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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.

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. 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,

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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 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.

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 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 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 > 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 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 accumulation is still prevention of moulds' growth in raw materials.

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. 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. 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. 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,

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