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Metabolite fingerprinting of barley whole seeds, endosperms, and embryos during industrial malting

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ABSTRACT

Samples of whole seeds, isolated endosperms including the aleurone layer and isolated embryos with attached scutellum from an industrial scale barley malting process (variety *Braemar*) were analysed for their water soluble metabolites by gas chromatography–mass spectrometry (GC–MS). 73 known metabolites and about 350 unknown signals were detected. Principal component analysis (PCA) showed a time dependent shift of sample profiles. Whole seeds and endosperm samples showed very similar patterns with nearly all compounds rising until the end of germination. In the embryos a maximum concentration of compounds was reached after 72–96 h of malting. Most concentrations decreased afterwards. The kilning step, namely the drying and roasting of germinated seeds, induced variable effects of increases, stability or decreases of metabolites and thereby separated kilned samples from germinated seeds in the PCA. A second barley cultivar (*Quench*) underwent the same malting and analysis procedures and gave nearly identical results.

Fructose, malate, *myo*-inositol and raffinose exhibited the potential to serve as markers for specific developmental stages of seeds in both varieties. Biological markers represent targets for industrial process control. Their potential application would meet the maltsters' demand to flatten variances in germination properties and to produce equal composed malt by directed malting management.

1. Introduction

Malt plays an important role for the brewing industry since it is the main ingredient of beer. Malt quality and homogeneity is essential for optimal and constant brewing performance. The annual global malt production actually ranges from 18 to 22 million tons. 94% of the malt served for beer production and most of it was made from barley (*Hordeum vulgare*) (Ullrich, 2011).

Beside the fermentation of milk, malting is one of the oldest biotechnological processes. Starch and proteins from barley seeds cannot be used by the brewing yeast directly. Therefore these polymers have to be degraded prior to fermentation, which is achieved by malt derived hydrolytic enzymes during mashing. During malting the nascent barley embryo triggers the induction of these enzymes. In contrast to an ordinary germination, the growth of the embryo is controlled in order to obtain optimal enzymatic action but avoiding the unnecessary growth of the seedling. The afterwards roasting of the germinated seeds conduces to stop further germination, to produce flavour compounds and to define the colour of the malt. Malt production involves three steps: steeping in water serves moistures seeds and induces the germination. The later germination part is carried out for several days at temperatures around 15 °C and is stopped by kilning with temperatures around 80 °C or higher – depending on the kind of malt required (Fig. 1).

The raw barley seed contains the endosperm as storage tissue with the surrounding aleurone layer. The dormant embryo makes up approximately 3% of the seeds dry weight and is separated from the endosperm by the scutellum. During steeping, the embryos' dormancy is broken. Upon signal transduction via phytohormones, proteolytic and amylolytic enzymes are released from the aleurone layer (Schoonheim et al., 2009). These enzymes degrade starch, storage proteins and cell walls in the endosperm. The products of this hydrolysis pass the scutellum, are taken up by the embryo, and used for growth and respiration. The biotechnology of malting uses these natural germination events to provide favourable mashing material. Intact enzymes, starch availability and viability of the seeds are the main criteria for malt quality concerning brewing houses. Therefore the aim of malting is to get the optimum point of maximal enzymatic induction without losing too much energy by embryo metabolism and growth.

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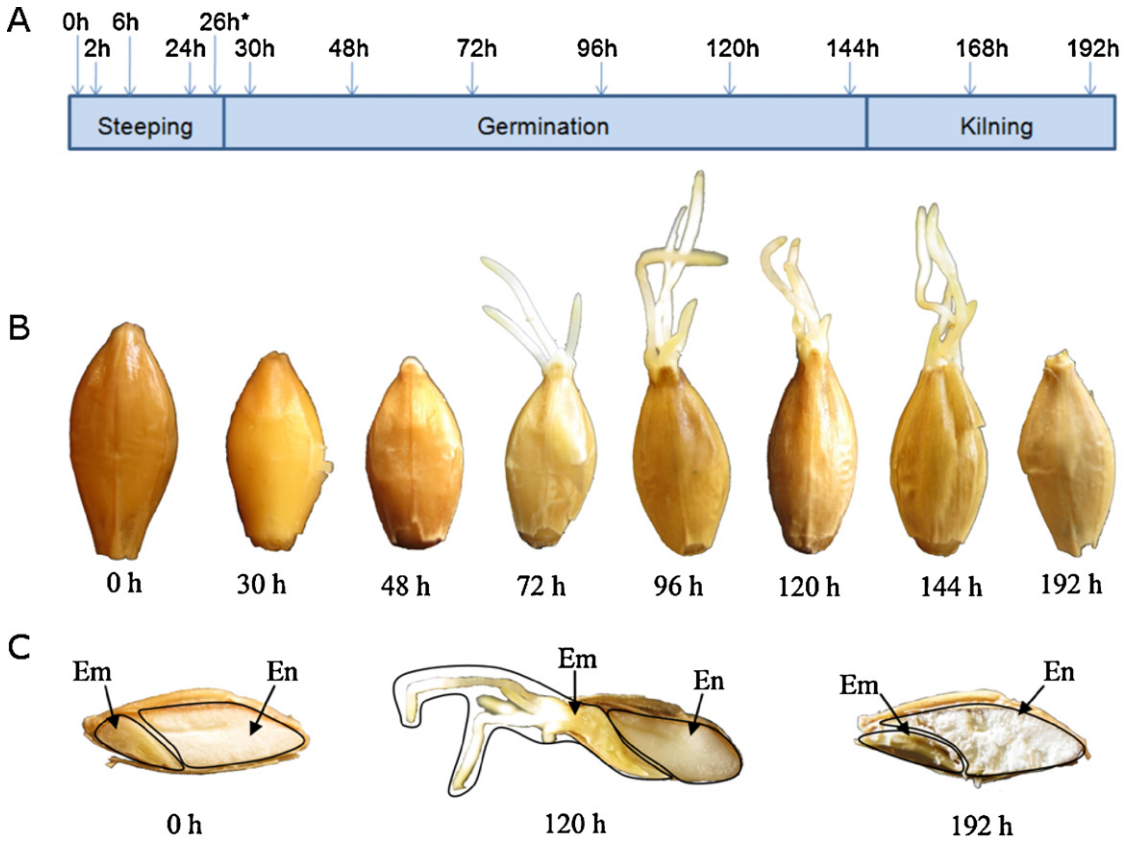

Fig. 1. Time points of sampling and optical impressions of seeds during malting of *Braemar* barley. (A) Schematic time scale of malting with sampling time points of *Braemar* and *Quench*; Sample 26 h* was left out in *Quench* analysis. (B) *Braemar* barley whole seeds during germination and after kilning. (C) Sections of ungerminated *Braemar* barley, after 120 h of germination and of ready malt. Numbers indicate sampling time point in hours after first water contact. Em, embryo; En, endosperm.

Problems in malting management occur because of the heterogeneous starting material. The raw barley seeds exhibit natural variability for example in germination power. This item is especially important for malting. Although at each delivery of barley to the industry all charges were checked for their germination power, variances remain and result in more or less different malt qualities. Until now, process control and adaptations depend mainly on the maltsters' experience. Standardized malt quality and composition would be desirable for breweries to assure reproducible brewing performance. To achieve this, molecular markers for malt quality and germination time points could facilitate malting management and the evaluation of end products.

Micro malting is the first choice to screen various barley cultivars for their malting properties under laboratory conditions. Little material is needed (kilograms or less) and malting conditions are well controlled, reproducible and adaptable. Frank et al. (2011) published the metabolome analysis of barley whole seeds ("*Maltasia*") during micro malting. Compared to micro malting, the industry deals with several hundred tons of barley. Delayed turnover, temperature adaptation, suboptimal oxygen support and other problems occur and could result in a different malt quality than estimated by the micro malting approaches. Most studies on biological events during malting were restricted to few compounds like special proteins or metabolite subsets (Narziss and Back, 2005). Gas chromatography–mass spectrometry (GC–MS) provides an ideal platform to screen many compounds simultaneously. Limitations depend on the extraction method and on polarity of compounds and their mass. Considering the aspects of energy lost to the embryo and of sugar and amino acid release in the endosperm during malting, water soluble metabolites were targeted by a hydrophilic extraction and derivatization. With the help of bioinformatic tools like the MeltDB-software (Neuweger et al., 2008) known and unknown compounds (TAGs) can be annotated and relative abundances monitored.

In this study industrial malting samples of two 300 tons productions were analysed. Additionally analysis of fractioned seeds was applied with seeds dissected into the embryo with attached scutellum and the endosperm with attached aleurone layer. This allowed a detailed insight into distributions of water soluble small compounds. Considering the problem of inhomogeneous starting material and the resulting diverse malt composition, known and unknown metabolites were reviewed for their marker potential. These markers define the biological germination progress. Industrial application could include fast quantification tests for germination or quality markers during ongoing malting. Based on these indicators further process modification and control measures could be applied. This would give the opportunity to produce equal composed high quality malt and enhance efficiency by guiding the process to the maximum margin.

2. Materials and methods

2.1. Malting

300 tons of barley seeds (varieties *Braemar* and *Quench*, malted separately) were steeped for two hours in water and air rest applied for one day (15 °C). After a second steeping for one hour, germination was carried out for five days at 15 °C in a special germination device (500 tons capacity). Kiln-drying (60 °C, upper floor) and kilning (80 °C, lower floor) were applied both for 24 h each on a two floor kiln. 12 (*Quench*) or 13 (*Braemar*) time points were sampled as follows: four (*Quench*) or five times (*Braemar*) during steeping