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Relationship of ergosterol content and fungal contamination and assessment of technological quality of malting barley preserved in a metal silo using the near-ambient method

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ABSTRACT

Environmental microbiologists frequently use ergosterol, a fungal-specific membrane lipid, as an indicator of fungal infection in grain and other plant materials. Microbiological loading and technological quality of barley was determined directly after harvest, after post-harvest drying, and during storage. The conventional plate count method was used to measure fungal contamination (CFU). Ergosterol concentration (ERG) was determined by extraction, saponification and quantification using high-performance liquid chromatography (HPLC) with UV detection. The laboratory malting method was used to determine technological quality of the malt. Results showed a significant correlation between ERG and CFU (the coefficient of correlation was 0.92). Analyses also indicated that the high germinative energy and technological quality of the malt produced from dried barley was retained.

1. Introduction

The microflora of the stored-grain mass ecosystem contains several groups of microorganisms, of which moulds are the principal spoiling agents (Multon, 1988; Jayas et al., 1995). Mould growth is associated with the modification of organoleptic quality, aspect and biochemical composition that may lead to reduced enzymatic activity, germinative capacity and energy of the grain (Chełkowski et al., 1983, 1991; Ramos et al., 1998; Knight and Wilkin, 2004; Krstanović et al., 2005). Some fungi are capable of producing toxic metabolites, i.e. mycotoxins, which may be transmitted to the human food chain, thus constituting a real threat to human health or even life (Pardo et al., 2004; Marín et al., 2005).

Grain for use as malt must meet very high-quality requirements, especially in terms of enzymatic activity, germinative energy (GE) and capacity (Briggs, 1998). In order to preserve high-quality grain and ensure its safe storage, the ecosystem of the harvested grain mass must be promptly converted into a state of anabiosis by proper drying and cooling (Karunakaran et al., 2001). It should be noted that malting barley for use as a raw material for malt production requires especially mild drying conditions (Wawrzyniak et al., 2006). Such conditions are guaranteed by convection drying and cooling using the near-ambient method, e.g. in metal silos, as used by grain producers to conserve freshly harvested grain. In this method, compressed air at a temperature close to ambient temperature is blown into a static grain bed ranging in depth from less than one to several meters (Nellist, 1998). Near-ambient drying is slow and in the climate of most European countries, it takes from 1 to 3 weeks in the post-harvest period, depending on the drying potential of the ambient air (Ryniecki et al., 1993). During that time the upper layer of grain remains wet and its moisture content (m.c.) is similar to the initial m.c. of the grain (Nellist, 1998), so there is a risk that the quality of barley in this layer may decline.

It has been postulated that crop spoilage as well as fungal growth and mycotoxin formation result from the interaction of several factors in the storage environment (Abramson et al., 1999; Pardo et al., 2004). These factors include moisture, temperature, time, insect vectors, seed damage, oxygen level, substrate composition, fungal infection level and prevalence of toxigenic strains of fungi (Abramson et al., 2005). It seems that the determination of fungal contamination of barley can be helpful for the quantification of deterioration.

The most common methods for the detection and quantification of fungi in grains are the serial dilution technique and the direct plating of grain (Castro et al., 2002). However, these methods are time-consuming and laborious. The use of a faster method is becoming increasingly necessary. The ergosterol (ERG) measurement method proposed by Seitz et al. (1977) makes it

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Nomenclature

CFU	colony-forming units of moulds per 1 g of grain (cfu g^{-1})
ERG	ergosterol concentration in cereal grain (mg kg^{-1})
GE	germinative energy (%)
T	temperature (°C)
T_{Atm}	ambient air temperature (°C)
T_{IN}	plenum inlet air temperature (°C)
T_{OUT}	grain temperature measured at the air outlet (°C)
ψ	relative humidity (r.h.), 100 kPa [water vapour in air], kPa^{-1} [water vapour in saturated air] or (%)
ψ_{OUT}	relative humidity measured in intergranular spaces at the air outlet (%)

Subscripts

Atm	ambient
IN	inlet
OUT	outlet

possible to determine fungal biomass in cereal grain and it is an alternative for estimating fungal growth. However, more information is needed on the relationship between ergosterol content and fungal growth as measured by the serial dilution technique, the method traditionally used to evaluate fungi in food (Castro et al., 2002), since there is no agreement among researchers on the utilization of ergosterol as a biomarker to monitor the microbiological condition of the grain. Some scientists believe that ERG analysis is a reliable method of monitoring the level of fungal microflora in cereal grain and point to a correlation between ERG and the level of microscopic fungal biomass (Seitz et al., 1977; Montgomery et al., 2000; Pronyk et al., 2006). Some of them claim that in the case of toxin-forming fungi that occur in the environment, the results of analyses of ergosterol content in cereal grain usually demonstrate a significant correlation with mycotoxin concentrations found in grain (Saxena et al., 2001; Srobarova et al., 2007). Other researchers stress the fact that different fungi produce different amounts of ergosterol and its level is also dependent on the growth medium used and incubation conditions, as well as on mycelium age (Zhaoa et al., 2005; Pronyk et al., 2006). They suggest caution when using ergosterol as a biomarker to estimate fungal contamination levels, without the simultaneous use of the additional methods, since it may lead to erroneous conclusions. Because, as Mille-Lindblom et al. (2004) suggested, there is a lack of agreement on the use of ergosterol as a biomarker to determine levels of fungal contamination, there is need for further studies on the subject. Thus, the aim of this study was to determine the efficiency of ergosterol as an indicator of fungal contamination in near-ambient drying of malting barley in a metal silo at a site in Poland providing a mixture of continental and maritime climates, immediately after drying and cooling and during storage. Simultaneously, the technological quality of malting barley was determined.

2. Materials and methods

2.1. Cooling and drying of grain

The experimental material was malting barley cv. *Annabell* harvested in 2005 at the Dłoń Experimental Station owned by the Poznań University of Life Sciences, Poland. Because of high m.c. (20.2% w.b.) grain was dried using the near-ambient method in a metal silo constructed at the Złotniki Poznań University of Life Sciences Experimental Farm (Fig. 1). The metal silo was 4.8-m-high and had a capacity of 36 m^3. The 10,500 kg of grain formed a layer 2 m deep. The silo was equipped with a fan giving a volumetric airflow of 0.9 $m^3 s^{-1}$ at 2 kPa pressure (2.2 kW motor), an electric air heater of power output 9 kW and a drying process controller ("BIT-04" type) with a set of measuring probes. The air and grain temperature probes were accurate to ±0.5 °C and the relative humidity (r.h.) probes to ±2.5%. The controller measured 21 process variables, including temperature and humidity values of: (a) ambient air, (b) plenum air blown into the grain bulk and (c) air in the intergranular spaces at the air outflow from the deep bed of barley. Data were recorded on computer. Grain temperature was also recorded at several levels, most importantly in the bottom and top layers (0.5- and 2-m-high). The locations for measurement of the most important parameters are shown in Fig. 1. The "BIT-04" unit controlled the fan and the air heater (dashed lines with arrows in Fig. 1). This automatic control method for near-ambient drying was selected in order to control random changes in the drying potential of the ambient air and was identical to the system considered to be optimal for wheat drying (Ryniecki et al., 1993; Ryniecki, 2005). In this system, the drying process is controlled indirectly by controlling the r.h. of the drying air. The controller forces the ambient air to flow continuously through a layer of grain (except during precipitation when the r.h. value of the ambient air exceeds 95%) and ensures that the air r.h. does not exceed the set-point humidity (a value of equilibrium r.h. reduced by a constant value, e.g. 12%). The equilibrium r.h. was calculated by the controller on-line from the Chung–Pfost equation (ASAE Standards, 2000) with coefficients for barley, taking into account the current temperature of the top layer of grain and the assumed final grain m.c. of 14.5% w.b. If the drying air r.h. was higher than the set-point humidity, the

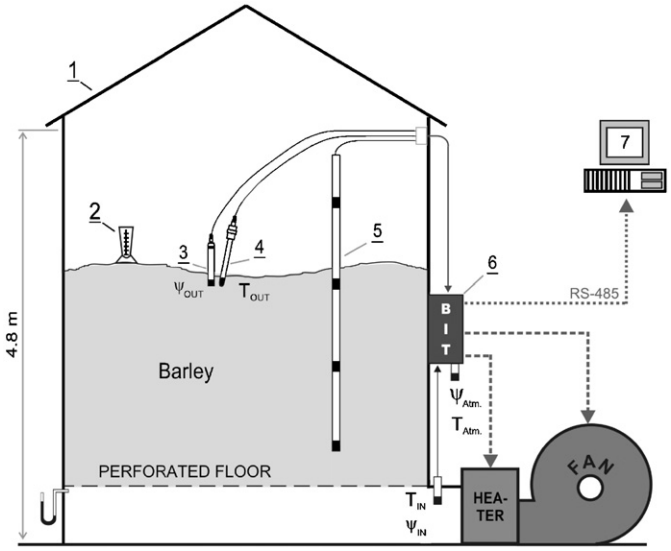

Fig. 1. On-farm testing facility for in-bin drying and storage: 1—metal silo (36 m^3) with fully perforated floor equipped with a fan (2.2 kW) and electrical heater (9 kW); 2—rotameter; 3—probe for measuring r.h. in intergranular spaces of grain mass; 4—portable grain temperature probe; 5—multi-sensor grain temperature probe (fixed); 6—the "BIT"-type air-ventilation controller for drying and cooling barley in bulk; 7—computer (PC) with "ViBIT" data acquisition program.