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The examination of parameters for lactic acid fermentation and nutritive value of fermented juice of beetroot, carrot and brewer's yeast autolysate

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Abstract: The conditions for lactic acid fermentation based on a mixture of beetoot juice (*Beta vulgaris* L.) and carrot juice (*Daucus carota* L.) and different content of brewer's yeast autolysate with *Lactobacillus plantarum* A112 and with *Lactobacillus acidophilus* NCDO 1748 has been studied. Both cultures showed good biochemical activity in these mixtures. The production of lactic acid has been stimulated using a higher content of brewer's yeast autolysate. In these mixtures, *L. plantarum* A112 showed better growth and lactic acid production than *L. acidophilus* NCDO 1748. From the data obtained through chemical analyses of the fermented products, it can be seen that the mixture of beetroot and carrot juice and brewer's yeast autolysate is richer in minerals (Ca, P, Fe) and β-carotene than fermented beetroot juice with the same content of brewer's yeast autolysate.

Keywords: beetroot, carrot, lactic acid fermentation, autolysate of brewer's yeast, nutrition.

INTRODUCTION

During the closing decades of the 20th century people became more and more interested in food, its composition and its role in the preservation of human health. The relation between nutrition and degenerative diseases has been studied and numerous recommendations for preferable consummation of certain nutrients has 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 a health effect exceeding the one characteristic for traditional food.[2] In order to accomplush this goal, one must start from the fact that no natural food raw-material satisfies all the nutritive and protective necessities of a human being. Therefore, the individual natural food raw

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materials are combined, in order to obtain the foods of preferable composition. Simultaneously, investigations have been carried out in order to establish technological processes which would enable the preservation of the value of starting raw-materials and, if possible, addition of the missing but preferable qualities. In modern technology of food 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 foods are lactic acid bacteria. Their selection depends on desired qualities of an end product, raw material properties and applied technological process.

Vegetables belong to the group of protective foods. The most important reasons for their use in human nutrition are as follows: attainment of good health condition, prevention of a series 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 vegetables, as the squeezing process destroys the fiber structure and releases bound phytonutrients.

In 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 advantageous to adopt a mixture of beetroot and carrot juice with brewer's yeast autolysate as 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 previous investigations,[5,6] it has been shown that the extract of brewer's yeast positively affects the rate of fermentation and activity of lactic acid bacteria in various substrates. This was explained by the fact that the extract of brewer's yeast is a rich source of nutrients and growth factors necessary for lactic acid bacteria for successful growth and activity. It is 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 this way, the obtained product would significantly approach the qualities of functional food.

EXPERIMENTAL

Substrate for fermentation

Beetroot and carrot juices were prepared by slicing the fresh vegetables, followed by separation of the juice in a juice maker. Subsequently, the juices were pasteurized at 70 ºC for 20 min. The brewer's yeast autolysate, produced in the process described in a previous paper,[7] was poured into the pasteurized mixture of vegetable juices. The following samples were prepared:

Sample 1 – A mixture of carrot and beetroot juices and brewer's yeast autolysate (1 part of the mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate calculated on the dry matter), with *L. plantarum* A112;

Sample 2 – A mixture of carrot and beetroot juices with brewer's yeast autolysate (2 parts of the mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate, calculated on the dry matter), with *L. 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 the dry matter), with *L. acidophilus* NCDO 1748;

Sample 4 – A mixture of carrot and beetroot juice with brewer's yeast autolysate (2 parts of the mixture of beetroot and carrot juice : 1 part of brewer's yeast autolysate, calculated on the dry matter), with *L. acidophilus* NCDO 1748.

Bacteria culture

For the fermentation of the above mentioned mixtures the following cultures were used: *Lactobacillus plantarum* A112 and *Lactobacillus acidophilus* NCDO 1748. The tested cultures were propagated in MRS broth. Starting number of bacteria in inoculated juices was of the order of magnitude 10^5–10^6/ml of substrate. The temperature of fermentation was 37 ºC, while the time of fermentation was 8 h.

The progress of fermentation was monitored by determining the increase in the number of cells on an MRS agar plate using the standard method of decimal dilution. The following parameters were determined: concentration of produced lactic acid (titrimetric), sugar concentration (spectrophotometry with anthrone) and concentration of free amino acids (spectrophotometry with ninhydrin).

Analysis of the chemical content was done in sample 1 (fermented product 1), which was the best regarding the production of lactic acid and bacterial growth, and was done as well in the individually fermented beetroot juice and individually fermented carrot juice with the same content of brewer's yeast autolysate (fermented products 2 and 3, respectively) under the same experimental conditions. The analysis was carried out using standard analytical methods.[9] The content of vitamins was analyzed by applying reverse-phase liquid chromatography[10] and the content of mineral matters was analyzed by applying atomic absorption spectrophotometry.[11]

RESULTS AND DISCUSSION

The content of lactic acid produced by the lactic acid fermentation, depends on type of lactic acid bacterial applied, the amount of available sugar present in the substrate and on other substances present in the substrate which support the production.[12,13] According to the results shown in Fig. 1, these bacterial cultures are characterized with a higher rate of acidification in the samples with the higher content of brewer's yeast autolysate. The best production of lactic acid was attained in sample 1, where the fermentation of beetroot and carrot juices with brewer's yeast autolysate was performed at a ratio of 1:1, with *L. plantarum* A112. There is a difference in the production of lactic acid between the two bacteria species that were tested. Thus, *L. plantarum* A112 in these mixtures of vegetable juices and various quantities of free amino acids produces a larger quantity of lactic acid than *L. acidophilus* NCDO 1748. In comparison to preliminary investigation,[5] *L. acidophilus* NCDO 1748 produces a larger quantity of lactic acid in the mixture of juices 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 the free amino acids content during fermentation (Fig. 2), no significant differences between the analyzed samples were observed. The greatest consumption of available nitrogen was observed in sample 3. The reason for the absence of a complete correlation between the growth and the consump-

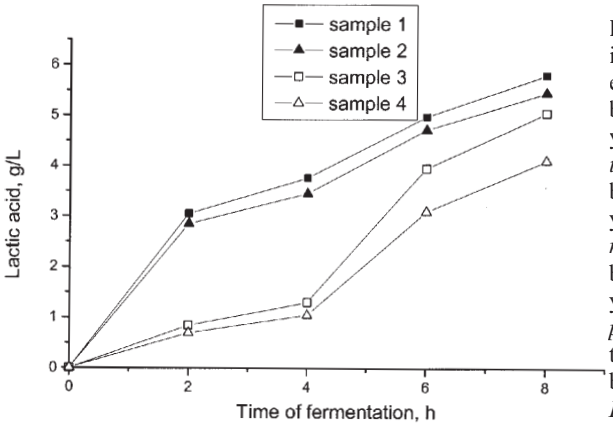

Fig. 1. The content of lactic acid during lactic acid fermentation in different samples: sample 1 – mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) with *L. plantarum* A112; sample 2 – mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1) with *L. plantarum*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

tion of nitrogen may be found in the fact that brewer's yeast undergoes the autolysis even at ferementation temperature of 37 ºC, forming free amino acids.

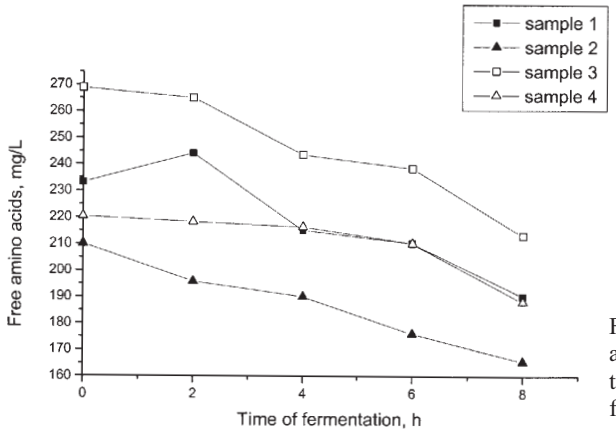

Fig. 2. The change of content of free amino acids during lactic acid fermentation in different samples. Samples as for Fig. 1.

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

From the results related to the increase in the number of cells during fermentation, noticeable increases in samples 1 and 2 in comparison with those in samples 3 and 4 can be observed (Fig. 4). The increase in the samples 1 and 2 was 1.6 and 1.4 log CFU/mL, respectively, while in the samples 3 and 4 it was 1.15 and 1.14 log CFU/mL, respectively, indicating that *L. plantarum* A112 grows better in these samples than *L. acidophilus* NCDO 1748.

For a better presentation of the comparative relationships of the increase in the number of cells and production of lactic acid using bacterial cultures *L. plantarum* A112 and *L. acidophilus* NCDO 1748, the Luedeking & Piret model was ap-

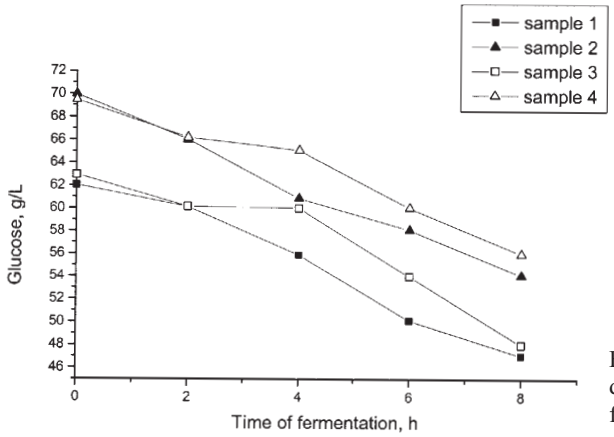

Fig. 3. The concentration of glucose during lactic acid fermentation in different samples. Samples as for Fig. 1.

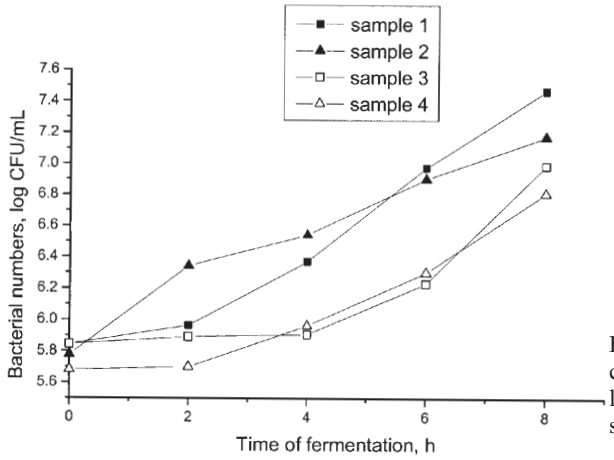

Fig. 4. The changes in the number of colony forming units (CFU) during lactic acid fermentation in different samples. Samples as for Fig. 1.

plied.[14–16] According to this model the instantaneous rate of lactic acid formation (dP/dt) can be related to the instantaneous rate of bacterial growth (dN/dt), and to the bacterial density (N), throughout a fermentation at a given pH, by the expression:

$$dP/dt = \alpha\, dN/dt + \beta N \tag{1}$$

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

After dividing by N the equation is modified to:

$$1/N \cdot dP/dt = \alpha/N \cdot dN/dt + \beta \tag{2}$$

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

$$q_P = \mu\alpha + \beta \tag{3}$$

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

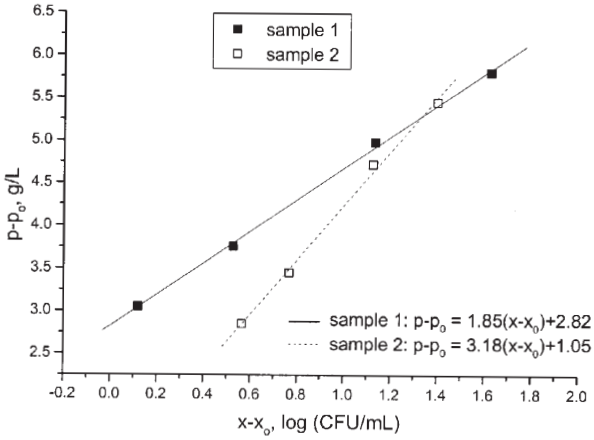

Fig. 5a. Production of lactic acid by *L. plantarum* A112 growing on: a mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) and mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1).

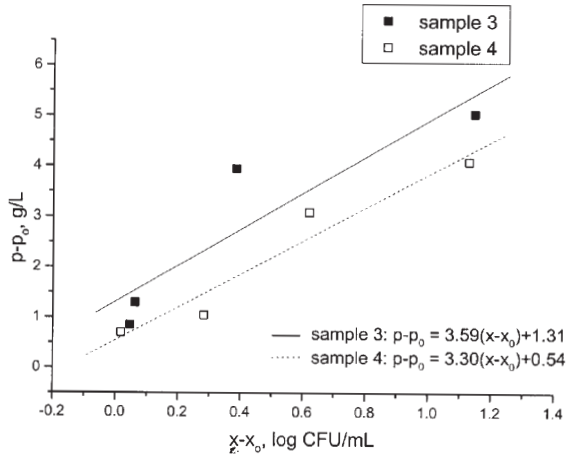

Fig. 5b. Production of lactic acid by *L. acidophilus* NCDO 1748 growing on: mixture of beetroot and carrot juice and brewer's yeast autolysate (1:1) and mixture of beetroot and carrot juice and brewer's yeast autolysate (2:1).

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

Simplified presentation of the above model relates to the linear part of Eq. (1) which is presented as:[16]

$$(p - p_0) = \alpha\ (x - x_0) \tag{4}$$

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

Graphical presentation of these data (Fig. 5) shows the connection between the lactic acid production and the lactic acid bacteria growth. In Figs. 5a and 5b one can observe that *L. plantarum* shows better linear correlation of the growth and the 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.[16]

Based on these results it can be concluded that the differences in the quantity of the autolysate of brewer's yeast did not significantly affect the process of fermentation of lactic acid bacteria, as there was 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 the B complex and minerals such as magnesium, manganese and iron.[17–19] The individual juices of beetroot and carrot have got different contents of certain minerals such as P, Ca, K, Na and Fe in comparison with a mixture of carrot and beetroot juice.[15] The higher content of autolysate of brewer's yeast in the samples 1 and 3 improved the production of lactic acid, which is in accordance with data from the literature.[3,4] Arasaratnam[3] states that yeast extract, in addition to the vitamins of the B complex, possesses other components that support the production of lactic acid. In addition to the effects of brewer's yeast, Aeschlimann[4] studied the effects of other extracts, such as corn steep liquor (CSL) and the extract of malt, and concluded that the extract of brewer's yeast provided the best results regarding the growth of biomass and the production of lactic acid.

The basic chemical analysis of the mixture of fermentation products of the mixture of beetroot and carrot juices with brewer's yeast autolysate and of the individual beetroot and carrot juices with brewer's yeast autolysate are given in Table I. The results show that there is no significant difference between the fermentation products. 34–40 % of the total dry matter consists of proteins and 40 % are carbohydrates. The content of proteins is 15 % higher in the fermented beetroot juice with brewer's yeast autolysate (fermented product 2) than in the fermented carrot juice with brewer's yeast autolysate (fermented product 3). The fermented carrot juice contains more cabohydrates and ashes than does the beetroot juice. This indicates that a mixture of carrot and beetroot juices with autolysate of brewer's yeast may represent a product of higher quality than the individual juices.

As far as minerals are concerned, fermented carrot juice with autolysate of brewer's yeast is richer in Ca, P and Fe (Table II). According to the recommendation of the Food and Nutrition Board, the needs of an adult are 800–1000 mg of Ca, 10–15 mg of Fe and 800–1000 mg of P, which may be found in fermented carrot juice with autolysate of brewer's yeast (1000 ml).[20] All the samples have a favorable ratio of K and Na (approx. 3–3.5:1). The content of Fe in fermented beetroot juice is lower than that in fermented carrot juice, due to the content of Fe and some other minerals (Ca and P) being lower in beet root than in beet leaf. The content of Mg in all samples is very close to the recommended daily consumption (280 mg), and is necessary for the metabolism of Ca and C, P, Na and K vitamins. The content of P in the fermented products mainly originates from the brewer's yeast, as it is higher than in fresh beetroot and carrot.

TABLE I. The chemical content of the fermented samples based on vegetable juice and brewer's yeast autolysate

Parameters	Fermented product 1	Fermented product 2	Fermented product 3
Water (g/100 mL)	90.02	90.06	89.97
Proteins (g/100 mL)	3.88	4.05	3.42
Carbohydates (g/100 mL)	4.18	4.13	4.25
Lipids (g/100 mL)	1.39	1.32	1.45
Ash (g/100 mL)	0.43	0.33	0.47

Fermented product 1 – fermented mixture of carrot and beetroot juice and brewer's yeast autolysate in the ratio 1:1
Fermented product 2 – fermented beetroot juice and brewer's yeast autolysate in the ratio 1:1
Fermented product 3 – fermented carrot juice and brewer's yeast autolysate in the ratio 1:1

TABLE II. The content of minerals and vitamins in the fermented samples based on vegetable juice and brewer's yeast autolysate

Parameters	Fermented product 1	Fermented product 2	Fermented product 3
Ca (g/L)	0.310	0.180	0.580
Mg (g/L)	0.241	0.236	0.270
Na (g/mL)	0.625	0.625	0.500
K (g/L)	1.850	1.875	1.720
Fe (g/L)	0.008	0.007	0.0095
P (g/L)	0.714	0.595	0.9523
Vitamin C (mg/L)	91.6 ± 10 %	102.5 ± 10 %	82.5 ± 10 %
Vitamin B_1 (mg/L)	1.66 ± 20 %	1.77 ± 20 %	1.7 ± 20 %
Vitamin B_2 (mg/L)	2.12 ± 10 %	1.84 ± 10 %	2.12 ± 10 %
Vitamin B_6 (mg/L)	0.28 ± 10 %	0.26 ± 10 %	0.29 ± 10 %
β-carotene (mg/L)	2.6 ± 10 %	1.3 ± 10 %	3.6 ± 10 %

Fermented product 1 – fermented mixture of carrot and beetroot juice and brewer's yeast autolysate in the ratio 1:1
Fermented product 2 – fermented beetroot juice and brewer's yeast autolysate in the ratio 1:1
Fermented product 3 – fermented carrot juice and brewer's yeast autolysate in the ratio 1:1

The content of B vitamins in the fermented products mainly originates from the brewer's yeast, as their presence in fresh beetroot and carrot is negligible. The contents of B_1 and B_2 vitamins in the fermented product satisfy the recommended daily needs. Lower values were observed for the content of B_6 vitamin, the values being 10–20 times lower than the content in the extract of brewer's yeast. Therefore, these products should be enriched with B_6 vitamin, as vitamins from the group

B manifest their effects only if they are quantity-balanced.[21] The content of β- carotene is highest in the individual carrot juice with brewer's yeast autolysate and is higher than the recommended daily consumption (2 mg).[22] The content of vitamin C is satisfactory, as the recommended daily needs for an adult are 60 mg and 100 mg for the smokers. Vitamin C in these samples mainly originates from the vegetables; its content is higher in the suspension with beetroot juice than with carrot juice, which is in accordance with the content of vitamin C in the fresh vegetable.[20]

The products obtained in this study might be used as functional additives in the form of a 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 the bacteria from *Lactobacillus* and *Bifidobacter* survive.[23]

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

CONCLUSION

Based on the results obtained by investigating the biochemical acitivty of bacteria for lactic acid fermentation, *L. plantarum* A112 and *L. acidophilus* NCDO 1748, in samples based on a mixture of beetroot and carrot juices with various contents of brewer's yeast autolysate, the folowing can be concluded. The production of lactic acid is better in samples with a higher content of brewer's yeast autolysate (samples 1 and 3), irrespective of which bacterial culture was used. *L. plantarum* A112 showed better growth and production of lactic acid than *L. acidophilus* NCDO 1748. Thus, priority was given to *L. plantarum* A112 and to a mixture of beetroot and carrot juices with brewer's yeast autolysate in the ratio of 1:1.

Based on the chemical analysis of the fermented products, the fermented product consisting of a mixture of beetroot and carrot juices with brewer's yeast autolysate can be considered to represent a nutritive valuable product. It contains all the essential components of the initial materials enriched with valuable fermentations products.

ИЗВОД

ОДРЕЂИВАЊЕ ПАРАМЕТАРА МЛЕЧНО-КИСЕЛЕ ФЕРМЕНТАЦИЈЕ И НУТРИТИВНЕ ВРЕДНОСТИ ФЕРМЕНТИСАНОГ СОКА ЦВЕКЛЕ, МРКВЕ И АУТОЛИЗАТА ПИВСКОГ КВАСЦА

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У раду су испитивани услови за млечно-киселу ферментацију смеше на бази сока цвекле (*Beta vulgaris* L.) и мркве (*Daucus carota* L.) и различитог садржаја аутолизата пивског квасца са *Lactobacillus plantarum* A112 и *Lactobacillus acidophilus* NCDO 1748. Обе културе су показале добру биохемијску активност у испитиваним смешама. Продукција млечне киселине била је стимулисана вишим садржајем аутолизата пивског квасца. У тој смеши, *L. plantarum* A112 је показао бољи раст и продукцију млечне киселине од *L. acidophilus* NCDO 1748. Резултати добијени хемијском анализом ферментисаних производа су показали да је смеша сока цвекле и мркве и аутолизата пивског квасца богатија у садржају минерала (Ca, P, Fe) и β-каротену од појединачно ферментисаног сока цвекле са истим садржајем аутолизата пивског квасца.

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