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CORRECTED: THE INFLUENCE OF BREWERS' YEAST ADDITION ON LACTIC ACID FERMENTATION OF BREWERS' SPENT GRAIN HYDROLYSATE BY *LACTOBACILLUS RHAMNOSUS*

KORIGOVANO: UTICAJ DODATKA PISVKOG KVASCA NA MLEČNO-KISELU FERMENTACIJU HIDROLIZATA PIVSKOG TROPA SA *LACTOBACILLUS RHAMNOSUS*

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ABSTRACT

Brewers' spent grain (BSG) hydrolysates were used for lactic acid (LA) fermentation by Lactobacillus rhamnosus ATCC 7469. The aim of this study was to evaluate possibilities of the BSG hydrolysate utilization as a substrate for LA fermentation as well as the effect of dry brewers' yeast addition in hydrolysate on lactic acid fermentation parameters (L-(+)-LA and reducing sugar concentration and number of viable cell-viability). Very high L. rhamnosus ATCC 7469 cell viability was achieved in all fermentations (9.26-9.52 log CFU/mL at the end of fermentation). L-(+)-LA concentration increased with an increase in brewers' yeast content in hydrolysate.

Key words: *Lactic acid fermentation; brewers' spent grain, brewers' spent yeast.*

REZIME

Mlečna kiselina ima široku primenu u prehrambenoj, farmaceutskoj, hemijskoj, tekstilnoj i industriji polimera. Primena jeftinih i obnovljivih sirovina u proizvodnji mlečne kiseline postupcima fermentacije je osnova ekonomične proizvodnje. U zavisnosti od količine najzastupljeniji sporedni proizvodi prizvodnje piva su: pivski trop i pivski kvasac. Pivski kvasac je relativno jeftin pa se koristi u proizvodnji ekstrakata za primenu u prehrambenoj industriji. Pivski trop čini najveći deo sporednih proizvoda proizvodnje piva (približno 85%). U istraživanju su hidrolizati pivskog tropa korišćeni u mlečno-kiseloj fermentaciji sa Lactobacillus rhamnosus ATCC 7469. Cilj ovog istraživanja je bio da se ispita mogućnost primene hidrolizata pivskog tropa u mlečno-kiseloj fermetnaciji kao i uticaj dodatka suvog pivskog kvasca u hidrolizat na parametre mlečno-kisele fermentacije (koncentraciju L-(+)-mlečne kiseline i redukujućih šećera i broj vijabilnih ćelija-vijabilnost). Pri svim ispitivanim sadržajima pivskog kvasca u hidrolizatima u toku mlečno-kisele fermentacije je ostvarena veoma visoka vijabilnost ćelija L. rhamnosus (9,26-9,52 log CFU/ml na kraju fermentacije). S porastom sadržaja pivskog kvasca u hidrolizatu za mlečno-kiselu fermentaciju povećala se i koncentracija L-(+)-mlečne kiseline.

Ključne reči*: Mlečno-kisela fermentacija, pivski trop, pivski kvasac.*

INTRODUCTION

In beer production, the major by-products are brewers' spent grain and brewers' spent yeast (*Suwanapong et al., 2013*). Spent yeast obtained after beer fermentation is relatively inexpensive so it is used in the production of extracts in food industry (*Tanguler and Erten, 2008*). Yeast cells contain plenty of protein, lipid, RNA, vitamins, and minerals. The brewers' yeast is an inexpensive nitrogen source and generally recognized as safe (GRAS) (*Chae et al., 2001*). Large quantities of brewers' spent yeast are obtained after beer fermentation. Brewer's spent grain (BSG) represents 85 % of the total by-products generated in beer production (*Mussatto, 2014*). Its chemical composition depends on barley variety, harvest time, malting and mashing conditions, and the quality and type of unmalted raw materials used in brewing. It is a lignocellulosic material rich in protein and fibre, which account for around 20 and 70 % of its composition, respectively, but it also contains starch, lipids, amino acids, vitamins, and minerals (*Mussatto et al., 2008*).

Lactic acid (LA) is used in the food industry as preservative, acidulant, flavoring agent or pH buffer. (*Datta and Henry, 2006*). LA is predominantly produced by fermentation in industrial facilities and implementation of the processes on renewable and cheap substrates is a base for cost-effective production (*Djukić-Vuković et al., 2013*). The annual world market for LA production is expected to reach 830,000 tonnes in 2020 (*Dreschke et al., 2015*). The production of LA from lignocellulosic materials can be performed by sequential steps of chemical and/or mechanical processing (in order to make the cellulose more accessible to the enzymes), enzymatic saccharification (for obtaining solutions containing glucose as main sugar) and finally, the hydrolysate fermentation by microorganisms, especially *Lactobacillus* species (*Mussatto et al., 2008*).

In this study brewers' spent grain (BSG) hydrolysates were used for LA fermentation by *Lactobacillus rhamnosus* ATCC 7469. The aim of this study was to evaluate possibilities of the BSG hydrolysate utilization as a substrate for LA fermentation as well as the effect of dry brewers' yeast (1.0, 3.0, and 5.0 %) addition in hydrolysate on lactic acid fermentation parameters (L-(+)-LA and reducing sugar concentration, and number of viable cell-viability).

MATERIAL AND METHOD

Brewers' spent grain composition

The following quality parameters and methods were used for the analysis of BSG: soluble extract, available residual extract and total residual extract (% dry matter) according to MEBAK (2013), starch content after by polarimetric method (% dry matter) (*International Standard, ISO 10520, 1997*), cellulose content (% dry matter) by Kirschner and Hoffer method, and protein content (% dry matter) by Kjeldahl method (*AACC, 2008*). BSG before enzymatic hydrolysis was dried and analyzed. After enzymatic hydrolysis liquid hydrolysate was separated from solid hydrolysate and used in LA fermentation. Solid residue after hydrolysis was dried and analyzed. All BSG analyses were carried out in triplicate. Results were represented as mean ± standard deviation.

Brewers' spent grain hydrolysate preparation

BSG obtained in a lager beer production was dried at 40 ºC for 12 h. Dried BSG was finely ground in a laboratory DLFU mill from Bühler-Miag (Braunschwieg, Germany). BSG hydrolysis was carried out as described previously (*Pejin et al. 2014, 2015*). After enzymatic hydrolysis obtained BSG hydrolysate was cooled to 20 °C and centrifuged (4000 rpm, 20 min, centrifuge: BOECO model C-28A, Hamburg, Germany). Liquid hydrolysate was separated from solid hydrolysate. Liquid hydrolysate was used in LA fermentations. Its pH was adjusted to 6.5 with the addition of 1M NaOH. Brewers' yeast content in hydrolysate was set to 1.0, 3.0, and 5.0 % with the addition of corresponding contents of dry brewers' yeast (AD BIP-Belgrade Beer Industry, Serbia). After this, liquid hydrolysate was sterilized at 121 °C for 15 min and used as a fermentation medium. Two series of experiments were conducted: without reducing sugar concentration correction and with reducing sugar concentration correction to 5.0 % in hydrolysate before fermentation. Dry brewers' yeast contained: proteins (minimum 45 g/100 g), carbohydrates (27.56 g/100 g), minerals (maximum 11 g/100g), lipids (3.0 g/100 g), vitamin B1 (7.0 mg/100 g), vitamin B2 (1.1 mg/100 g), vitamin B6 (1.1 mg/100 g), vitamin B3 (41 mg/100 g), phosphorus (1610 mg/100 g), and magnesium (300 mg/100 g).

Microorganism

A homofermentative L-(+)-LA strain, *Lactobacillus rhamnosus* ATCC 7469, was obtained from American Type Culture Collection (ATCC, Rockville, USA). *L. rhamnosus* culture was stored and activated as described previously (*Pejin et al., 2015*). Inoculum was prepared by taking 3 mL of the activated culture and transffering it to 60 mL of MRS broth. Inoculum was incubated for 24 hours at 37 °C to reach high LA bacteria cell number.

LA fermentation

All LA fermentations were performed as batch cultures with shaking (150 rpm, Biosan model ES-20, Biosan Ltd., Lithuania). The fermentations were performed for 72 h in 300 mL Erlenmayer flasks with 200 mL of BSG hydrolysate. The fermentation was initiated by the addition of inoculum (5.0 % v/v). Fermentations were performed at 37 °C. Reducing sugar and L-(+)-LA concentration, and number of viable cell were analyzed every 12 h during fermentation.

Analytical methods

Reducing sugar concentration, calculated as glucose, was determined by 3,5-dinitrosalicylic acid method (*Miller, 1959*). A calibration curve was set at 570 nm using standard glucose solutions. L-(+)-LA concentration was determined by enzymatic method (L-(+)-LA assay, Megazyme, Wicklow, Ireland). Proteins were removed from samples by precipitation method prior to the LA determination (*Methods of Enzymatic BioAnalysis and Food Analysis, 1997*). Pour-plating method was used for determination of the number of viable *L. rhamnosus* cell. Microaerophilic conditions were maintained during incubation in Petri plates using double MRS medium layer. Samples were incubated at 37 °C for 48 hours. Total viable cell number was expressed as log CFU/mL. All chemicals used in experiments were of analytical and microbiological grade.

Statistical analysis

The experiments were done in triplicates. All values are expressed as means±standard deviation. Mean values of treatments were compared by the analysis of variance (one-way ANOVA) followed by Duncan test for mean differences testing (*IBM SPSS Statistica 20*). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

BSG composition before and after enzymatic hydrolysis is given in Table 1. Soluble and available residual extract content and cellulose content decreased during hydrolysis. Starch was not determined after the hydrolysis in the solid residual.

Table 1. Brewer's spent grain (BSG) composition before and after enzymatic hydrolysis (Pejin et al., 2015)

Parameter	Before enzymatic hydrolysis	After enzymatic hydrolysis
Soluble extract, % dry matter	5.84 ± 0.27	0.23 ± 0.11
Available residual extract, % dry matter	4.47 ± 0.33	1.63 ± 0.14
Total residual extract, % dry matter	10.31 ± 0.37	1.86 ± 0.16
Proteins, % dry matter	26.48 ± 0.35	26.23 ± 0.34
Starch, % dry matter	4.10±0.27	Not determined
Cellulose, % dry matter	15.15 ± 0.38	3.25 ± 0.09

BSG hydrolysate contained 2.5 % of reducing sugars. With dry brewers' yeast addition in hydrolysate reducing sugars content increased since brewers' yeast contained carbohydrates (27.56 g/100 g). With the increase in brewers' yeast content reducing sugars content increased and was 4.4 % in fermentation with the addition of 5.0 % of dry brewers' yeast.

In fermentations without reducing sugars correction L-(+)-LA concentration increased significantly ($p<0.05$) by 11.01 % (1.0 % of brewers' yeast) - 73.46 % (5.0 % of brewers' yeast) (Fig. 1) while in fermentations with reducing sugar correction it increased significantly by 16.14 % (1.0 % of brewers' yeast) - 30.64 % (5.0 % of brewers' yeast) with brewers' yeast addition (Fig. 2). With reducing sugar concentration correction L-(+)-LA concentration increased by 128 % compared to the concentration achieved in fermentation without brewers' yeast addition and reducing sugar concentration correction. L-(+)-LA concentration increased by 198 % when reducing sugar concentration was corrected and 5.0 % of brewers' yeast was added compared to the concentration achieved in fermentation without brewers' yeast addition and reducing sugar concentration correction.

In all fermentations *L. rhamnosus* cell viability significantly ($p<0.05$) increased until the end of the fermentation. *L. rhamnosus* cell viability was significantly higher ($p<0.05$) in fermentations with brewers' yeast addition and without reducing sugar correction (2.89 % (1.0 % of brewers' yeast) – 5.00 % (5.0 % of brewers' yeast) than in fermentation without brewers' yeast

addition and without reducing sugar correction. *L. rhamnosus* cell viability was also significantly higher (p<0.05) in fermentations with brewers' yeast addition and with reducing sugar concentration correction (1.41 % (1.0 % of brewers' yeast) – 3.03 % (5.0 % of brewers' yeast) compared to the fermentation without brewers' yeast addition and with reducing sugar concentration correction (Figs. 3 and 4). *L. rhamnosus* requires a complex nutrient composition for its growth because it lacks enzymes to self-synthesize B vitamins and amino acids (*Cui et al., 2012*). However, most *Lactobacillus* require an exogenous nitrogen source of amino acids or peptides to meet cell growth (*Manca de Nadra, 2007*).

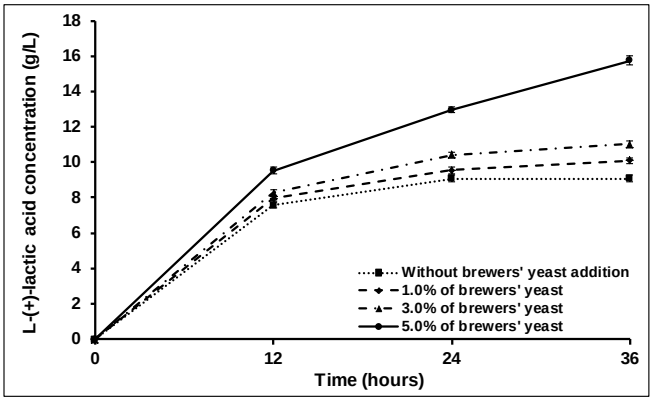

Fig. 1. L-(+)-LA concentration in BSG hydrolysate fermentations without and with brewers' yeast (1.0, 3.0, and 5.0 %) addition and without reducing sugar concentration correction

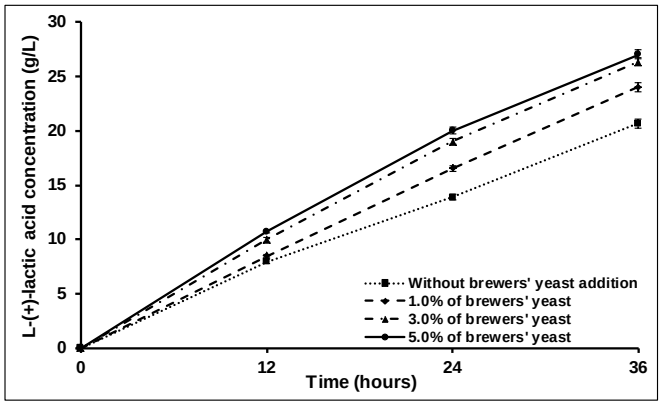

Fig. 2. L-(+)-LA concentration in BSG hydrolysate fermentations without and with brewers' yeast (1.0, 3.0, and 5.0 %) addition and with reducing sugar concentration correction to 5.0%

Brewers' yeast addition increased L-(+)-LA yield by 6.85 % (5.0 % of brewers' yeast, without reducing sugar concentration correction) – 9.71 % (5.0 % of brewers' yeast, with reducing sugar concentration correction). The highest L-(+)-LA yield (89.01 %) was achieved in fermentation of hydrolysate with 5.0 % of brewers' yeast and reducing sugar concentration correction (Fig. 5).

Hydrolysates obtained from BSG (*Mussatto et al., 2008*) and other cellulosic materials (*Garde, et al., 2002; Ali et al., 2009*; *Laopaiboon et al., 2010*), required additional nutrients (yeast extract, MRS medium, petone, different salts, etc.) for lactic acid production by *Lactobacillus* strains. Results obtained in this study showed that brewers' yeast addition significantly increased L-(+)-lactic acid yield and *L. rhamnosus* cell viability.

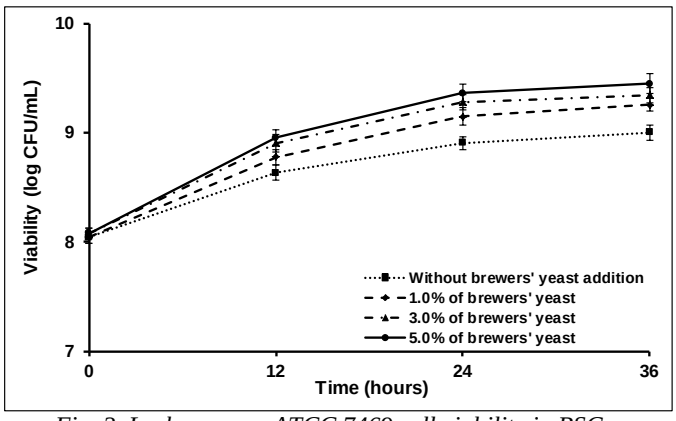

Fig. 3. L. rhamnosus ATCC 7469 cell viability in BSG hydrolysate fermentations without and with brewers' yeast (1.0, 3.0, and 5.0 %) addition and without reducing sugar concentration correction

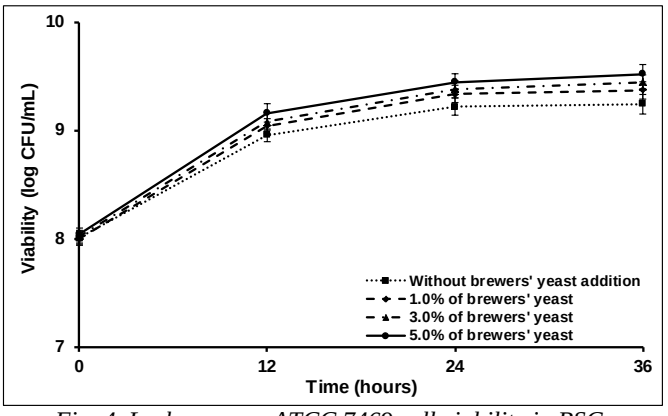

Fig. 4. L. rhamnosus ATCC 7469 cell viability in BSG hydrolysate fermentations without and with brewers' yeast (1.0, 3.0, and 5.0 %) addition and with reducing sugar concentration correction to 5.0 %

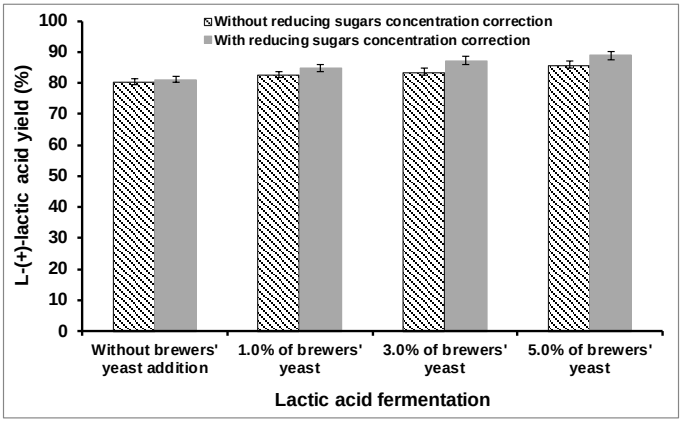

Fig. 5. L-(+)-LA yield in BSG hydrolysate fermentations

CONCLUSION

In fermentations without reducing sugar correction L-(+)-LA concentration increased by 11.01-73.46 % while in fermentations with reducing sugar correction it increased by 16.14-30.64 % with brewers' yeast addition. With reducing sugar concentration correction L-(+)-LA concentration increased by 128 % compared to the concentration achieved in fermentation without brewers' yeast addition and reducing sugar concentration correction. L-(+)-LA concentration increased by 198 % when reducing sugar concentration was corrected and 5.0 % of brewers' yeast was added compared to the concentration

achieved in fermentation without brewers' yeast addition and reducing sugar concentration correction. Brewers' yeast addition increased L-(+)-LA yield by 6.85–9.71 %. The highest L-(+)-LA yield (89.01 %) was achieved in fermentation of hydrolysate with 5.0 % of brewers' yeast and reducing sugar concentration correction.

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