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Proteomic analysis of differences in barley (*Hordeum vulgare*) malts with distinct filterability by DIGE☆

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ABSTRACT

Filterability is an essential quality parameter of barley malt and significantly impacts productive efficiency and quality of beer. In the study, differences of metabolic capability, rather than of initial contents of macromolecules in barleys, were found to be the main reason for malt filterability gap between the widely used cultivars Dan'er and Metcalfe in China. Comparative proteomics based on fluorescent difference gel electrophoresis (DIGE) was employed to quantitatively analyze proteins of four commercial malts belonging to the two cultivars, and 51 cultivar-differential spots were identified to 40 metabolic proteins by MALDI-TOF/TOF mass spectrometry, mainly including hydrolases and pathogen-related proteins. According to their function analysis and abundance comparison between cultivars, filterability-beneficial and -adverse proteins were putatively proposed. Two most remarkable differential proteins, β-amylase and serpin Z7, were further investigated to verify their effects on Dan'er malt filterability. These results provide biological markers for barley breeders and maltsters to improve malt filterability.

Biological significance
To the best of our knowledge, this is the first report of comprehensive investigation of metabolic proteins related to wort filterability of barley malts, and sheds light on clues for filterability improvement. Visible differences in the expression level of metabolic proteins between Dan'er and Metcalfe malts using 2D-DIGE signify a valuable tool for cultivar comparison, illustration of key proteins responsible for filterability and even other qualities of barley malts. And with these explorations on biomarkers of malt filterability and other aspects, there will be higher efficiency and quality of beer brewing, less application of exogenous hydrolases and more expending market for Chinese malting barleys.
This article is part of a Special Issue entitled: Translational Plant Proteomics.

1. Introduction

Malt derived from germinated barley (*Hordeum vulgare*) is a basic ingredient for beer brewing. Through the sequential processes of malting and mashing, endogenous enzymes synthesized or activated in barley germination stage, catalyze the degradation of macromolecules, mainly including cell wall polysaccharides, starch and proteins into maltoses, amino acids and others. At

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the end of mashing, the mash is filtered to remove spent grains from wort (or malt extract solution), which contains micromolecular nutrients for brewer's yeast fermentation. Filterability, including wort separation rate, viscosity and clarity, is an important aspect of malt quality and plays a significant role in the efficiency of beer production and even the quality of beer. Wort with high viscosity and low separation rate would delay the production process, and turbid wort may lead to the filter membrane blocking and the beer haze [1,2].

For the pronounced defects in wort filterability of the domestic malts produced from some cultivars grown in the downstream area of Yangtze River in China, their use in beer brewing was limited [3]. To overcome this deficiency, addition of exogenous hydrolases during malting and mashing processes of these malts, or mixing them with imported barley malts of superior filterability was prevalently adopted [4]. However, this increases production cost and cannot go on for long.

Historically, certain macromolecules without complete degradation, including β-glucan, arabinoxylan (AX) and prolamine, were considered as the direct effectors of wort filterability [2,5]. The incomplete degradation of these macromolecules was attributed to complex reasons, such as low expression levels of endogenous hydrolases, differential expression of isozymes among cultivars, affections of enzyme regulators, and even the excessive contents of macromolecules in barleys [6–10]. It has also been proved that oxidative cross-linking of polyphenols with proteins and polysaccharides acted unfavorably on the wort clarity [11,12]. Due to its complexity, no attempt to systematically characterize filterability-related metabolic proteins in the barley malt has been carried out, except of our previous proteomic study on barley malts of cultivars Gangpi and Baudin with distinct malt quality, including filterability [13]. Differential 2DE profiles were observed between the two cultivars, and some differentially-expressed metabolic proteins such as amylases, pathogen-related proteins and enzymes involving in glycolysis, were identified.

In the present study, we have sought to investigate metabolic proteins responsible for filterability using 2D-DIGE, and tried to gain an understanding of interactions among them. Malts of cultivars Dan'er and Metcalfe with distinct filterability, were selected for this systematical proteomic study. Dan'er is widely grown in Jiangsu Province which is one of the top three malting barley grown areas in China and located in the lower reach of Yangtze River. However, the filterability of malts from Dan'er barleys is generally unsatisfactory by domestic maltsters and brewers [14], in contrast to the superior filterability of the imported Canadian barley cultivar Metcalfe [15,16]. Proteomic analysis followed by some key metabolic proteins verification, in combination with the constitution determination of macromolecules in barleys and malts, was employed to ferret out the differentially expressed proteins responsible for malt filterability deficiency of Dan'er and even other domestic cultivars.

2. Materials and methods

2.1. *Commercial malt samples*

Dan'er malt I and Metcalfe malt I were provided by a Chinese commercial malting company, and malts II of both cultivars were from another one. The malting conditions I and II for both cultivars were listed in Table 1. Barley of cultivar Dan'er (winter barley of two-row regular-hulled), released in 1997and widely grown in Jiangsu Province of China by now [17], was harvested in May 2011 in Jiangsu. And barley of Metcalfe cultivar (spring barley of two-row regular-hulled) received full registration in 1997 [18], then extensively adapted to western Canada, and widely used in Chinese breweries since about 2000 [16], was harvested in November 2011 in Western Canada. Malt samples were stored at 4 °C following receipt.

2.2. *Measurement of malt quality*

The European Brewery Convention (EBC) Congress mash was carried out, and the malt quality parameters, including filterability that represented by separation rate, viscosity and clarity of the Congress wort, were measured by EBC official analytical methods [19]. Specially, separation rate was represented by wort volume filtered for 30 min after recirculation of the initial turbid wort, viscosity was determined at 20 °C by a falling ball viscometer (Hoppler, Germany) and turbidity was measured according the 90° scattering method on an EBC turbimeter.

Table 1 – Malting conditions for barleys of cultivars Dan'er and Metcalfe.

Malting conditions	Malting condition I [a]		Malting condition II [a]	
Cultivars	Dan'er	Metcalfe	Dan'er	Metcalfe
Steeping temperature (°C)	17	17	16	16
Ex-steep moisture (%)	43	45	42	43
Germination times (h)	108 ± 3	84 ± 3	120 ± 2	96 ± 2
Germination temperature (°C)	17 °C and 18 °C for the last day	17 °C and 18 °C for the last day	16 °C and 17 °C for the last day	16 °C and 17 °C for the last day
The lowest moisture during germination (%)	46	46	46–47	46–47
Kilning procedures	45 °C for 1 h, 50 °C for 1 h, 55 °C for 1 h, 60 °C for 4 h, 65 °C for 5 h, 68 °C for 1 h, 70 °C for 1 h, 77 °C for 1 h, 85 °C for 3 h		45 °C for 8 h, 65 °C for 5 h, rising from 65 °C to 85 °C for 4 h, 85 °C for 3 h	
Equipments	Malting box		Malting tower	

[a] In the same malting company, the malting condition was controlled by the same maltster and equipment, and slightly optimized according to the specific malting quality (such as water sensitivity and germination capacity) for each cultivar.