UDC 663.81:543.94
APTEFF, 36, 1-266 (2005) BIBLID: 1450–7188 (2005) 36, 71-80
Original scientific paper

LACTIC ACID FERMENTATION IN VEGETABLE JUICES SUPPLEMENTED WITH DIFFERENT CONTENT OF BREWER'S YEAST AUTOLYSATE

Marica B. Rakin, Josip K. Baras and Maja S. Vukašinović

The work is concerned with the conditions for lactic acid fermentation in a mixture of beetroot (Beta vulgaris L.*) juice and carrot (Daucus carota* L.*) juice and different content of brewer's yeast autolysate with Lactobacillus plantarum A112 and with Lactobacillus acidophilus NCDO 1748.Both cultures showed good bioc hemical activity in these mixtures. The production of lactic acid has been stimulated using the higher content of brewer's yeast autolysate. In these mixtures, L. plantarum A112 has shown better growth and lactic acid production than L. acidophilus NCDO 1748.*

KEYWORDS: Beetroot; carrot; lactic acid fermentation; autolysate of brewer's yeast

INTRODUCTION

During the closing decades of the 20th century people became more and more interested in food, its composition and role in preservation of human health. The relation between nutrition and degenerative diseases has been studied and numerous recommendations for preferable consumption of certain nutrients have been given (1). According to the Food and Nutrition Board of the American Academy of Sciences, the group of functional foods consists of food-industry products with potentially favorable effects, which may provide health effect exceeding the one characteristic for traditional food (2). In order to accomplish this goal, one must start from the fact that no natural food raw-material satisfies all of the nutritive and protective needs of a human being. Hence, the individual natural food raw materials are combined in order to obtain the foods of preferable composition. At the same time, the investigations have been carried out in order to establish the technological processes which would enable preservation of the value of starting raw-materials and, if possible, addition of the missing but preferable qualities. In modern technology of food

Dr. Marica Rakin, Senior Teaching Assist., Dr. Maja S. Vukašinović, Assist. Prof., Dr Josip Baras, Prof., Faculty of Technology and Metallurgy, 11000 Belgrade, Karnegijeva 4, Serbia and Montenegro

production, a significant contribution to this goal may be accomplished by applying biological processes of production and transformation.

Fermentation of foods is one of the oldest known biotechnologies. The most important bacteria used for the fermentation of foodstuffs are lactic acid bacteria. Their selection depends on desired qualities of the end product, raw material properties and applied technological process.

Vegetable belongs to the group of protective foods. The most important reasons for its use in human nutrition are as follows: attainment of good health condition, prevention of a number of diseases, attainment of balanced nutrition, rich and inexpensive source of vitamins, minerals and carbohydrates. Vegetable juices are more easily assimilated in an organism than fresh vegetable, as the process of squeezing destroys the structure of fibers and releases bound phytonutrients.

In the present work, we started from the point of view that, from the aspect of nutritive and protective quality of the product obtained, it would be suitable to adopt a mixture of beetroot and carrot juice with brewer's yeast autolysate for a starting raw material, and to subject this mixture to lactic acid fermentation for the sake of further improvement of quality and stabilization of the mixture. According to the data available in the literature (3,4) and based on our investigations (5,6), it has been proven that the extract of brewer's yeast positively influences the rate of fermentation and activity of lactic acid bacteria in various substrates. That is explained by the fact that the extract of brewer's yeast is a rich source of nutrients and growth factors needed for the lactic acid bacteria successful growth and activity. It was important that a fermented product should contain an adequate number of viable cells of the lactic acid bacteria, which would ensure their positive effect on human health. In that way, the product obtained would significantly approach the qualities of functional food.

EXPERIMENTAL

Substrate for fermentation

Beetroot and carrot juices were prepared by slicing fresh vegetable, followed by separation of juice in a juicer. After that the juices were heated at 70°C for 20 min. Brewer's yeast autolysate produced in the process described in a previous paper (7), was poured into mixture of vegetable juices. The following samples were prepared (Table 1).

Bacteria culture

For fermentation of the above mixtures the following cultures were used: *Lactobacillus plantarum* A112 and *Lactobacillus acidophilus* NCDO 1748. Tested cultures were propagated in MRS broth. Starting count of bacteria in the inoculated juices was of the order of magnitude 10^5-10^6/ml of substrate. Temperature of fermentation was 37°C, while the time of fermentation was 8 h.

Methods

The progress of fermentation was observed by determining the increase in the cell count on MRS agar plate using standard method of decimal dilution (8). The following parameters were determined: concentration of produced lactic acid (titrimetric), sugar con-

Table 1. Samples of vegetables juices and brewer's yeast autolysate used in experiments

Sample 1	A mixture of carrot and beetroot juices and brewer's yeast autolysate (1 part of mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate calculated on dry matter), with *Lb. plantarum* A112
Sample 2	A mixture of carrot and beetroot juices with brewer's yeast autolysate (2 part of mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate, calculated on dry matter), with *Lb. plantarum* A112
Sample 3	A mixture of carrot and beetroot juices with brewer's yeast autolysate (1 part of mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate, calculated on dry matter), with *Lb. acidophilus* NCDO 1748
Sample 4	A mixture of carrot and beetroot juice with brewer's yeast autolysate (2 part of mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate, calculated on dry matter), with *Lb. acidophilus* NCDO 1748

centration (spectrophotometry with anthrone) and concentration of free amino nitrogen (spectrophotometry with ninhydrin) (9).

RESULTS AND DISCUSSION

The content of lactic acid produced by the lactic acid fermentation, depends on type of lactic acid bacterial applied, available sugar and other substances present in the substrate, which support that production (10,11). According to the results (Fig. 1), these bacterial cultures are characterized with higher speed of acidification in the samples with higher content of brewer's yeast autolysate. The best production of lactic acid was attained in sample 1, where fermentation of beetroot and carrot juices with brewer's yeast autolysate was carried out at a ratio of 1:1, with *L. plantarum* A112. There is a difference in the production of lactic acid between the bacteria species that were tested. Thus, *L. plantarum* A112 in these mixtures of vegetable juices and various quantities of free amino acids produced larger quantity of lactic acid than *L. acidophilus* NCDO 1748. In comparison to our preliminary investigation [5], *L. acidophilus* NCDO 1748 produced larger quantity of lactic acid than obtained by individual fermentation of beetroot juice and carrot juice with brewer's yeast autolysate.

The growth and biosynthesis of the cell components require a source of nitrogen. Based on the presented results of the change in free amino nitrogen content during fermentation (Fig. 2), no significant differences between analyzed samples were observed. The greatest consumption of available nitrogen was observed in sample 3. The reason for the absence of complete correlation between the growth and consumption of nitrogen may be found in the fact that brewer's yeast undergoes the autolysis even at fermentation temperature of 37°C, forming free amino acids.

Production of lactic acid during fermentation is proportional to sugar catabolism. Utilization of sugar during fermentation varied from 19.4 to 24.1%. Maximum utilization was shown in sample 1, with higher content of autolysate of brewer yeast and with *L. plantarum* A112 (Fig. 3).

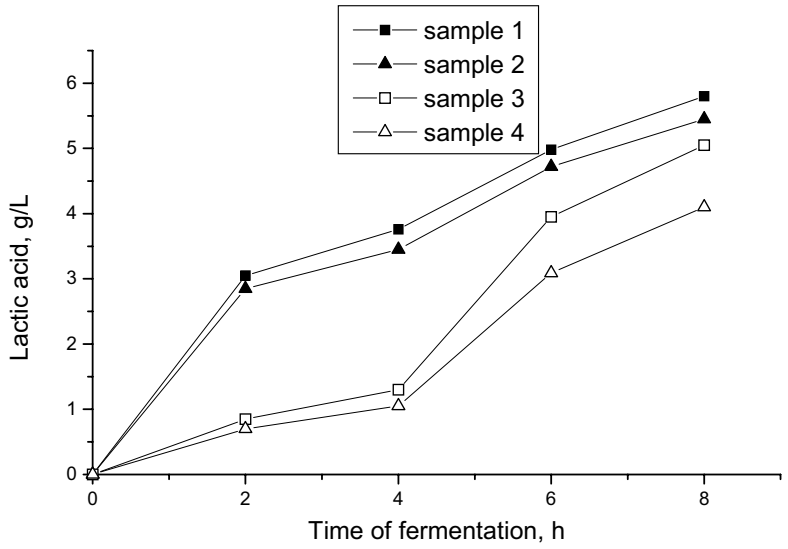

Fig. 1. The content of lactic acid during lactic acid fermentation in different samples: sample 1- mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) with *L. plantarum* A112, sample 2 – mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1) with *L. plantarum* A112, sample 3- mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) with *L. acidophilus* NCDO 1748, sample 4- mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1) with *L. acidophilus* NCDO 1748

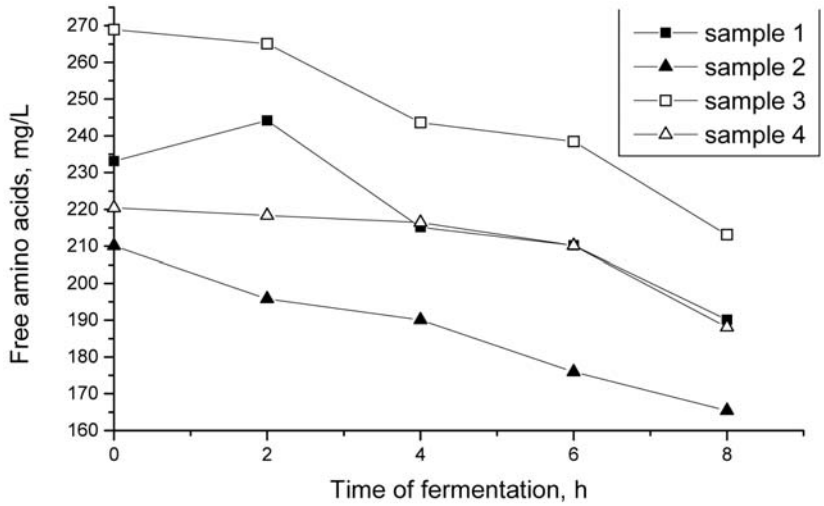

Fig. 2. The change of free amino acids during lactic acid fermentation in different samples; samples as in Fig. 1

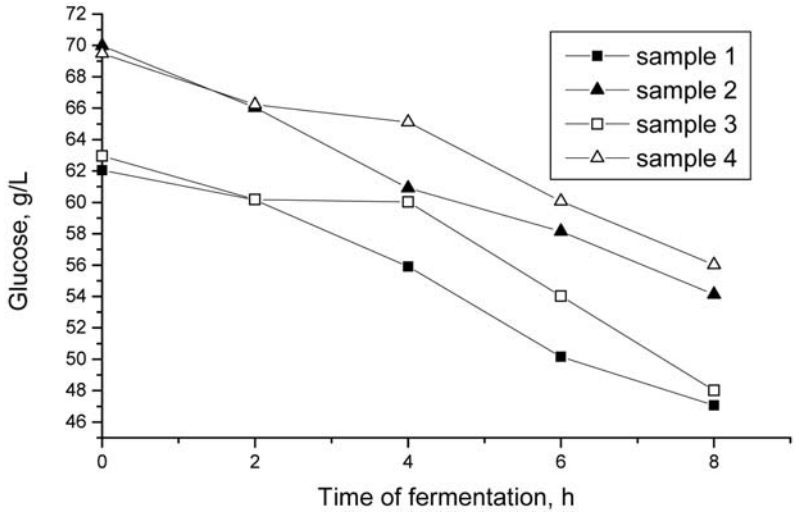

Fig. 3. The concentration of glucose during lactic acid fermentation in different samples; samples as in Fig. 1

From the results related to the increase of the cell count during fermentation, one can observe an increase in the samples 1 and 2 in comparison with that in the samples 3 and 4 (Fig. 4). The increase in the samples 1 and 2 was 1.6 and 1.4 log CFU/mL, while in the samples 3 and 4 it was 1.15 and 1.14 log CFU/mL, indicating that *L. plantarum* A112 grows faster in these samples than *L. acidophilus* NCDO 1748.

For a more straightforward of the comparative relationships of the growth and production of lactic acid using bacterial cultures *L. plantarum* A112 and *L. acidophilus* NCDO 1748, the Luedeking & Piret model was applied (12,13,14). According to this model, the rate of lactic acid formation (dP/dt) could be related to the rate of bacterial growth (dN/dt), and to the bacterial density (N), throughout the fermentation time at a given pH, by the expression:

$$dP/dt = \alpha\, dN/dt + \beta N \qquad [1]$$

where the constants α and β are determined by the pH of the fermentation.

After dividing by N, the equation is modified to:

$$1/N \cdot dP/dt = \alpha/N \cdot dN/dt + \beta \qquad [2]$$

Since, by definition $\mu = 1/N \cdot (dN/dt)$ the equation finally reduces to

$$q_P = \mu\alpha + \beta \qquad [3]$$

where q_p – specific rate of lactic acid production (g N^{-1} h^{-1}), μ - specific growth rate (h^{-1}), dN/dt – rate of bacterial growth, dP/dt – rate of lactic acid formation, α and β - constants.

For measuring bacterial growth, optical density that measures bacterial density is preferred to plate counting. However, in this work it was not possible because the substrate was colored. Therefore, plate counting technique was used.

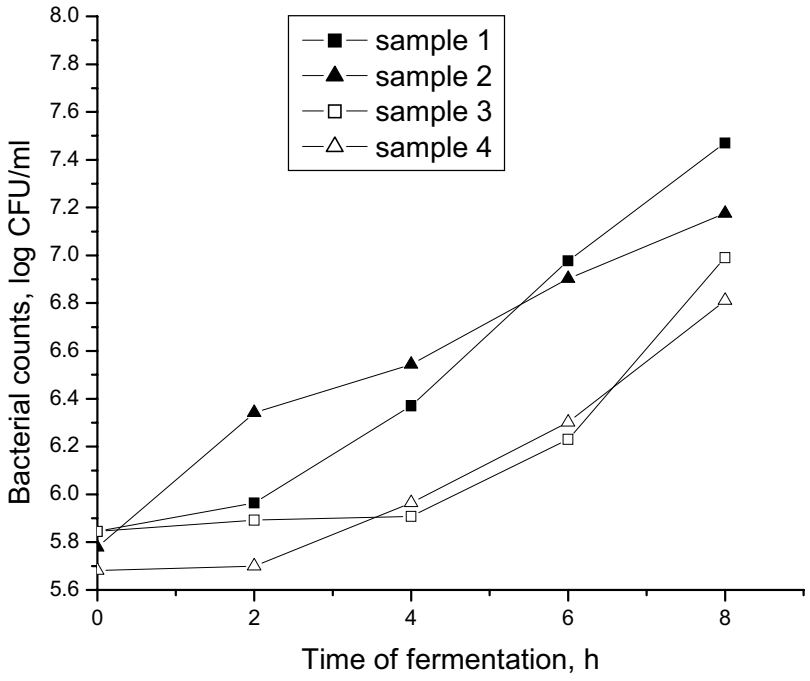

Fig. 4. The changes in the count of colony forming units (CFU) during lactic acid fermentation in different samples; samples as in Fig. 1

Simplified presentation of the above model relates to the linear part of equation [1] which is presented as:

$$(p\text{-}p_0) = \alpha\ (x\text{-}x_0) \qquad [4]$$

where p_0 and p are concentrations of lactic acid (g/L) in the initial period of time and at time t, and x_0 and x are the increases of biomass (log CFU/mL) in the initial period of time and at time t.

By graphic presentation of this dependence, we obtained the data showing the correlation between the production of lactic acid and growth of lactic acid bacteria. In Figs. 5a and 5b one can observe that *L.plantarum* shows better linear correlation of the growth and production of lactic acid than *L.acidophilus*. The deviations from the linear dependence are mostly caused by nutritive limitations of the substrates, and are related to the specific bacterial species (14).

Based on these results we can conclude that the differences in the quantity of autolysate of brewer yeast did not significantly affect the process of fermentation of lactic acid bacteria, as there was a sufficient amount of available free amino acids in both samples. Slightly slower growth of *L. acidophilus* NCDO1748 in the samples can be explained by their very specific growth requirements. *Lactobacilli* have very complex growth requirements regarding the content of sugar, proteins, thioamino acids, vitamins of B complex and minerals such as magnesium, manganese and iron (15,16,17). Individual juices of beetroot and carrot have different contents of certain minerals such as P, Ca, K, Na and

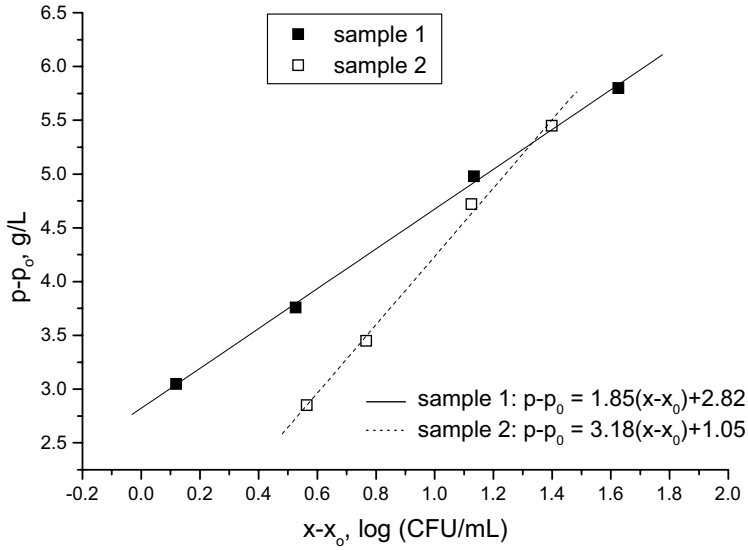

Fig. 5a. Production of lactic acid by *L. plantarum* A112 growing on: mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) and mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1)

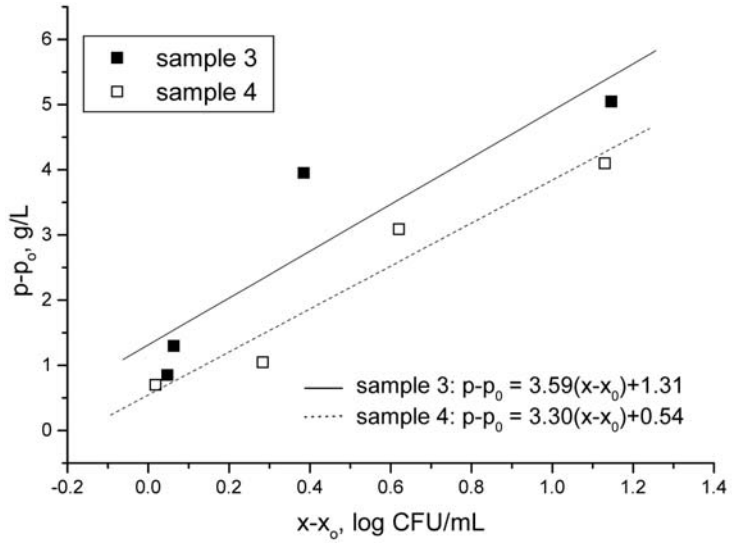

Fig. 5b. Production of lactic acid by *L. acidophilus* NCDO 1748 growing on: mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) and mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1)

Fe in comparison with their mixture. Higher content of autolysate of brewer's yeast in the samples 1 and 3 enhanced the production of lactic acid, which is in accordance with the data from the literature (3,4). Arasaratnam (3) states that the extract of yeast, in addition to the vitamins of B complex, contains other components that support production of lactic acid. Besides, brewer's yeast, Aeschlimann (4) studied the effects of other extracts such as corn steep liquor (CSL) and malt extract, and concluded that the extract of brewer's yeast provided best growth of biomass and production of lactic acid.

The products obtained in this study might be used as functional additives in the form of soft drink after fermentation, or in some other, more convenient technological form. Our previous investigations showed that similar products might be dried using the technique of spout-fluid bed drying, when approx. 30% of bacteria from *Lactobacillus* and *Bifidobacter* survive (18).

In this study the priority is given to the mixture of beetroot and carrot juice with brewer's yeast autolysate in the ratio 1:1, calculated on dry matter. Such product is of a particular importance, as, in addition to lactic acid and lactic acid bacteria, it contains valuable matters from beetroot and carrot juices and brewer's yeast autolysate, as well.

CONCLUSION

Based on the results obtained by investigation of biochemical activity the bacteria of lactic acid fermentation, *L. plantarum* A112 and *L. acidophilus* NCDO 1748, in the samples based on a mixture of beetroot and carrot juices with various contents of brewer's yeast autolysate, it can be concluded that better production of lactic acid is achieved in the samples with higher content of brewer's yeast autolysate (samples 1 and 3), no matter which bacterial cultures were used. *L. plantarum* A112 showed better growth and production of lactic acid than *L. acidophilus* NCDO 1748. Thus the priority is given to *L. plantarum* A112 and to the mixture of beetroot and carrot juices with brewer's yeast autolysate in a ratio of 1:1.

REFERENCES

1. Select Committee on Nutrition and Human Needs, Dietary Goals for the United States, United States Senat, Washington, DC, US Government Printing Office, 1977, pp 104.
2. Roberfroid M.B.: Prebiotics and probiotics: are they functional food. Am. J. Clin. Nutr. **71** (2000) 1682-1687.
3. Arasrtnam V., A. Sentthuran and K. Balasubramaniam: Suplementation of whey with glucose and different nitrogen sources for lactic acid production by *Lactobacillus delbrueckii.* Enzyme Microb.Tech. **19** (1996) 482-489.
4. Aeschlimann A. and U. von Stocar: The effect of yeast extract supplementation on the production of lactic acid from whey permeate by *Lactobacillus helveticus.* Appl. Microbiol. Biotechnol. **32** (1990) 398-404.
5. Rakin M., J. Baras, M. Vukašinović: The influence of brewer's yeast autolysate and lactic acid bacteria on the production of functional food additive based on beetroot juice fermementation. Food Technol. Biotech. **42** (2004) 109-113.

6. Dimitrijević S. and J. Baras: Comparative study on biochemical activity of the intestinal isolate Lactobacillus sp.V3 and Bifidobacter sp. A71 in different substrates. J. Serb. Chem. Soc**. 66** (2001) 581-587.
7. Baras J., M. Maslić i L. Turubatović: II.deo Kinetika hidrolize pivskog kvasca Pivarstvo **29** (1998) 23-28.
8. Herbert D., P.J. Phipps, R.E. Strange: Chemical analysis of microbial cells. Methods Enzimol. **5B** (1971) 265-308.
9. Trajković J., M. Mirić, J. Baras, S. Šiler: Analiza zivotnih namirnica, Tehnološko-metalurski fakultet, Univerzitet u Beogradu, 1983.
10. Gardner N.J., T. Savard, P. Obermeier, G. Caldwell and C. P. Champagne: Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. Int. J. Food Microbiol. **64** (2001) 261-268.
11. Carr F.J., D. Chill, N. Maida: The Lactic Acid Bacteria: Literature Survey. Crit. Rev. Microbiol. **28** (1998) 281-370.
12. Amarane A., Y. Prigent: Growth and lactic acid production coupling for *Lactobacillus helveticus* cultivated on supplemented whey: Influence of peptidic nitrogen deficiency. J. Biotechnol. **55** (1997) 1-9.
13. Amarane A., Y. Prigent: Analysis of growth and production coupling for batch cultures of *Lactobacillus helveticus* with help of anunstructurated model. Process Biochem. **34** (1999) 1-10.
14. Schepers A.W., J. Thibault, C. Locroix: *Lactobacillus helveticus* growth and lactic acid production during pH-controlled batch cultures in whey permeate/yeast extract medium. Part I. Multiple factor kinetic analysis. Enzyme Microb. Tech. **30** (2002) 176-186.
15. Gomes A.M.P. and F. X. Malcata: Bifidobacterium spp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotic. Trends Food Sci.Tech. **10** (1999) 139-146.
16. Sharpe M.E.: Lactic Acid Bacteria in the dairy industry. J. Soc.of DairyTechnology **32** (1979) 9-17.
17. Dziezak J.D.: Yeast and Yeast Derivates: Definitions, Characteristrics and Processing. Food Technol. **41** (1987) 104-114.
18. Baras J., S. Dimitrijević, D. Povrenović and L.Turubatović: Biotechnological processes in modification of agricultural products - 6th International Conference, Moscow, 5-6 December, 279-284 (2002).

МЛЕЧНО-КИСЕЛА ФЕРМЕНТАЦИЈА СОКОВА ПОВРЋА ОБОГАЋЕНИХ РАЗЛИЧИТИМ САДРЖАЈЕМ АУТОЛИЗАТА ПИВСКОГ КВАСЦА

Марица Б. Ракин, Јосип К. Барас и Маја С. Вукашиновић

У раду су испитивани услови за млечно-киселу ферментацију са *Lactobacillus plantarum* A112 и *Lactobacillus acidophilus* NCDO1748 смеша сока цвекле (*Beta vulgaris* L.) и сока мркве (*Daucus carota* L.) са различитим садржајем аутолизата пивског квасца.

Обе културе показале су добру биохемијску активност у тим смешама. Производња млечне киселине била је стимулисана вишим садржајем аутолизата пивског квасца. У смешама сокова поврћа са вишим садржајем аутолизата пивског квасца *L. plantarum* A112 показао је бољи раст и производњу млечне киселине него *L. acidophilus* NCDO1748.

Received 22 April 2005
Accepted 6 September 2005