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# Electrophoretic and HPLC methods for comparative study of the protein fractions of malts, worts and beers produced from Scarlett and Prestige barley (*Hordeum vulgare* L.) varieties

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**Abstract**

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reversed-phase high performance liquid chromatography (RP-HPLC) methods were used for studying the protein fractions (hordeins; albumins and other soluble proteins) of Scarlett and Prestige barley malts and to follow changes of the protein profile of worts and beers from these two malt varieties. Similar industrial brewing conditions were applied for both varieties.

Statistical analyses of RP-HPLC data showed that hordeins were exposed to a proteolytic process during germination, which reduced its content and originated less hydrophobic peptides. In contrast, albumins and other soluble proteins increased during the germination process. Some malt water-soluble proteins result from the hordein proteolysis. Quantitative differences were observed between the protein fractions of the two malt varieties.

SDS-PAGE patterns indicate that most of the components present in the worts were also detectable in final beers. However, chemometric analysis of the HPLC data showed quantitative differences between Scarlett and Prestige worts quantitative protein profiles. Scarlett wort contained more protein than Prestige worts. However, final beer samples presented a quantitative protein profile more similar than the respective worts.

The optimized methodologies can be successfully used to compare the protein fractions of malts produced from two barley varieties, to follow the evolution of protein fraction during germination and the evolution of protein fraction content of worts and beers.


*Keywords:* *Hordeum vulgare* L.; Malt hordeins; Malt albumins; RP-HPLC; SDS-PAGE; Wort; Beer

## 1. Introduction

Barley is the most important raw material for beer production. It is a widely grown cereal crop, used for human and animal feed and for brewing, due to the high enzymatic content, that is conversion of starch into fermentable sugars and being a cereal with a husk that protects the embryo during the handling of the grain and is an important aid during the wort filtration. In fact, the aim of the malting process is the production and activation of enzymes. These molecules also contribute to the hydrolysis of β-glucans and hordeins (water insoluble proteins), which would otherwise restrict access of enzymes to the starch granules (Hughes & Baxter, 2001).

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Barley grain germination is initiated by the uptake of water. The grain imbibes water during controlled cycles of water spraying, or water immersion, followed by aeration, until the water content of the grain reaches 42–48%. Water enters the grain via the embryo, and after approximately 24 h, the first visible sign of germination is the appearance of the root, as a white 'chit'. Germination is typically allowed to proceed over a period of around 5 days to obtain green malt, which is then stopped during the Dewatering and Kilning phase by forced flow of hot air. Hydrolases produced during malting are partially inactivated during this process. The malt is stable for storage and has a friable texture suitable for the milling process, which precedes brewing.

Breeders are involved in the development of high-quality value barley cultivars for the malting and brewing industries. Barley selected for use in the brewing industry must meet special quality requirements and be must be approved for malting and beer production. The malt quality of a given barley variety is determined by its genetic background and the physical conditions during growth, harvest and storage. The validation process for brewing includes trials in micro and pilot malting and brewing plants before introduction at production scale (Østergaard, Melchior, Roepstorff, & Svensson, 2002).

Proteins are among the barley components that are essential for the quality of malt and beer. Thus, the total protein quantity in the barley grain is a crucial factor for final beer quality. High protein contents decrease available carbohydrates, with a negative influence on the brewing process (Fox, Onley-Watson, & Osman, 2002; Peltonen, Rita, Aikasalo, & Home, 1994). The protein content in barley grains represents, approximately, 8–15% of its total mass. Hordeins are the most abundant proteins (40–50%) found in a barley grain (Osman et al., 2002). In addition to hordeins, other proteins have been identified, including albumins, glutelins (globulins), friabilin, enzymes, serpins and other inhibitors, chaperones and other proteins with unknown functions (Borén, Larsson, Falk, & Jansson, 2004; Finnie, Melchior, Roepstorff, & Svensson, 2002; Fox et al., 2002; Osman, Coverdale, Onley-Watson, Bell, & Healy, 2003; Østergaard, Finnie, Laugesen, & Roepstorff, 2004; Østergaard et al., 2002).

Hordeins, as the main storage protein fraction in barley seeds, accounts for up to half of the total protein in the mature grains, and may be classified into four groups named B, C, D and γ hordeins based on their electrophoretic mobilities. The B (30–45 kDa) and C (45–75 kDa) fractions account for ~70–80% and ~10–12%, respectively, of the total hordeins, while the D and γ fractions are minor components. Hordeins exist both in monomeric and aggregated forms. (Brennan, Smith, Harris, & Shewry, 1998; Fox et al., 2002; Lookhart, Bean, & Jones, 1999; Molina-Cano et al., 2001; Peltonen et al., 1994; Schmitt, Gille, Gaucher, & Montembault, 1989; Shewry, Kreis, Parmar, Lew, & Kasarda, 1985).

Information about albumins, found in the literature, is mainly related to protein Z and lipid transfer protein 1 (LTP1). Protein Z is a 40 kDa hydrophobic glycoprotein, with an isoelectric point of 5.5–5.8 (barley form) or 5.1–5.4 (beer form) (Curioni, Pressi, Furegon, & Peruffo, 1995; Hejgaard & Kaersgaard, 1983; Hejgaard, 1982; Leiper, Stewart, & McKeown, 2003; Lusk, Cronan, Chicoye, & Goldstein, 1987; Sørensen, Bech, Muldbjerg, Beenfeldt, & Breddam, 1993; Yokoi, Maeda, Xiao, Kamada, & Kamimura, 1989). In barley and malt, protein Z can be found in two isoforms: Protein Z4 (80%) and Z7 (20%) (Evans, Nischwitz, Stewart, Cole, & MacLeod, 1999). On the other hand, LTP1, which was initially named probable amylase/protease inhibitor (PAPI) (Leiper et al., 2003), is a 9.7 kDa glycoprotein, with an isoelectric point of 9 (Jégou, Douliez, Mollé, Boivin, & Marion, 2000; Jones & Marinac, 1997; Vaag, Bech, Cameron-Mills, & Svendsen, 1999). LTP1 contains 91 amino acid residues (Lindorff-Larsen & Winter, 2001), organized into four α-helix segments, which are stabilized by four disulphide bonds (Bech, Vaag, Heinemann, & Breddam, 1995).

The majority of beer protein lies in the 10–40 kDa size range (Leiper et al., 2003). Mostly, the origin of this protein is malted barley (Hughes & Baxter, 2001). Some beer proteins appear to have no function in beer except their contribution to mouthfeel, flavor, texture, body, color, and nutritional value (Leiper et al., 2003; Osman et al., 2003). Protein Z, LTP1, and other proteins present in beer have been associated to foam formation and/or stabilization (Evans & Sheehan, 2002; Ferreira, Jorge, Nogueira, Silva, & Trugo, 2005; Lusk, Goldstein, & Ryder, 1995; Nierop, Evans, Axcell, Cantrell, & Rautenbach, 2004; Perrocheau, Rogniaux, Boivin, & Marion, 2005). Protein Z has also been related to beer haze (Curioni et al., 1995).

Scarlett and Prestige barleys were two rowed spring varieties. Scarlett has very good brewing quality and is considered a standard variety, by the European Brewery Convention (EBC). Presently, Prestige is also proposed as standard variety for some regions, it presents good agronomic characteristics, including high plague resistance. A comparative study of the protein fractions (hordeins; albumins and other soluble proteins) of Scarlett and Prestige malts is important to contribute to the characterization of these barley varieties. Chromatographic (Osman et al., 2003; Schmitt et al., 1989) and electrophoretic (Echart-Almeida & Cavalli-Molina, 2001; Leisegang & Stahl, 2005; Molina-Cano et al., 2001; Villiers & Laubscher, 1989) techniques are frequently used to study malt proteins, thus, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reversed-phase high performance liquid chromatography (RP-HPLC) techniques were chosen for comparison of malt proteins from Scarlett and Prestige varieties. Studies were performed for two different germination times (60 and 120 h).

In a posterior phase, both malts were used to produce beer. Similar industrial brewing conditions were separately applied to Scarlett and Prestige malts. Sodium dodecylsul-