures to activate all the enzymatic equipment present (inactivated during kilning) and to allow the conversion of starches into fermentable sugars. Two types of enzymes are mainly present: ones acting on sugars and others acting on proteins. The physical conditions applied while mashing are aiming to maximize the efficiency of the enzymes according to their different optimal temperatures (Tse, Boswell, Nienow, & Fryer, 2003). Four temperature scales are, usually, hold for some time in order to allow the following changes: 45 to 50 °C for β-glucans and protein hydrolysation, 62 to 65 °C for maltose production, 70 to 75 °C for saccharification and 75 to 78 °C for α-amylases activation and finishing of mashing (Briggs, Boulton, Brookes, & Stevens, 2004; Kunze, 2006). An alternative

mashing process exists called “decoction mashing”, where different temperatures are achieved by removing repetitively a part of the mash, boiling it and mixing it back (Pires & Branyik, 2015). During this step, it is important to control all possible parameters, starting with temperature and heating time and continuing with pH (optimal being pH = 5.2), oxygenation level and stirring speed.

An infection of the malting barley with *Fusarium* genera (*Fusarium* Head Blight disease) may lead to a further protein digestion realized by fungal proteases which will affect beer colour, flavour, texture and foaming characteristics (e. g. beer gushing related to DON presence) (Inoue et al., 2013; Wolf-Hall, 2007). Concerning the influence on mycotoxin levels, at this production step, there is a possible release of DON conjugate to protein structures (due to physico-chemical and biochemical conditions: *T* = 40 °C and/or enzymatic changes) and, as a result, increase in total DON concentration (Wolf-Hall, 2007), but according to another study performed later by Kostelanska et al. (2011) the key factor in DON levels in the final product still remain the initial barley contamination as, probably, these conjugated toxins are present in malt but are not extractable using common procedures used for their analysis. Inoue et al. (2013) have identified an almost 20% reduction in all analysed mycotoxin levels (14 analysed mycotoxins) which was mainly due to their elimination with the spent grains. Similar is the case of enniatins (Vaclavikova et al., 2013), where 64 to 91% of the initial charge was removed with spent grains, and of ZEN (~~more than~~ ≥ 60% was quantified in the spent grains) (Wolf-Hall, 2007). A particular attention has to be given to unmalted ingredients added at this step, especially the ones coming from maize, which is known to be an important source of AFs, FUM and DON (Benešová et al., 2012; Torres, Sanchis, & Ramos, 1998). However, no other references were found concerning this aspect of mashing.

~~3.5.~~3.5 Wort separation and boiling

Wort separation and boiling is performed after the separation of the solid particles, and at this step hops are added. The wort can also be enriched by adding sugars and syrups but also “seasoning” like coriander seeds, orange peel etc. (Pires & Branyik, 2015). The hop boiling typically lasts for 45–60 ~~minutes~~min or more. The process varies as a function of the hops used, hoping rate, boiling time, the moment the hopes are introduced (in the beginning, in the middle or at the end of the boiling process) (Briggs et al., 2004). The main processes taking place during wort boiling are: enzyme inactivation, evaporation of water and volatile compounds (mainly represented by dimethyl sulphides, undesirable in the final product), proteins precipitation, sterilization, isomerization of hop α-acids, Maillard reactions and thus flavour modulation (Briggs et al., 2004). The wort after all is cooled down, filtered and transported to fermentation tanks.

Concerning the impact of wort boiling on mycotoxin content, the ingredients added might represent a source of mycotoxins if not controlled (Inoue et al., 2013) but the study performed by Kostelanska et al. (2009) did not identify it to be of a significant importance. Hops are reported to be susceptible to fungal contamination but as the amount added to the wort is low, their impact may be discarded (Vaclavikova et al., 2013). The effect of temperature and beer filtration on mycotoxin levels probably has not to be totally discarded. Taking into account that wort boiling temperature is above 100 °C and that the average boiling time is about ~~one hour~~1 h, a decrease of mycotoxins concentration may occur. Also, filtration residues may contain a certain amount of mycotoxins. However, no studies exist on this particular aspect.

~~3.6.~~3.6 Fermentation

Fermentation of wort is a process initiated by yeasts of *Saccharomyces* genus. Different yeast strains are used according to the type of beer. Two most common technologies are known: ale or top fermentation ~~realised~~realized by *Saccharomyces cerevisiae*, and lager or bottom fermentation using *Saccharomyces pastorianus*. The yeasts will transform sugars into alcohol and carbon dioxide but also a range of secondary compounds such as esters, higher alcohols, volatile compounds etc. The initial yeast concentration at inoculation must be 10^7 cells/mL. The metabolic activity of yeasts is possible at a temperature range of 2 to 30 °C. Usually, the fermentation temperature is 18–25 °C for ale beers and 7–15 °C for lager beers during 7–9 days (Lewis & Young, 1995).

A recently performed study has investigated the adsorption of mycotoxins on beer fermentation residue (BFR), very high ratio of adsorption being observed in the case of ZEN (75.1%) but also for AFB1 (48.1%) and OTA (59.4%) (Campagnollo et al., 2015). The reduction of DON did attain only 11.6%. According to the authors and other previously published researches (Jouany, Yiannikouris, & Bertin, 2005), the adsorption is due to the binding of the toxins (especially ZEN) to β-glucans from yeast cell wall (hydrogen and Van der Waals bonds being involved together with the proportion of β-1,3-~~D~~D-glucans and β-1,6-~~D~~D-glucans in the product). Barley is also known as containing a quite high β-glucan content (2.5 to 3.5%). However, by the fifth day of germination, almost 95% of it is broken down losing its binding properties (Agu & Palmer, 2001).

The effect of mycotoxin contaminated raw material on alcoholic fermentation volatile by-products was studied (Kłosowski, Mikulski, Grajewski, & Błajet-Kosicka, 2010). It was found that some of the mycotoxins (mainly AFB1 and DON) may inhibit the activity of alcohol dehydrogenase, which is in accordance with Reiss (1973), and results in the decrease in carbon dioxide production. It also implies an increase in acetaldehyde concentration and other undesirable volatile compounds synthetized during alcoholic fermentation but no effect on total ester content was identified (Kłosowski & Mikulski, 2010). However, in a recently performed study by Nathanail et al. (2016), the presence of mycotoxins in wort, even at high concentration (10,000 µg/L), do not influence fermentation parameters, such as alcohol production, pH, sugar utilisation and cell viability. The study did not find any impact of different mycotoxin combinations on yeasts' activity and a concentration of 10,000 µg/L of DON was needed to obtain a significant reduction of cell viability. The differences between the two studies concerned with the impact of mycotoxins on yeast activity may be

explained by different yeast strains used which can possess a different resistance to mycotoxin action.

~~3.7.~~3.7 Maturation and stabilization

Maturation and conditioning are aiming to improve and stabilize the beer taste after fermentation (CO_2 elimination and removal of some undesirable volatile compounds). During this step, other processes take place such as beer clarification, yeasts sedimentation and flavour formation of the final product. The process of maturation usually takes from 1 to 3 months and involves lowering of the temperature (cold break) to around 0 °C. A secondary fermentation is often practiced ($2 \cdot 10^6$ cells/mL) and addition of priming sugars is acceptable.

During conditioning stage, protein and tannins combination takes place (sedimentation of high mass molecules) resulting in beer clarification. Proteins may be also removed by adding enzymes, introducing additional tannins or adsorption on surface (nylon membranes, silica gels etc.) (Lewis & Young, 1995). The clarification process can be accelerated by filtration or centrifugation (also at low temperatures, 0 to 1 °C). Yeasts are removed from the beer volume by filtration and the product is transferred to aging tanks for more prolonged storage. The next steps in beer production are aiming to stabilize physically (colloidal stabilization) and microbiologically (filtration and pasteurization) the product before its packaging.

Related to the impact of the stabilization on mycotoxin levels in beer, the use of inorganic adsorbents for clarification is doubted to be involved in mycotoxin removal from the product. The adsorption on the surface is a function of mycotoxin polarity, water solubility, molecule's size, etc. Belajová, Rauová, and Daško (2007) have found that -Cl and -CN modified silica gel were very effective in bounding the OTA and the FUMs. A dosage of 2.5–6.5 g/L has a good adsorption potential and does not alter beer organoleptic properties. However, there are no studies regarding the impact of inorganic adsorbents in the case of multi-mycotoxin contamination of beer which would take into account possible interferences.

~~4.~~4 Decontamination strategies

In some harvesting years, fungal infestation is almost inevitable due to weather conditions and the specificity of field treatment. Also, inappropriate barley storage conditions may take place because of equipment fail which could lead to microorganism activation and growth. In 2003, (This is the citation for the uncited reference Robens and Cardwell 2003.)Robens & Cardwell published a report on economic losses from mycotoxin contamination of food commodities in USA. They have found that the annual damage on malting barley retail market, due to the contamination with DON brought a loss of 406 million US dollars (from 1993 to 1998) and probably more, taking into account barley availability resulting from crop rotation. Still, no available reports on economic losses in EU in money equivalent was found but, in 2009, a survey on cereal traders from 11 European countries showed that 37% of mycotoxin contaminated batches are recalled because of an official control on mycotoxins and 19% were recalled due to consumer complaint (Siegel & Babuscio, 2011).

Thus, in order to minimize economical losses, the need of fungal and mycotoxin decontamination becomes obvious. Chemical treatment, such as ozonation, is of a promising future in barley and beer detoxification of mycotoxins (mainly because, compared to other chemicals, it does not leave residuals of any type) (Piacentini et al., 2015). Allen, Wu, and Doan (2003) have studied the fungicidal effect of ozonation on barley considering the applied ozone dose, the ozonation time, the water activity and the temperature of barley. A 96% of spores were inactivated after a 5 minutes treatment with 0.16 mg of ozone per gram of barley and minute at 20 °C and 0.98 a_w without having an impact on kernel germination (fungal mycelia being less resistant to ozone action).

Kottapalli and Wolf-Hall (2008) studied the effect of hot water treatment of *Fusarium* infected malting barley and have found a 65 to 92% reduction of fungal infection after the malting of the treated grains. Also, a reduction of 79–93% in DON was observed after a treatment with water at 45 °C.

Lactic Acid Bacteria (LAB) have been many times reported as spoilage microorganisms in brewing, mainly being concerned the genera *Lactobacillus* and *Pediococcus* (Suzuki, 2015). However, according to some authors, LAB starter cultures added during malting and brewing could represent an efficient strategy in mitigation of fungal and maybe mycotoxin contamination (Halasz & Lasztity, 2009; Lowe, Arendt, & Brew, 2004; Oliveira et al., 2015). Lowe et al. (2004) review describes the reported use of LAB in malting and brewing as an antifungal agent. Oliveira et al. (2015) have proved the ability of *Lactobacillus reuteri* R29 to reduce *Fusarium* growth by 23% and attenuate DON accumulation by 83%. Besides, the pH drop due to the presence of lactic acid is able to stimulate enzymatic activity during malting and fermentation.

Fermentation yeasts, *Saccharomyces cerevisiae* isolated from different fermented products, were also proven to be able to decontaminate partially the product by binding mycotoxins, particularly OTA (Bejaoul, Mathieu, Taillandier, & Lebrihi, 2004), ZEA (Wolf-Hall, 2007) and AFB1 (Shetty, Hald, & Jespersen, 2007). Bejaoul et al. (2004), Shetty et al. (2007) and Campagnollo et al. (2015) proved that the fermentation residue (non-viable yeast cells) are of a better performance in mycotoxin binding compared to viable cells and the pH of the product had a significant impact on complexation reactions.

As it was mentioned in the previous section, mycotoxins can be adsorbed on the surface of clarification agents. However, no actual application on industrial level with the aim of decreasing the charge of mycotoxins in the product is known. Further studies are needed in order to justify the technological and economical efficiencies of the procedure.

Although there are decontamination methods, the best way to preserve the food and the consumer is prevention. However, the aforementioned methods give a possibility to reduce food waste and stimulate a sustainable production. Also, further studies concerning the economical viability of the mentioned decontamination measures and their applicability on a large industrial scale are needed.

~~5.~~5 Conclusion

Mycotoxin contamination of cereals is of a great concern for both food and feed industries. Beer production is also concerned with this issue, especially taking into account the possibility of mycotoxin carry-over, and its worldwide high consumption rate.

The most studied mycotoxins in barley and beer are DON and its derivatives, ZEA, FBs, HT-2 and T-2 toxins, AFs. The most important stages of beer production process having an inhibitory impact on mycotoxin levels are steeping, kilning, mashing, fermentation and clarification. During these stages, the mycotoxins are either removed with drainage water, spent grains and fermentation residue, diluted or destroyed as a result of thermic treatment, or adsorbed on the surface. Germination do not actually impact DON levels in beer but promote its transformation into its glycosylated derivate (DON-3-Glc). During mashing, the enzymes stimulate the release of conjugated DON from protein structures but also decrease the initial toxin concentration due to dilution. This step can be a source of AFs and FUM- contamination because of maize based unmalted adjuncts added to increase the amount of fermentable sugars. Hops added while boiling might be contaminated with mycotoxins, but they are added in a too small amount to be considered significant for the final product. ZEN is mainly removed with spent grains (approximately 60%).

Strategies of mycotoxin decontamination and prevention can be applied at all production stages: fungicide treatments on the field, lactic acid bacteria during malting and brewing, special yeast strains (known to be able to bind mycotoxins), ozonation, hot water treatment of barley grains etc. They are needed because sometimes commodities contamination is inevitable and the economic loss of it is too high to be discarded.

Further studies are needed on the impact of beer production process on mycotoxin levels in order to better understand the risk to the population and to animals (many secondary products resulting from beer fabrication process are used in animal feeding). Also, an economic evaluation of losses and possible benefits brought by decontamination strategies need to be identified for a better view of the situation.

Uncited references

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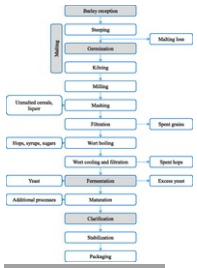
Graphical abstract



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Highlights

- Beer in one of the most consumed beverages in the world
- The mainly present mycotoxins are deoxynivalenol, fumonisins and aflatoxins
- Brewing operations ~~leading to the~~ that decrease ~~in~~ mycotoxin contamination are steeping, kilning, roasting, fermentation and clarification.

- Promising decontamination strategies exist such as hot water treatment, ozonation and lactic acid bacteria cultures

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