Journal of Stored Products Research 77 (2018) 166–176

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Evaluation of critical points of mould growth and mycotoxin production in the stored barley ecosystem with a hazardous initial microbiological state of grain

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ARTICLE INFO

Article history:
Received 6 May 2017
Received in revised form
1 March 2018
Accepted 23 April 2018

Keywords:
Malting barley
Grain ecosystem
Mycotoxins
Mould growth
Safe storage time

ABSTRACT

The development of effective control systems for post-harvest preservation and storage processes seems to be one of the best ways to reduce the activity of fungi and thus the risk of mycotoxin contamination of grain during its storage. A crucial step towards developing such a system requires the understanding of ecological relationships between environmental factors, fungal development and mycotoxin production in bulk of grain treated as holistic systems. The aim of this study was to estimate the critical points associated with mould development and mycotoxin production in the ecosystem of malting barley grain with a hazardous initial microbiological state (provided by the inoculation of naturally contaminated grain with *Aspergillus ochraceus* and *Penicillium verrucosum*) stored in different temperature and water activity conditions ($t = 12–30\,^{\circ}C$ and $a_w = 0.78–0.96$, where a_w corresponds to equilibrium relative air humidity in inter-grain spaces). The most intensive fungal growth occurred in the ecosystems of barley grain with $a_w = 0.91–0.93$ stored at 24 and 30 °C. The biosynthesis of ochratoxin A (OTA) was observed at 0.91–0.95 a_w at 18–30 °C, whilst penicillic acid and citrinin were detected only at temperatures of 24 and 30 °C. The toxicogenic ability of fungi at 12–24 °C and $a_w \leq 0.80$ was significantly limited. In the ecosystem of barley grain, the threshold of fungal concentration (expressed in colony forming units of moulds, CFU g^{-1}), for which the amount of OTA exceeded binding limits, was 10^5 CFU g^{-1} of grain. The study indicates that the duration of the lag phase of fungal development (comprising spores germination and the beginning of exponential mould growth), which in all tested ecosystems was shorter than the time, after which admissible levels of OTA were exceeded, may be used to evaluate the safe period, at which grain should be subjected to preservation processes.

1. Introduction

Postharvest preservation and storage processes, apart from environmental conditions in which cereal plants grow and ripen, have an essential influence on nutritive and technological quality of cereal grain. One of the most crucial factors causing the deterioration of technological quality of preserved and stored grain is connected with the development of microscopic fungi. The growth of moulds may lead not only to losses in cereal processing, but also causes the risk of contamination of grain with toxic metabolites of these microorganisms - mycotoxins. Literature survey reveals that in European countries many plant origin products are significantly contaminated with ochratoxin A (SCOOP, 2002) - the mycotoxin that has been shown to be nephrotoxic, hepatoxic and that is known to be carcinogenic and mutagenic (JECFA, 2002; Heussner and Bingle, 2015; Kőszegi and Poór, 2016). It needs to be emphasized that ochratoxin A (OTA) is a threat to consumer health not only through the consumption of contaminated plant origin products, but also through consumption of animal origin products, to which it penetrated via contaminated animal feed (Cabañes et al., 2010). In order to secure consumer health safety the European Community Commission with a decree no. 466/2001 (dated 8 March 2001 with following changes) defined maximum legislative limits for ochratoxin A of 5 μg kg^{-1} in unprocessed cereals and 3 μg kg^{-1} in cereal products for direct human consumption (EC, 2002; EC, 2004; EC, 2005; EC, 2006; EC, 2012).

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https://doi.org/10.1016/j.jspr.2018.04.008
0022-474X/

The presence of OTA in the stored grain is mainly related to the activity of fungi of *Aspergillus* spp. and *Penicillium verrucosum* (Abramson, 1991; Magan, 2006; Cabañes et al., 2010). In countries with temperate climate *P. verrucosum* has been found to be the main OTA producer in stored cereal grain (JECFA, 2002; Cairns-Fuller et al., 2005; Cabañes et al., 2010). Apart from OTA in stored bulk of grain, other mycotoxins, i.e. citrinin (CIT) or penicillic acid (PCA) may also appear (Chełkowski and Goliński, 1983). The presence of CIT may be a consequence of the toxigenic ability of *P. verrucosum*, which is capable of forming both OTA and CIT, while the presence of PCA may result from the toxigenic profile of *Aspergillus ochraceus* (Wawrzyniak and Waśkiewicz, 2014; Schmidt-Heydt et al., 2015).

Grain deposited at a warehouse, containing contaminants, microorganisms and colonising it pests is a system of live organisms, which together with abiotic factors, constitutes the ecosystem of stored grain (Sinha, 1995). It is known that fungal growth and mycotoxin production in the ecosystem of stored grain is affected by a wide variety of complex interactions between abiotic and biotic factors. The most important abiotic factors are temperature and water availability. It is worth mentioning that the most favourable form of water for the growth of microorganisms in stored grain is the water that may form a film on the surface of kernels as a result of condensation of water vapour contained in the air of inter-kernel spaces. For this reason, as it is indicated in literature, the best measure of the availability of water for microbial activity, enabling a comparison of microbial growth in different environments, is the water activity in grain (a_w), rather than the absolute content of water in grain, referred to as grain moisture content (MC), (Lacey and Magan, 1991). Water activity in bulk of grain corresponds to relative humidity of air in inter-kernel spaces during thermodynamic equilibrium state ($ERH = 100 \cdot a_w$, %). It is essential from a practical point of view, as both water activity and temperature are parameters that can be easily measured.

Among cereal raw materials special attention is required for malting barley, which due to the specific character of malt production technology should meet the strictest quality requirements. This material requires exceptionally mild conditions during postharvest preservation and storage. This results from the need to maintain high germinability and germination energy of grain, which are parameters of key importance in malt production.

A primary method of postharvest preservation of cereals raw materials that runs under mild conditions is convection drying and cooling grain in bulk, known as near-ambient methods. In the near-ambient method of drying the temperature of drying air is close to ambient temperature and it changes stochastically depending on climatic conditions (Ryniecki et al., 2006). Due to the relatively low temperature of drying air, this process is long and may last from several to around a dozen days (Gawrysiak-Witulska et al., 2008). Over most of the drying process grain moisture content in the top layer of the deep-bed is close to its initial moisture content, which creates a risk of mould development and the appearance of mycotoxins in the bulk of grain. This is a particularly substantial problem in countries of Northern and Central Europe and other regions of the climate, which is the mixture of maritime and continental climates, where moisture content of freshly harvested cereal sometimes may reach 19–20% wet basis (w.b.), (Scudamore et al., 2003; Gawrysiak-Witulska et al., 2008). As a result, in such climatic conditions cereal is at a great risk of formation of OTA and other mycotoxins. Since, there is no universal or rapid method allowing the determination of the level of fungal population and its toxigenic metabolites without the involvement of skilled workers and specialized as well as expensive equipment, the best method of limiting the fungal invasion of grain, and thus preventing its mycotoxin contamination is the continuous monitoring and automatic control of postharvest drying and cooling of grain.

Numerous researches are devoted to postharvest control strategies in order to minimise mycotoxins in the food chain (Magan, 2006; Ryniecki et al., 2007; Wawrzyniak, 2008). A crucial step towards preventing mycotoxin formation in stored grain ecosystem is the knowledge of relationships between environmental factors such as temperature, water availability, kernel maturity, intergranular gas composition, insects and the development of fungal population and accumulation of mycotoxins. There are many studies that deal with the impact of various factors on mould development and biosynthesis of mycotoxins (Ramos et al., 1998; Lee and Magan, 2000; Pardo et al., 2004b, 2006a; Schmidt-Heydt et al., 2007; Medina and Magan, 2011). Its main drawback is the fact that these researches were usually carried out on agar media or for individual strains, while the bulk of grain is a complex dynamic system, in which many life processes and interactions take place and which should be treated as an integral whole (Magan et al., 2003). It should be emphasized that incomplete understanding of ecological relationships occurring in the bulk of stored grain can lead to inappropriate management strategies. In view of the wide variety of interactions occurring in a bulk of grain, there is still a need for studies aimed at defining the conditions in which there is a risk of mould development and mycotoxins accumulation. These investigations should be conducted in the systems as close as possible to natural ecosystems of stored grain. Sinha (1995), defining the ecosystem of stored grain, emphasizes that the size and boundary for an ecosystem can be arbitrary, and thus a bag of grain, a silo from which the grain is drawn, or the region where many silos and grain-handling equipment are located can all be considered ecosystems.

There are few reports considering the ecosystems of stored grain as holistic systems, whilst only knowledge of the processes occurring in these ecosystems can be the basis for developing a safe control system for the postharvest drying, cooling and storage of grain (Magan and Aldred, 2007), especially for the near-ambient drying. Our previous study on the kinetic of mould growth in the stored barley ecosystem has showed that water activity in grain is the main factor affecting mould development (Wawrzyniak et al., 2013). However, this research was carried out for a limited temperature range ($t = 23–30\,^{\circ}C$) and the effect of unfavourable temperature and humidity storage conditions was examined only with regard to mould growth and, what is very important, without consideration of mycotoxins contamination of grain ecosystems. Taking into account the fact that the average temperature during grain storage in bins varies depending on outside temperatures between 12 and 26 °C, causing moisture migration in the grain mass (Ryniecki et al., 2006; Wontner-Smith et al., 2014), it becomes essential to extend the data of kinetic of mould growth on a wider range of temperatures. Moreover, the determination of conditions in which there is a risk of mycotoxin formation in the ecosystem of stored grain is also desirable. Therefore, the objective of this study was to evaluate the critical points - associated with mould development and mycotoxin production – being decisive for safe-storage of the ecosystem of malting barley grain with a hazardous initial microbiological state (reflecting the microbiological contamination of grain during vegetation and harvest at adverse weather conditions) stored at different temperature (12–30 °C) and water activity ($a_w = 0.78–0.96$) conditions. Both temperature and water activity can be easily measured on-line, which is essential in the design of practical control devices for grain preservation processes. The decisive points of moulds growth and mycotoxin formation, estimated for the ecosystem of malting barley, treated holistically, can be the basis for the future studies on the development of improved control systems for postharvest preservation processes in the climate that is a mixture of maritime