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## The Influence of Brewer's Yeast Autolysate and Lactic Acid Bacteria on the Production of a Functional Food Additive Based on Beetroot Juice Fermentation

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### Summary

The importance of »functional foods« in the world is increasing, and the procedures for their production are under intense development. The goal of this paper is to optimise the production of a functional food additive based on beetroot juice (*Beta vulgaris* L.) using brewer's yeast autolysate. In order to improve the nutritive properties of the product and to preserve it, the possibility of beetroot juice fermentation using a *Lactobacillus* species has been investigated. Comparative investigations of three bacteria cultures (*L. plantarum* A112, *L. acidophilus* BGSJ15-3 and *L. acidophilus* NCDO1748) during fermentation in two media, beetroot juice and a mixture of beetroot juice with an autolysate of brewer's yeast, have been performed. The poorest fermentative activity and growth in both substrates was observed using the *L. acidophilus* NCDO1748 culture. The two cultures demonstrated better fermentative activity in the mixture of tested substrates, while acidifying activity (production of lactic acid and a decrease in pH) of the *L. acidophilus* BGSJ15-3 culture was considerably better than that of the *L. plantarum* A112 culture. *L. plantarum* A112 culture showed better growth than *L. acidophilus* BGSJ15-3. From the results obtained, it has been concluded that the *L. plantarum* A112 and *L. acidophilus* BGSJ15-3 can be successfully used for fermentation of the mixture of beetroot juice and brewer's yeast autolysate in order to obtain a functional food additive.

*Key words:* beetroot, lactic acid fermentation, brewer's yeast autolysate

### Introduction

The aim of the so-called functional foods is to ensure satisfactory nutritive properties and prevention of illness, while promoting and extending active human life (1–3). In order to obtain these goals, it is necessary to make substantial modifications in an entire chain of food production, controlling the production according to the EU concept of »farm to table« (4–7). Important contribution to this goal could be achieved by biological processes, *i.e.* biological procedures of food production and transformation (4,8).

As no natural raw material for food production satisfies total nutritional requirements, the principle of combining two natural raw materials and a bacterial fermentation has been applied in this work in order to improve nutritional value, the shelf life and the safety of the food. In this way, desirable properties have been enhanced.

The goal of this work was to develop the procedures for biological processing of beetroot (*Beta vulgaris* L.) through fermentation using lactic acid bacteria. Beet-

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root was chosen as a starting substance for the production of biologically highly valuable food, as there are numerous publications describing its favourable nutritive and protective benefits on humans (9,10).

Fermentation using lactic acid bacteria is a wide-spread tradition. However, processing beetroot has not been considered in detail. Our previous investigations (11–13) showed that beetroot juice could be successfully fermented by using lactic acid bacteria, and that successful fermentation depended on the selection of bacterial cultures. It has also been established that brewer's yeast extract favourably promotes fermentative activity of lactic acid bacteria, because it is a rich source of nutrients and biofactors needed by numerous microbial species, and especially lactobacilli (14,15).

In this paper we have used a mixture of beetroot juice and autolysate of brewer's yeast in equal proportion as a starting raw material and subjected this mixture to lactic acid fermentation using the *Lactobacillus* species for the sake of improvement of quality and preservation. These bacteria were chosen based on our previous investigations, as well as on the fact that they are probiotics (13,16). The obtained fermented product could therefore be categorised as functional food.

## Materials and Methods

### Bacteria cultures

For fermentation of selected substrates the following cultures have been used: *Lactobacillus plantarum* A112 (Greece), *Lactobacillus acidophilus* NCDO1748, and a natural isolate, *Lactobacillus acidophilus* BGSJ15-3, from the collection of lactic acid bacteria from the Laboratory for Microbiology of the Faculty of Technology and Metallurgy in Belgrade. Tested cultures were propagated in MRS broth. Initial inoculated numbers of viable cells in the substrates used were  $10^5$ – $10^7$  CFU/mL. Fermentation temperature was 37 °C, while the time of fermentation was 8 h.

### Fermentation substrates

Substrate  $S_1$  was beetroot (*Beta vulgaris* L.) juice, obtained by chopping the fresh vegetable, and separating the juice in a juice maker. It was then pasteurised at 70 °C for 20 min. Substrate  $S_2$  was a mixture of beetroot juice and brewer's yeast autolysate prepared in a 1:1 mass ratio per dry matter. Brewer's yeast was first debittered with 4 % NaCl, washed (17) and then autolysed at 55 °C for 3 h. Single cultures of *L. plantarum* A112, *L. acidophilus* BGSJ15-3 and *L. acidophilus* NCDO1748 were propagated in MRS broth. A volume fraction of 2 % of selected cultures grown in MRS broth was used as inoculum for the substrates  $S_1$  and  $S_2$ . After the 8-h fermentation satisfactory lactic acid production and viable counts in samples were obtained, as described in our previous investigations (12,13). A longer time of fermentation would not be economical for industrial application. The experiment was conducted with five combinations of substrates and starter cultures: sample 1 – Substrate  $S_1$  (beetroot juice) with *L. plantarum* A112; sample 2 – Substrate  $S_1$  with *L. acidophilus* BGSJ15-3; sample 3 – Substrate  $S_1$  with *L. acidophilus* NCDO1748; sample 4 – Substrate  $S_2$  (beetroot juice and brewer's yeast autolysate) with *L. plantarum* A112; sample 5 – Substrate  $S_2$  with *L. acidophilus* BGSJ15-3.

strate  $S_2$  (beetroot juice with autolysate of brewer's yeast) with *L. plantarum* A112; sample 5 – Substrate  $S_2$  with *L. acidophilus* BGSJ15-3.

### Assays

The progress of fermentation was monitored by determination of the number of colony forming units on MRS agar plates using standard decimal dilution method after incubation at 37 °C for 48 h under microaerophilic conditions (18). The following parameters were determined over time: change of pH (pH meter), concentration of lactic acid (potentiometer) (19), sugar content (spectrophotometry, with anthron) (20) and content of free amino acid nitrogen (spectrophotometric method with ninhydrin) (20).

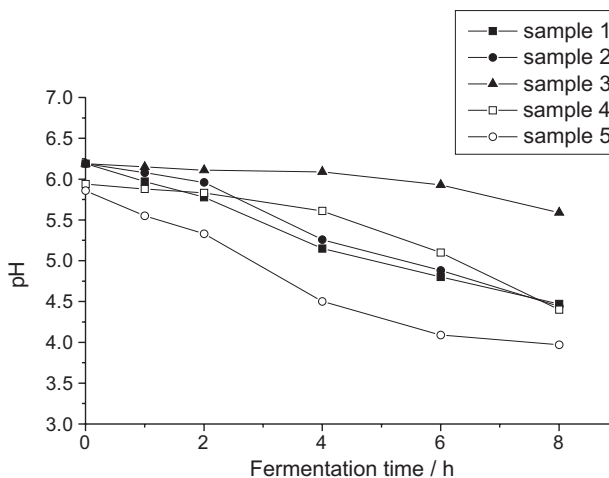
The results are expressed as the average values of three independent measurements.

## Results and Discussion

The data obtained show that the tested bacterial cultures, *L. plantarum* A112, *L. acidophilus* BGSJ15-3 and *L. acidophilus* NCDO1748, in two types of substrates,  $S_1$  and  $S_2$ , significantly differ in fermentative activity and growth (Figs. 1–5).

*L. acidophilus* NCDO1748 showed very poor fermentative activity in  $S_1$ . This was shown by the low utilisation of glucose (Fig. 3), small pH drop during the 8-h fermentation (only 0.5 units) and equivalently low production of lactic acid (Figs. 1 and 2). Assimilation of sugar and free amino acid nitrogen was also rather low. As the goal of investigation was to attain successful acidification of beetroot juice (production of lactic acid and decrease of pH value in the tested substrates), this culture was excluded from further investigations.

The other two bacterial cultures exhibited successful fermentative activity in both tested substrates, and they showed differences expressed in all tested parameters.



**Fig. 1.** Changes in pH during fermentation in: sample 1 – beetroot juice ( $S_1$ ) with *L. plantarum* A112; sample 2 –  $S_1$  with *L. acidophilus* BGSJ15-3; sample 3 –  $S_1$  with *L. acidophilus* NCDO1748; sample 4 – beetroot juice and brewer's yeast autolysate ( $S_2$ ) with *L. plantarum* A112; sample 5 –  $S_2$  with *L. acidophilus* BGSJ15-3

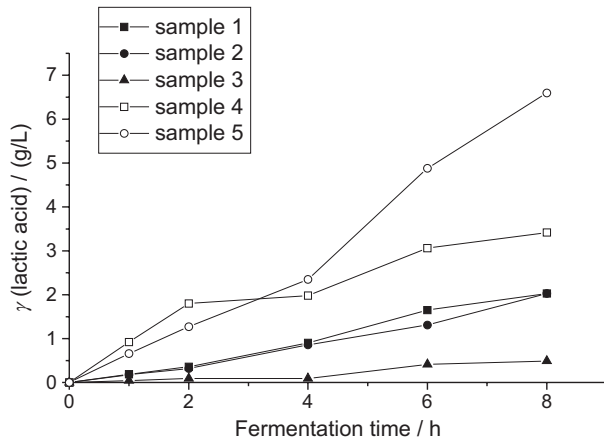


Fig. 2. Lactic acid production during fermentation. Samples as in Fig. 1

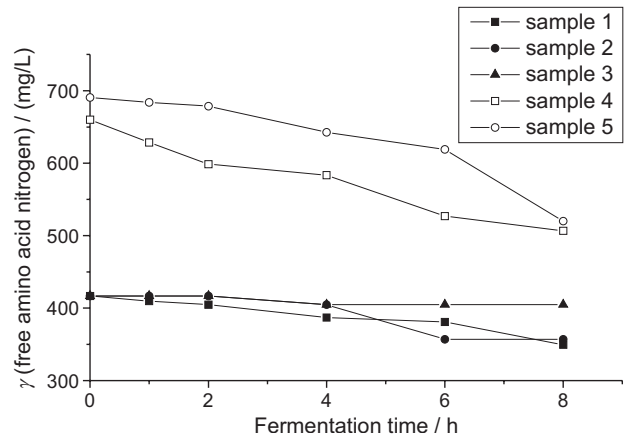


Fig. 4. Changes in free amino acid nitrogen during fermentation. Samples as in Fig. 1

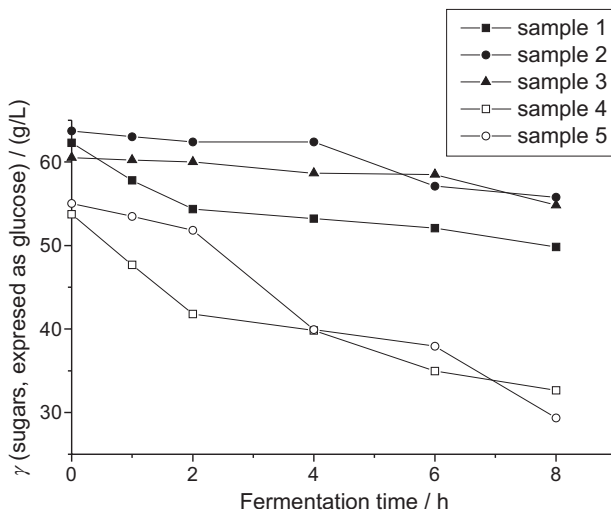


Fig. 3. Utilisation of sugars (as glucose) during fermentation. Samples as in Fig. 1

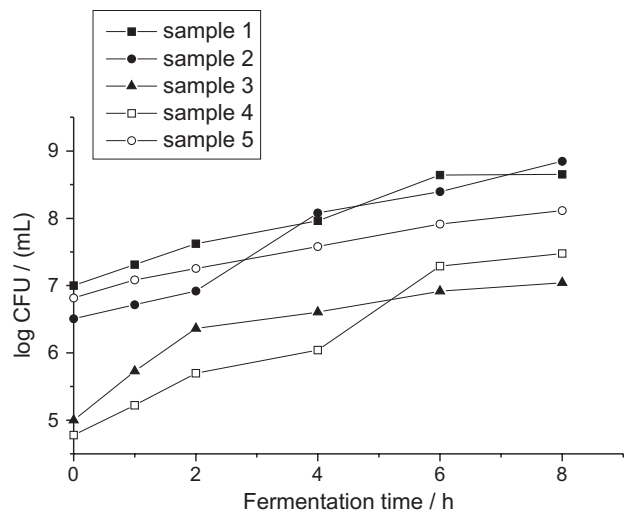


Fig. 5. Changes in number of colony forming units (CFU) during fermentation. Samples as in Fig. 1

The most successful acidifying activity expressed by the decrease of pH (from 6 to 4, Fig. 1) and production of lactic acid (6.6 g/L, Fig. 2) was exhibited by the *L. acidophilus* BGSJ15-3 culture in substrate  $S_2$ . This was accompanied by equivalently high utilisation of sugar from the substrate (over 45 %, Fig. 3) and good assimilation of free amino acid nitrogen (24.6 %, Fig. 4). In the same substrate, this culture showed considerable growth (1.3 log CFU/mL, Fig. 5), while it exhibited poorer fermentative activity in substrate  $S_1$ , where the pH decreased by 1.6 units (Fig. 1) and the produced lactic acid was 2 g/L (Fig. 2). Consequently, utilisation of sugar and free amino acid decreased (12.5 %, Fig. 3, and 14.3 %, Fig. 4, respectively).

*L. plantarum* A112 exhibited lower fermentative activity than *L. acidophilus* BGSJ15-3 in both tested substrates. The decrease in pH of about 1.6 units (Fig. 1) and the production of 2 g/L of lactic acid (Fig. 2) in substrate  $S_1$  were measured. They were accompanied by lower utilisation of sugar and free amino acids from the substrate, which resulted in poor growth of this culture.

On the contrary, considering the utilisation of sugar and free amino acid in substrate  $S_2$ , this culture exhibited almost the same activity as the *L. acidophilus* BGSJ15-3 culture. High utilisation of nutrients resulted in considerable growth of this culture (2.7 log CFU/mL, Fig. 5).

As it can be seen from the data, substrate  $S_2$  was better regarding all tested activities of *L. plantarum* A112 and *L. acidophilus* BGSJ15-3 cultures. The tested cultures showed similar acidifying activity in substrate  $S_1$ . They are similar in assimilation of sugar and free amino acids, as well as in growth. However, these cultures differed considerably in acidifying activity and growth in substrate  $S_2$ . In it, the acidifying activity of *L. acidophilus* BGSJ15-3, expressed through the production of lactic acid, is 49 % higher (Fig. 2) than that of the *L. plantarum* A112. On the contrary, *L. plantarum* A112 in this substrate grows better than the *L. acidophilus* BGSJ15-3 culture by 1.4 log CFU/mL (Fig. 5).

By consumption of sugar lactic acid is produced and bacterial biomass,  $CO_2$  and energy are created. It is obvious that *L. acidophilus* BGSJ15-3 consumed the sub-

strate for the production of lactic acid (Fig. 2) in the first place, while *L. plantarum* A112 consumed it for the growth of cells.

The results obtained through investigations in this paper have confirmed that the extract of brewer's yeast has a stimulating effect on acidifying activity and growth of lactic acid bacteria. This is in accordance with our previous results and literature data as well (13,21,22). The positive influence of brewer's yeast autolysate can be explained by the fact that it contains many nutrients and biofactors used by most microorganisms, especially those like lactic acid bacteria, which are dependant on the organic sources of nitrogen, and vitamins, particularly those from the B group (14,15,23).

The product obtained in this investigation can be used as a functional additive in a form of a beverage after pasteurisation, or else it may be dried and used in form of powder or tablets. Our previous investigations showed that similar products could be dried using the technique of fluidised bed drying, when about 30 % of bacterial cells of genera *Lactobacillus* and *Bifidobacterium* survive (5). Such a product is of a particular importance, as the strains from the above mentioned bacterial species possess probiotic properties important for human health. Further investigations initiated in this paper have as their goal an investigation of the fermentation of combined beetroot juice and brewer's yeast autolysate with mixed cultures of *L. plantarum* A112 and *L. acidophilus* BGSJ15-3, as well as the investigation of the possibility of drying the obtained product using the technique of fluidised bed drying.

## Conclusion

Based on the results obtained by comparative investigation of fermentative activity of three lactic acid bacteria cultures, *L. plantarum* A112, *L. acidophilus* BGSJ15-3 and *L. acidophilus* NCDO1748, in substrates  $S_1$  and  $S_2$ , the following has been established: good fermentative activity and growth in both substrates were shown by two cultures, *L. plantarum* A112 and *L. acidophilus* BGSJ15-3; considerably better results were obtained in substrate  $S_2$ , which confirms that brewer's yeast autolysate stimulated the activity of those bacterial cultures. Therefore, bacterial cultures *L. plantarum* A112 and *L. acidophilus* BGSJ15-3 can be successfully used for fermentation of a mixture of beetroot juice and brewer's yeast autolysate in order to obtain a functional food additive.

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## Utjecaj autolizata pivskoga kvasca i laktobacila na vrenje soka cikle za proizvodnju dodatka funkcionalnoj hrani

### Sažetak

Budući da funkcionalna hrana u svijetu ima sve veće značenje i da se intenzivno razvijaju postupci za njezinu proizvodnju, svrha je ovoga rada bila doprinos optimiranju postupka proizvodnje dodatka funkcionalnoj hrani na bazi soka cikle (*Beta vulgaris* L.) koristeći autolizat pivskoga kvasca. Da bi se poboljšala hranjiva i zaštitna svojstva proizvoda te da bi se on mogao konzervirati, ispitana je mogućnost fermentacije soka cikle bakterijama mliječno-kiselog vrenja iz roda *Lactobacillus*. Provedena su komparativna ispitivanja triju bakterijskih kultura *L. plantarum* A112, *L. acidophilus* BGSJ15-3 i *L. acidophilus* NCDO1748, tijekom fermentacije u dvije podloge, u soku cikle te mješavini soka cikle i autolizata pivskoga kvasca u jednakim omjerima. Najslabiju fermentativnu aktivnost i rast u oba supstrata pokazala je kultura *L. acidophilus* NCDO1748. Ostale su dvije kulture imale bolju fermentativnu aktivnost u smjesi ispitivanih supstrata, pri čemu je acidifikacijska aktivnost (proizvodnja mliječne kiseline i snizivanje pH-vrijednosti) kulture *L. acidophilus* BGSJ15-3 bila kudikamo bolja od kulture *L. plantarum* A112. Što se tiče rasta navedene su se kulture ponašale obrnuto. Na osnovi dobivenih rezultata zaključeno je da se ispitivane kulture *L. plantarum* A112 i *L. acidophilus* BGSJ15-3 mogu uspješno koristiti za fermentaciju mješavine soka cikle i autolizata pivskoga kvasca radi dobivanja dodatka funkcionalnoj hrani.