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Research Paper

Influence of phytase and supportive enzymes applied during high gravity mash preparation on the improvement of technological indicators of the alcoholic fermentation process

D. Mikulski, G. Kłosowski, A. Rolbiecka*

Department of Biotechnology, Kazimierz Wielki University, 85-667 Bydgoszcz, ul. Chodkiewicza 51, Poland

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ABSTRACT

Phytic acid is one of the compounds that can limit the availability of biogenic substances responsible for the environmental stress response of the yeast. The aim of the study was determining the influence of hydrolysis of the phytic acid complexes in the HG maize mashes on the availability of free amino nitrogen (FAN) released from hydrolyzed proteins and the resulting change in the fermentation activity of the yeast. Prospects for the optimization of the alcoholic fermentation process by combined application of phytase, protease and augmented set of amylolytic enzymes were also assessed. The application of protease phytase resulted in an increase of FAN concentration during subsequent hours of the alcoholic fermentation process which indicated an increased availability of proteins susceptible to hydrolysis. A positive effect of combined application of phytase, protease and amylolytic enzymes (with pullulanase) on technological indicators of the fermentation process was observed. The final ethanol concentration increased by 1% v v^{-1} and the yield increased by 6 L EtOH 100 kg^{-1} of starch. The optimization of the mashing process, involving the application of additional enzymes (phytase, pullulanase, protease), improved the yield of fermentation in relation to the theoretical yield to the level of 93%. A combined application of α-amylase, pullulanase, glucoamylase, protease and phytase during HG mashes preparation lowered the concentration of higher alcohols by 450 mg L^{-1} EtOH, on average, as compared to the control variant. At the same time, the concentration of acetaldehyde in the spirits increased above 480 mg L^{-1} EtOH.

* *Corresponding author*. Tel.: +48 052 341 32 86; fax: +48 052 341 91 84.
E-mail address: klosowski@ukw.edu.pl (G. Kłosowski).
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1. Introduction

One of the methods for improving the productivity of the alcoholic fermentation is an increase in the initial nutrient concentration in the medium, especially saccharides. High gravity (HG) mashes contain more than 20% w v^{-1} of dissolved substances [1]. Better economic indicators can be obtained by increasing the ethanol yield per fermentation tank in a given time unit when fixed cost are simultaneously reduced. High gravity technology is associated with some benefits like lower water usage, considerable energy savings in the distillation process (because of a higher ethanol concentration in the mashes), reduced amounts of industrial wastewater, and even a lower susceptibility to bacterial infections [2–4]. It has to be stressed that the aforementioned benefits can be obtained only when the high yeast activity is maintained throughout the fermentation process, which in turn depends on the availability of substances minimizing the influence of stress factors.

Antinutrients present in high gravity (HG) mashes that are natural components of the raw material can negatively influence the biological availability of some substances. Phytic acid which is a main phosphorus storage in cereals can limit the amount of biogenic compounds available to yeast during the ethanol fermentation process [5]. Due to its structure, phytic acid (myo-inositol hexakisphosphate) chelates strongly divalent metal cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}), starch and proteins forming insoluble salts referred to as phytates (Fig. 1). Phytic acid binds to proteins directly or indirectly (via divalent cations) over a wide pH range and therefore it can inhibit the proteolysis. *In vitro* studies showed that protein-phytate complexes are formed due to electrostatic interactions [6–8]. Compounds bound to phytic acid are unavailable for organisms that do not produce phytates (enzymes enabling phytate hydrolysis). A microbial phytase (E.C. 3.1.3.8) derived from *Aspergillus* is often used for enzymatic hydrolysis of phytates, mainly in the animal feed industry [9]. Studies of the influence of phytate hydrolysis on the availability of biogenic compounds in fodder showed unequivocally that phytase improved the digestibility of the animal feed. It was demonstrated that the supplementation of animal diet with phytase increased the availability of phosphorus to livestock [10,11]. Moreover, it was shown that utilization of phytic acid in the plant material improved the availability of proteins susceptible to hydrolysis as well as particular amino acids such as arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, valine, serine and glycine [12–14].

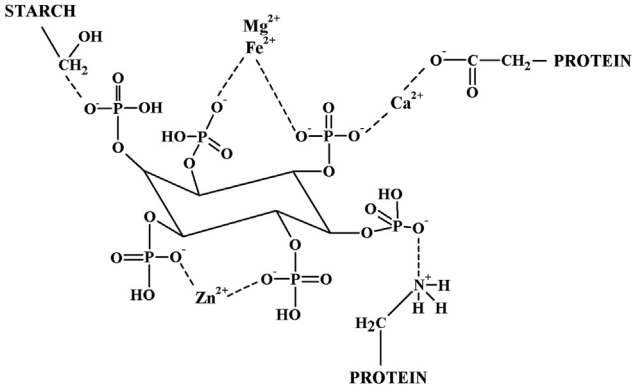

Fig. 1 – Chemical structure of phytates [8].

Since the yeast cells are not capable of protein or long polypeptide assimilation, a high level of free amino nitrogen (FAN) in the mash is very important for the fermentation to be effective [15]. A supplementation of fermentation media with selected amino acids, mainly alanine, arginine, tryptophan, aspartic and glutamic acid, results in a higher ethanol concentration, faster fermentation and better attenuation, as compared to the media without amino acids [16]. A similar effect was observed when proteins were enzymatically degraded during the mashing, but an effective protease application depended on the availability of proteins in the mash and the activity of the biocatalyst used [17]. The presence of selected amino acids in the fermentation media also influences the amount of volatile by-products of the process. Amino acid metabolism changes the concentrations of higher alcohols produced by the yeast. This can be explained by the Ehrlich pathway mechanism which involves deamination and decarboxylation of amino acids. Such obtained aldehydes are then reduced with by alcohol dehydrogenase (ADH) to corresponding alcohols. During the Ehrlich pathway valine is metabolized to isobutanol, leucine to 3-methyl-1-butanol, isoleucine to 2-methyl-1-butanol, etc. [18]. The increase in the amino acid availability also changes the concentration of acetaldehyde, esters and organic acids in the fermentation media [19–21].

It is expected that the application of phytase for the utilization of phytic acid present in HG mashes, which limits the availability of compounds it binds to, combined with the enzymatic protein hydrolysis will result in a significant increase in the concentration of mineral substances and the free amino nitrogen (FAN) obtained from the proteolysis. An improved availability of mineral substances, i.e. phosphorus, magnesium, zinc and calcium, which are components of many enzymes and cellular structures as well as regulators of the yeast cell cycle, should also increase the fermentation activity of the yeast [22]. The aim of the study was to examine the influence of hydrolysis of phytic acid complexes present in HG mashes, resulting in a better availability of FAN released by proteolysis, on the indicators of the ethanol fermentation process. Hydrolysis of phytates was carried out using an exogenous phytase applied either before or after the mashing process. The prospects of optimization of the alcoholic fermentation process by simultaneous application of phytase (for hydrolysis of phytates), pullulanase (for better starch hydrolysis) and a protease (for hydrolysis of proteins released from phytates) during the HG mash preparation were also assessed. The influence of proposed solutions on the ethanol yield and the course of the fermentation process was analyzed. The optimization of ethanol fermentation technology with HG mashes aiming at a better utilization of substances present in the raw material is important to reduce the cost of alcohol production.