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Brewer spent yeast susceptibility to protein hydrolysis: Effect of serial repitching and yeast strain

Gabriela Vollet Marson, Ruann Janser Soares de Castro, Mariana Teixeira da Costa Machado and **Miriam Dupas Hubinger**
School of Food Engineering, Brazil

Many different yeast strains and cultivars are available for beer production because each one may result in a different flavor profile. They are chosen considering brewing conditions and beer style. Brewer spent yeast (BSY) is the second most relevant sub-product generated from the brewing industry but it is usually discarded or used as inexpensive animal feed. However, this material is rich in proteins and may be a source of bioactive peptides, which can be obtained through proteolytic treatment. The aim of this study was to investigate the susceptibility to hydrolysis of two commonly used yeast strains for Lager Pilsen production (*Saccharomyces cerevisiae* and *Saccharomyces pastorianus*) using Alcalase™ 2.4L, Protamex™ (Novozymes, Denmark) and a commercial protease mixture for yeast cell hydrolysis, Brauzyn® 100L (Prozyn, Brazil). Three samples of brewer spent yeast from Lager Pilsen beer production were collected after 11 days of maturation: Repitched *Saccharomyces pastorianus* (RSP), non-repitched *Saccharomyces pastorianus* (NSP) and non-repitched *Saccharomyces cerevisiae* (NSC). Firstly, protease activity of the commercial proteases was determined for each studied condition using azocasein as substrate. Then, the effect of serial repitching (no repitching and 5 times repitching) and yeast strain on the degree of hydrolysis (DH*) with those three enzymes was studied, at the same hydrolysis conditions, using an automatic titrator. Protease activity results show that maximum Brauzyn® activity was achieved at low pH (5.6) and high temperature (74°C), but this enzyme showed 17 and 2 times less protease activity per mL when compared to Alcalase™ and Protamex™. In order to take into account the different protease activities of the enzymes, enzyme/substrate ratio (E:S) was determined in U g protein-1. When comparing non-repitched yeasts from different strains, NSC samples presented 18.5% higher DH* than NSP samples, when hydrolyzed using Brauzyn®. The effect of serial repitching of *Saccharomyces pastorianus* showed that non-repitched yeast samples were more easily hydrolyzed than the repitched ones. At the same hydrolysis conditions (pH, temperature and E:S) RSP samples took 3.5× more time to achieve the same DH* (3.2%) using Brauzyn®. Very low DH* was achieved using Brauzyn®, for a wide range of E:S, from 50 to 1500U g protein-1, which would indicate that this enzyme could not effectively break RSP yeast cells. Using Alcalase™, higher DH* could be obtained, but RSP had to be diluted 1.4 times and a higher E:S was needed to reach the same degree of hydrolysis of NSP yeast during 2h of hydrolysis. These results show that repitched cells seemed to be more difficult to break down. Indeed, although all fermentation yeasts are imposed to stressful conditions during beer production, the successive reuse of cells in repeated cycles of fermentation makes them more exhausted in terms of its cell components and their cell wall get thicker and more resistant to rupture treatments such as enzymatic hydrolysis. In conclusion, technologies and approaches proposed to add value and reuse BSY must contemplate yeasts differences in terms of its characteristics and susceptibility to break down so that they can be successfully transformed and processed.

mhub@unicamp.br

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