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# Beer proteomics analysis for beer quality control and malting barley breeding

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ABSTRACT

A series of proteomic procedures has been applied to analyze beer and wort proteomes including two-dimensional gel electrophoresis combined with mass spectrometry (MS), pre-fractionation followed by gel electrophoresis and MS, and gel-free based shotgun liquid chromatography–MS/MS. These approaches have detected a number of protein species in beer and wort, from barley and yeast, and occasionally from rice and maize. Of these beer proteins, barley dimeric alpha-amylase inhibitor-1 (BDAI-1), barley trypsin inhibitor-CMe precursor (BTI-CMe) and yeast thioredoxin have been identified as factors responsible for beer quality traits of foam and haze. In addition, a number of hordein derived polypeptides involved to celiac disease have also been identified. Among these proteins and peptides, the modifications such as glycation and partial digestion in several proteins such as lipid transfer protein (LTP) and protein Z have been characterized. These findings may provide novel tools to improve quality control during beer processing and they may enhance selection in malting barley breeding.



## 1. Introduction

Beer is one of the oldest and the most widely consumed alcoholic beverages throughout the world. A method for beer production was written on a clay tablet by Sumerians in 3000 B.C., which described the production of "beer bread" by mixing and baking barley flour with water, then adding water to the "beer bread." Subsequently, a primitive type of beer was produced by alcoholic fermentation. The Babylonians sometimes added herbs to their beer, including wild hops. The methods and recipes have been improved over time. At present, beer is produced by raw materials such as barley malt, hops and adjuncts such as barley grain, rice, and corn.

Barley (*Hordeum vulgare*) is grown all over the world, except the tropics, and 123 million tons of barley grain were produced in 2009 (http://faostat.fao.org/). Other than malting and brewing, barley is utilized as food and animal feed. Omics studies of barley such as genomics, transcriptomics, and proteomics have been conducted extensively because barley is a model genome for Triticeae, which includes wheat, rye, and triticale (Finnie & Svensson, 2009; Sreenivasulu, Graner, & Wobus, 2008; Sreenivasulu et al., 2008b). Although barley has a large diploid genome of 5 giga bases, majority of the barley genome sequence has been published (International Barley Sequencing Consortium, 2012). These data sets have highly impact on these omics analysis of barley.

Beer production process starts by malting the barley grain, which is followed by mashing, wort boiling, fermentation, and maturation. During malting, the water content of the barley grain is approximately 38%–45% after steeping. Subsequently, the grains germinate within 4–6 days. During these steps, several enzymes are activated including protease, amylase, and β-glucanase, and some proteins are modified. The green malt is then kiln treated. The temperature of kilning depends on the malt type, e.g., pilsner (approximately 80 °C), caramel (approximately 140 °C), or black malts (>200 °C), depending on their level of Maillard reaction. During mashing, the ground malt is mixed with water, and sometimes enzymes such as β-glucanase, amylase, and pullanase may be added at a controlled temperature to make the wort. Mashing has two main steps, i.e., protein rest and saccharification rest. During protein rest (45 °C–60 °C), proteins are modified and degraded by malt proteases into amino acids and low molecular weight peptides, which provides a nitrogen source for the brewing yeast. During saccharification rest (approximately 65 °C), the starch is degraded into maltose by β-amylase to provide carbon sources for the brewing yeast. The optimal temperature for β-amylase activity is 55 °C–63 °C and the thermostability of β-amylase influences its enzymatic activity. Different levels of thermostability are known among barley cultivars (Kihara, Kaneko, & Ito, 1998). The maltose content directly affects fermentability; therefore, cultivars with higher β-amylase thermostability may have higher fermentability. The "mashing-in temperature" affects several quality traits such as the foam stability and fermentability because the degree of protein degradation depends on the "mashing-in temperature." After filtration, the wort is boiled. The main objectives of wort boiling are protein coagulation, deactivation of the malt enzymes, and extraction of the hop components. During this step, some proteins are coagulated, denatured, and modified. The wort boiling temperature may affect the beer foam stability (van Nierop, Evans, Axcell, Cantrell, & Rautenbach, 2004). During fermentation,

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alcohol is produced by brewing yeast and some yeast proteins are released into the beer. During the steps used to process beer, the protein profiles change greatly in terms of degradation, coagulation, and modification including partial digestion, glycation, and acylation (Fig. 1).

Proteome analysis has been applied in a number of food investigations. This method initially used two-dimensional gel electrophoresis (2DE) and/or liquid chromatography (LC) combined with mass spectrometry (MS). Recent high sensitivity MS techniques such as matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) have accelerated the use of proteome analysis to various organisms and foods including beer. During the analysis, the protein and nucleotide sequences of target genes are of primary importance for identifying protein species. From this viewpoint, genomics research has promoted the application of proteomics in food science. During beer proteome analyses, several proteins have been identified that are related to beer quality traits. These results may be applied to beer quality assurance, quality control during malting and brewing processes, and malting barley breeding.

In this review, we discuss recent results achieved by beer proteomics analysis. Advances in barley genomics, which is the basis of beer, wort, malt, and barley proteomics, are also summarized.

## 2. Beer proteomics

One-dimensional (1D) analyses such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), lab-on-a-chip, high performance liquid chromatography (HPLC), and fast protein liquid chromatography (FPLC) are useful for analyzing the protein profiles of samples. These methods have been used for beer protein analysis (Evans et al., 2003; Klose, Thiele, & Arendt, 2010; Silva et al., 2008). Beer protein profiles appear to be simple in SDS–PAGE images, where two major bands are observed at approximately 40 kDa and 10 kDa. These protein bands are known as protein Z and LTP1, respectively. Thus, it is well known that these two proteins are abundant in beer. In some cases, beer proteome has been analyzed by protein separation using 1D gel electrophoresis. Hao et al. (2006) separated beer and foam proteins by 1D SDS–PAGE and identified the protein species by capillary liquid chromatography–electrospray ionization–ion trap MS (LC ESI–IT–MS). In total, they identified 21 and 24 proteins in beer and beer foam, respectively, based on database entries. Although this study identified a substantial number of proteins, the resolution of 1D gel electrophoresis was insufficient to compare beer protein profiles.

Earlier beer 2DE analyses have been reported in several papers (Gorinstein et al., 1999; Marshall & Williams, 1987; Williams & Marshall, 1995). However, the protein species detected in the spots on 2DE gel were not identified until recently. Advances in MS techniques and protein/nucleotide sequence databases make it possible to identify the protein species in beer and wort. Beer and wort proteomes have been analyzed using mainly three methods, i.e., SDS–PAGE or 2DE-based proteome analysis, the same analysis with pre-fractionation, and gel-free shotgun proteome analysis using HPLC–MS/MS. These proteome analyses have shown that a number of proteins derived from barley and brewing yeast are present in beer.

Perrocheau, Rogniaux, Boivin, and Marion (2005) analyzed beer proteins by 2DE with a p*I* range of 3–10 and identified a total of 30 protein spots using nanoscale capillary liquid chromatography combined with hybrid quadrupole orthogonal acceleration time of flight MS (Q-TOF MS). Iimure et al. (2010) separated beer proteins by 2DE with p*I* ranges of 4–7 and 6–9, and identified proteins by peptide mass finger printing (PMF) using MALD-TOF MS. Of the 199 spots, 85 were identified and categorized into 12 protein species. They proposed this comprehensive 2DE protein profile to call as a proteome map. They also used this beer proteome map to compare the beer protein profiles among barley cultivars under different levels of malt modification and found that the cultivar difference and the level of malt modification affected the profiles of foam-, haze-related proteins and yeast-derived proteins. Konečná et al. (2012) identified more beer proteins by 2DE and MS analysis. They separated beer proteins by 2DE and detected over 300 protein spots, and identified 52 proteins in beer using MALDI–MS/MS and LC–MS/MS, which were derived mostly from barley (20 proteins) and yeast (25 proteins). In addition, they compared the 2DE images of four beer samples from Czech lager beer and imported beer, and indicated that several yeast-derived proteins such as enolase, glyceraldehydes-3-phospahte dehydrogenase, and coproporphyrinogen III oxidase were absent from Czech lager beer. To make wort proteome map, Iimure, Nankaku, Kihara, Yamada, and Sato (2012) detected 202 wort protein spots by 2DE and identified 63 spots which were categorized into 20 protein species by PMF using MALDI–TOF MS. They compared the protein profiles of sweet wort, boiled wort, and trub, and suggested that the degree of coagulation and precipitation caused by proteins depended on different protein species. Jin et al. (2009) investigated changes in the physicochemical properties and structure of proteins from two barley cultivars during wort boiling by SDS–PAGE, 2DE, differential scanning calorimetry, gel filtration chromatography, and circular dichroism spectroscopy. They concluded that changes in the protein structure caused by wort boiling were largely independent of the malting cultivar. These 2DE-based proteome analyses have the potential to compare beer and wort protein profiles of different samples, as demonstrated by Iimure et al. (2010), Klose et al. (2010) and Konečná et al. (2012).

Pre-fractionation, i.e., fractionation prior to gel electrophoresis, is a powerful method for detecting minor beer protein components. Fasoli et al. (2010) successfully identified minor proteins, particularly those derived from yeast, by prior capture with combinatorial peptide ligand libraries (CPLL) (ProteoMiner and a homemade library with reduced polydispersity) at three different pH levels. CPLL technology has been applied to several beverages other than beer to detect low to very-low abundance proteins or polypeptides (D'Amato, Fasoli, Kravchuk, & Righetti, 2011; Fasoli, D'Amato, Kravchuk, Citterio, & Righetti, 2011). Fasoli et al. (2010) reported that CPLL treatments can detect minute traces of casein in white wine with a 50,000 times higher sensitivity than MS methods. CPLL consists of a library of hexapeptides, which bind with specific proteins or polypeptides. When a beer is treated with CPLL, abundant proteins such as protein Z and LTP1 are saturated

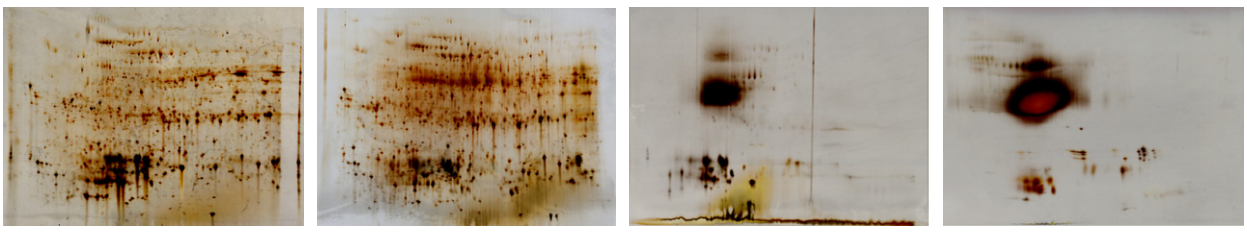


**Fig. 1.** The proteome changes observed by two-dimensional gel electrophoresis (2DE) from barley grain to beer. The p*I* range of 4–7 in first dimensional gel electrophoresis were used for the analyses of barley and malt, and that of 3–10 were used for the analyses of wort and beer.