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Effect of a milling pre-treatment on the enzymatic hydrolysis of carbohydrates in brewer's spent grain

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ABSTRACT

Millions of tonnes of brewer's spent grain (BSG) are annually produced worldwide as a by-product of the brewing industry. BSG has the potential to be a valuable source of food, chemicals and energy if cost-efficient fractionation methods can be developed. A 2-fold improvement in carbohydrate solubilisation could be achieved through the introduction of a milling step prior to enzymatic hydrolysis. Course and fine milled fractions were characterized by particle size distribution and light microscopy. Fine milling decreased particle size down to the micron level and this in turn improved the carbohydrate solubility yield by a multi-enzyme mixture from 23% up to 45%. Carbohydrate solubilisation could be further increased through the supplementation of this enzyme preparation with additional cellulases. The physical degradation caused by the milling also liberated soluble carbohydrates without the requirement of any enzymatic treatment.

1. Introduction

Brewer's spent grain (BSG) is the most abundant side stream generated by the brewing industry. BSG is the insoluble residue that is separated from the mash before fermentation. In 2010, 1.69 billion hl of beer from barley was produced worldwide (FAOSTAT, 2010). Based on current technologies, approximately 15–20 kg of BSG is produced per every hectolitre of beer, resulting in annual production of 30 million tonnes of BSG worldwide. Thus BSG is a universally abundant, low-cost material and available throughout the year. Current use has been limited mainly to low-cost ruminant feed. Nevertheless, as BSG is rich in arabinoxylan, cellulose, lignin and protein, it could provide important ingredients and precursors for the food, chemical and energy industries (Mussatto et al., 2006), if cost-efficient fractionation methods are developed.

There have been many reports over the last 10 years about the potential utilization of BSG. As almost all components of BSG could be used in more valuable ways than just animal feed, methods to isolate the individual components of BSG while retaining their functionality are required. Several studies on solely enzymatic solubilisation of BSG using carbohydrate and protein degrading enzymes have been already reported (Faulds et al., 2008; Forssell et al., 2008; Treimo et al., 2009). The protein in BSG is fairly easily degradable: up to 80% of protein can be removed from the insoluble biomass by a simple proteolytic treatment (Treimo et al., 2009). However, the carbohydrates are more resistant to enzymatic hydrolysis; the degree of solubilisation with one-step hydrolysis is only 28–30% (Forssell et al., 2008; Treimo et al., 2009). The resistance of BSG carbohydrates to hydrolytic enzymes is probably due to several factors, including inaccessibility of the carbohydrates to the enzymes caused by the cross-linking and substitution of the polymers in a complex, lignin-rich matrix. The xylan backbone in cereals is substituted with arabinose, xylose, galactose, glucuronyl and acetyl residues (Collins et al., 2010), which restrict the action of xylanase. Some of the arabinose residues are further substituted with ferulic acid either in mono or dimeric, and higher oligomeric forms (Bunzel, 2010). Ferulic acids form diferulate cross links between arabinoxylan molecules and also between arabinoxylan and lignin (Ralph et al., 1995), and such cross linking hinders the access of enzymes to the cell wall polysaccharides (Grabber et al., 1998). In addition, cellulase activity is inhibited by the presence of lignin through the non-specific binding of the enzyme to the polymer (Palonen et al., 2004), and this has been shown to negatively affect the hydrolysis of BSG (Mussatto et al., 2008).

Some attention has already been paid to the effects of possible pre-treatments of BSG prior to the enzymatic hydrolysis. Pre-treatments that decrease the particle size, open up the cell wall structures and reduce cellulose crystallinity make the biomass more accessible to enzymes and thus improve the digestibility (Hendriks and Zeeman, 2009). Previously, BSG has been pre-treated, for example, by coarse milling, using a 0.5 mm (Forssell et al., 2008; Beldman et al., 1987) or 1 mm (Treimo et al., 2009) sieve, but that

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