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How various malt endoproteinase classes affect wort soluble protein levels

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Abstract

During the germination of seeds, storage proteins are degraded and the resulting amino acids are utilized by the growing seedling. In barley, this process is commercially important because it forms the basis for the malting and brewing industries. In this study, barleys and malts were mashed in the presence of compounds that specifically inhibited the four common proteinase classes. The efficacies of the proteinases in solubilizing proteins were in the order cysteine ≈ metallo > aspartic > serine ≈ 0, which roughly reflected how the inhibitors affected the mash endoproteolytic activities. It was previously believed that only the cysteine enzymes were involved. All four enzyme classes affected the free amino nitrogen concentration but none altered any of the other measured wort characteristics. With either single inhibitors or inhibitor mixtures, the effect of pH was as expected, based on earlier studies that indicated that cysteine and aspartic proteinases were most active at low pH values and the metalloproteinases were only active at high pH. At the North American commercial mashing pH of 6.0, about one third of the soluble protein of a typical wort came from ungerminated barley, half was solubilized during malting and the remaining 22% was released during mashing.
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Keywords: Hordeum vulgare; Barley; Proteinases; Soluble protein; Free amino nitrogen; Malting; Brewing; Germination

1. Introduction

During malting and the mashing phase of brewing, a portion of the insoluble proteins of barley must be converted into 'soluble protein' (SP) if good brews are to be obtained. This SP fraction comprises a mixture of amino acids, peptides and dissolved proteins, and a major portion of it arises by proteolysis of barley proteins. To more efficiently produce malting barleys that have improved SP quality and to develop more effective malting and brewing methods, we need to ascertain which proteolytic enzymes are involved and how they operate. The same processes presumably occur during naturally occurring seed germination. Several researchers have shown that the endoproteinases, not the exoproteinases, are the rate limiting enzymes for the formation of soluble protein (Burger and Schoeder, 1976b; Sopanen et al., 1980). These, then, are the enzymes whose activities will need to be altered to vary the SP levels in the final brewing worts.

Until recently, it was thought that only a few endoproteinases were active during mashing and malting, but we have detected at least 40 different endoproteinases in green malt using a two-dimensional isoelectric focusing×polyacrylamide gel electrophoresis method (Zhang and Jones, 1995a). It was also thought that only the cysteine class of endoproteinases were involved. This seems unlikely, now that it has been shown that multiple enzymes belonging to each of the four classical protease classes are present in green malt (Zhang and Jones, 1995a). There is little or no inactivation of these enzymes during malt kilning (Jones et al., 2000) or the protein rest phase of mashing (Jones and Marinac, 2002) so all have considerable opportunity to hydrolyse the storage proteins and other proteins of the barley.

This study was aimed at determining which of the kilned malt endoproteinase classes were, in fact, involved in solubilizing protein during the mashing phase of brewing

Abbreviations: ASBC, American Society of Brewing Chemists; FAN, free amino nitrogen; *o*-phen, 1,10-phenantholine; HGASBC, high gravity ASBC; PMSF, phenylmethylsulfonyl fluoride; E-64, *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane; SP, soluble protein.

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and to measure what proportion of the SP and free amino nitrogen (FAN) fractions of worts were released during the malting and mashing processes. Compounds that specifically inhibited members of the various classes of proteases were added to mashes. Mashes were made at pH 3.8, 6.0 and 8.0, because it has been shown that there are two groups of proteinases in malt that have widely differing optimal pH values (Zhang and Jones, 1995a) so that by comparing the results obtained with the low and high pH mashes it was possible to deduce which of the enzyme groups was catalyzing the observed protein hydrolyses.

2. Experimental

2.1. Preparation of kilned malt

Seeds (170 g, dry basis), cleaned to remove seeds not retained on a 5/64 in screen, of both Morex and Harrington barleys (the six- and two-rowed American Malting Barley Association malting quality standards, respectively) were steeped at 16 °C for 36 h, to 45% moisture, with four 4-h couchings. The steeped seeds were germinated in the dark, with intermittent (3 min each 30 min) rotation at 17 °C and near 100% humidity, for 5 d. The resulting 'green malt' was kilned to around 4% moisture using a schedule (Jones et al., 2000) that started at 49 °C (10 h), finished at 85 °C (3 h) and that conformed closely to US industry practices. The malt samples produced were stored at room temperature until mashed.

2.2. Mashing

The terms 'mash' and 'extract' are used interchangeably in this paper, because they both refer to essentially the same process; taking a sample of malt or barley and putting it though an aqueous extraction process to obtain a solution of soluble molecules that can be measured. The extraction method applied to the ungerminated barley and ASBC samples would normally result in an 'extract' sample, and it differs somewhat from that used for the 'commercial' samples, whose resultant solution would be called 'wort'.

2.2.1. ASBC 'congress' or 'ASBC' mashes

Fine-grind malt or barley samples were prepared using a Miag laboratory cone mill adjusted as specified in the ASBC Malt-4 (American Society of Brewing Chemists, 1992) method. These samples were extracted according to the ASBC Methods, Malt-4 procedure, except that all weights and volumes specified for the method were halved.

2.2.2. High gravity ASBC congress or 'HGASBC' mashes

HGASBC malt mashes were made using the ASBC Malt-4 method, except that the initial malt concentrations in the extracts were increased from 25 g/100 ml to 60 g/200 ml. It would have been preferable to use a 60 g/100 ml mixture, which would have been closer to commercial mashing conditions, but such mixtures were too viscous to stir. To compensate for this, no water was added when the mash temperature reached 70 °C and the stirrers and containers were rinsed sparingly at the conclusion of mashing. By taking these precautions, the final extract volumes were reconstituted to exactly 200 ml, the normal volume. During extraction, these samples were 1.2 times as concentrated (25 g/100 ml vs 60 g/200 ml) as normal ASBC mashes. The high gravity filtrates that were analyzed were 2.4 (60 g malt vs 25 g/200 ml) times more concentrated than the traditional ASBC mash filtrates, which were diluted to 200 ml before filtration.

2.2.3. 'Commercial' mashes

Kilned malt was ground and mashed according to a schedule that generally conforms to US industry practices (Jones and Marinac, 2002). Since only the malt mash was of interest, no cooker (adjunct) mash was prepared. The malt mash was made by stirring 62 g of ground malt into 200 ml of 50 °C water and mixing it for 30 min at 50 °C. The temperature was then raised to 68 °C over 18 min and held at 68 °C for 30 min. It was then increased to 77 °C at 1.5 °C/min, maintained at 77 °C for 5 min, and cooled to room temperature over 15 min. The cooled samples were adjusted to 200 ml with water, mixed, and filtered though Ahlstrom fluted grade 509, 32 cm, filter paper. The resultant solutions were analyzed for their extract, SP and FAN values.

2.2.4. Adjusting the pH values of mashes

In experiments conducted at pH values that were either higher or lower than normal, the pH was adjusted downward by adding acetic acid or upwards with NaOH as described in Jones and Budde (2003)

2.3. Measuring the malt extract, SP, free amino nitrogen and (1→3,1→4)-β-glucan values

Variations of standard ASBC methods were used to measure the extract, SP, FAN and (1→3,1→4)-β-glucan levels of the various samples.

2.3.1. Extract

The densities of filtered mashes were measured with an Anton/Parr DMA 5000 density meter. The density data were used to calculate the amounts of soluble material present in the filtrates, and thus the percentage of the malt weight that had been dissolved (ASBC method Wort-2B) (American Society of Brewing Chemists, 1992).

2.3.2. Soluble protein

The SP levels of the worts were originally determined using two different methods. In the first, UV absorbance method (ASBC method Wort-17), the absorbances of the worts were measured at 215 and 225 nm and the differences in these absorbance values were used