Enzyme and Microbial Technology 49 (2011) 38–43

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Characterization of technological features of dry yeast (strain I-7-43) preparation, product of electrofusion between *Saccharomyces cerevisiae* and *Saccharomyces diastaticus*, in industrial application

Katarzyna Kotarska [a], Grzegorz Kłosowski [b,*], Bogusław Czupryński [a]

[a] Institute of Agricultural and Food Biotechnology, Independent Distillery Laboratory, 85-090 Bydgoszcz, Powstańców Wielkopolskich 17, Poland
[b] Department of Biotechnology, Laboratory of Fermentation Technology, Kazimierz Wielki University, 85-064 Bydgoszcz, ul. Chodkiewicza 51, Poland

ARTICLE INFO

Article history:
Received 7 March 2011
Received in revised form 11 April 2011
Accepted 12 April 2011

Keywords:
Amylolytic yeasts
Amylolytic enzymes
Starch hydrolysis
Fermentation yield
Ethanol production
Volatile fermentation by-products
Rye mashes

ABSTRACT

The aim of the study was to verify the technological usability and stability of biotechnological features of active dry distillery yeast preparation (strain I-7-43 with amylolytic abilities) applied to full-scale production of agricultural distillery. Various reduced doses of glucoamylase preparation (San-Extra L) were used for starch saccharification, from 90% to 70% in relation to the full standard dose of preparation. The dry distillery yeast I-7-43 were assessed positively in respect to fermentation activity and yield of ethanol production. Application of the dry yeast I-7-43 preparation in distillery practice lowers the costs of spirit production by saving the glucoamylase preparation (up to 30%) used in the process of mash saccharification. Concentrations of the volatile fermentation by-products in raw spirits obtained from fermentations with application of I-7-43 strain were on the levels guaranteeing good organoleptic properties of distillates.

1. Introduction

In distillery industry, application of the appropriate yeast strains, which couple the fermentation activity with the ability to hydrolyse starch, can result in improvement of the economy of spirit production by reduction of the dose of enzymatic preparations used for the starch saccharification.

Recently, the studies on preparation of the yeast strains characterized by the stable amylolytic abilities and their application in agricultural distilleries have been carried out in research centres worldwide [1–4]. Several advantages of using amylolytic yeast in industrial processes have been recently described by Tubb [5].

Conversion efficiency of batch fermentation with *Saccharomyces diastaticus* was improved by using a mixed culture with *Schwanniomyees castelli*, thus supporting the idea that the *S. diastaticus* amylolytic system was limiting fermentation efficiency and could be supplemented by using an additional, α-amylase producing yeast [6]. Wilson and Ingledew [7] described the associative fermentation of starch, using *Schwanniomyces alluvius* for its amylolytic activity and *Saccharomyces uvarum* for ethanol production. Dostalek and Häggström [8] used a mixed culture of *Endomycopsis fibuligera* and *Zymomonas mobilis* for alcoholic fermentation of soluble starch. In their system, glucose production from starch was a rate-limiting reaction.

Yeast ability to synthesise amylolytic enzymes is a desired feature for industrial fermentation of mashes from starchy raw materials, as it lowers the cost of spirit production.

The absence of the starch binding domain (SBD) in glucoamylases from *Saccharomyces cerevisiae* (*var. diastaticus*) does not reduce the efficiency of the enzyme in soluble dextrins hydrolysis, but it poses a problem in the hydrolysis process of larger insoluble starch molecules [9–11]. In industry, efficient hydrolysis of starch into fermentable sugars for its bioconversion into ethanol by yeast *S. cerevisiae* requires addition of exogenous enzymes. Another solution of the problem is application of genetically modified yeast strains (with *Bacillus subtilis* α-amylase and *Aspergillus awamori* glucoamylase activities) that are able to produce amylolytic enzymes [12–14]. However, in some countries direct transfer of this solution to the industrial practice can be difficult because of legal regulations forbidding to introduce GMO to open industrial systems.

The yield of the batch fermentation with *S. diastaticus* was improved by using a coculture with α-amylase producing yeast *Schwanniomyces castellii*, *S. alluvius* [6,7] or mixed culture of *E. fibuligera* and *Z. mobilis* [8]. However, to improve productivity and time

* Corresponding author. Tel.: +48 52 341 32 86.
E-mail address: klosowski@ukw.edu.pl (G. Kłosowski).

0141-0229/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2011.04.008

of fermentation at industrial scale, starch hydrolysis to mono- and disaccharides with amylolytic preparations obtained from mould or bacteria was required [15–17].

Publications describing the biotechnological features of the strains obtained by a fusion of protoplasts, i.e., *S. cerevisiae*, *S. diastaticus* and others, pointed out that not all of them comply with the technological requirements [1,18,19].

The aim of our study was to verify the technological usability and technological indices stability of active dry distillery yeast preparation (I-7-43 strain with amylolytic abilities) in full-scale commercial production under common technological conditions of agricultural distillery. The quality of raw spirits obtained was assessed based on the composition of volatile fermentation by-products.

2. Materials and methods

2.1. Yeast strains and microbiological methods

The new yeast strain (I-7-43 fusant) characterized by the amylolytic activities and high fermentative efficiency was obtained in the Department of Technical Microbiology and Biochemistry of Institute of Agricultural and Food Biotechnology in Warsaw as a result of electrofusion of protoplasts between the parent yeast strains *S. cerevisiae* (As-4 pro$^-$) and *S. diastaticus* (S.di/2-1 arg$^-$). The yeast strain I-7-43, characterized by an increased amylolytic activity and higher ability to produce alcohol compared to the parental strains [20], was obtained.

The test batch of active preparation of dry yeast I-7-43 with amylolytic abilities was produced by Lesaffre Bio-Corporation (Poland, Maszewo Lęborskie), and applied for technological assessment under agricultural distillery conditions [21]. The second dry yeast preparation, *S. cerevisiae* D-2 strain, thermophilic (up to 39 °C) and alcohol tolerant (over 12%, v/v), adapted to fermentation of starch hydrolysates, was employed for control fermentations [22].

Dry yeast preparations I-7-43 and D-2 were prepared for inoculation of mashes. As a result of stirring, a homogeneous yeast cells suspension was obtained and left for rehydration for 20 min in 10-fold volume of water (v/w). During rehydratation, disinfection was also performed for 10 min using concentrated sulphuric acid added to yeast cream in amount of 5 ml per each 1 l of water (pH of yeast cream was about 2.5). Such obtained yeast cream was introduced into the mashes (in the yeast starter vat) at the temperature of 30–32 °C and pH 3.0–3.3. Both yeast strains were applied in doses of 100 g per each 1000 l of sweet mash. After 24 h, the technologically mature yeast culture from the yeast vat was introduced into the first sweet mash prepared at a given day of production. The amount of yeasts introduced into mashes was equal to 5% of four mashes fermented volume (one fermentation tank) at the given day.

2.2. Enzymatic preparations

For liquefaction of starch in the mashing process, heat-stable bacterial α-amylase preparation Termamyl 120 L (Novozymes Company, Denmark, Bagsvaerd) was used in the standard dose (140 ml per ton of starch). Activity 120 KNU/g; KNU – One Kilo Novo alpha-amylase Unit is the amount of enzyme which hydrolyzes 5.26 g of starch per hour in standard conditions (the Novozymes standard method for the determination of alpha-amylase). For starch saccharification SAN Extra L preparation (Novozymes Company, Denmark, Bagsvaerd) of glucoamylase from genetically modified *Aspergillus niger* with the activity of 400 AGU/g (460 AGU/ml), (Amylo Glucosidase Units-1 amyloglucosidase unit is the amount of enzyme which catalyzes the conversion of 1 μmol of maltose per minute under given conditions), was used at standard dose of 0.6 l per ton of starch.

2.3. Technological process

Each cycle of experimental fermentation (four mashes per fermentation tank) was prepared using 1000 kg of good quality rye grain for each mash derived from one lot of grains (4000 kg of grain in total). Concentration of starch in the raw material was 55.2%.

Starch contained in rye grains was liberated from cells by barothermal method using Henze's steamer, by steaming the raw material at 150 °C and steam pressure of 0.4 MPa. Steamed mass was treated with amylolytic enzymes. For each of the four mashes (the whole fermentation tank) a dose of 80 ml of Termamyl 120 L preparation at the temperature of 90 °C was applied. After 10 min break and cooling down the mash to the temperature of 60 °C, saccharifying preparation San-Extra L was added. In the case of mashes destined for the control fermentations with application of the distillery strain D-2, the standard doses (100%) of amylolytic preparations were used. For saccharification of mashes fermented by yeast I-7-43, various doses of the glucoamylase preparation were applied (from 100% to 70% of the standard dose), i.e., Variant I 100% of standard dose – 330 ml per one mash; Variant II 90% of standard dose – 300 ml per one mash; Vari-

Table 1
Course of fermentations led by dry amylolytic yeast I-7-43 after application of full and reduced doses of glucoamylase.

Research variants	Dose of glucoamylase [%]	Amount of mash [L]	Apparent extract after hours [°Blg]			pH of mash after hours			Concentration of ethanol after hours [%, v/v]			Saccharification and liquefaction of starch after hours		Yield of ethanol [L]
			24	48	72	24	48	72	24	48	72	48	72	
Variant I	100	17566.7a ± 115.5	6.1c ± 0.1	1.9a ± 0.2	1.4a ± 0.1	4.6ab ± 0.1	4.4b ± 0.1	4.2a ± 0.1	5.83b ± 0.02	8.08a ± 0.09	8.33a ± 0.4	++	++	1457.3ab ± 25.4
Variant II	90	17433.3a ± 28.9	6.1c ± 0.2	2.0a ± 0.1	1.4a ± 0.1	4.6ab ± 0.0	4.6a ± 0.0	4.3a ± 0.1	5.79bc ± 0.12	8.04a ± 0.12	8.33a ± 0.04	++	++	1452.2ab ± 8.4
Variant III	80	17633.3a ± 57.7	6.7b ± 0.1	1.9a ± 0.2	1.4a ± 0.0	4.7ab ± 0.1	4.7a ± 0.1	4.3a ± 0.1	5.62c ± 0.00	8.06a ± 0.02	8.34a ± 0.04	+	++	1471.2a ± 6.3
Variant IV	70	17633.3a ± 57.7	7.2a ± 0.2	1.9a ± 0.1	1.4a ± 0.1	4.7a ± 0.0	4.6a ± 0.1	4.3a ± 0.1	5.11d ± 0.08	8.02a ± 0.05	8.33a ± 0.04	+	++	1469.4a ± 7.6
Control variant	100	17066.7b ± 104.1	4.5d ± 0.1	1.9a ± 0.1	1.4a ± 0.1	4.5b ± 0.1	4.4b ± 0.1	4.4a ± 0.0	6.46a ± 0.03	8.12a ± 0.04	8.36a ± 0.04	++	++	1426.8b ± 4.6

The mean values given in columns with different letter indexes are significantly different ($\leq$0.05).
Degree of saccharification and liquefaction of starch: (++) very good; (+) good.