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Survival of probiotic bacteria during lactic acid fermentation of vegetable juices

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Abstract

The general objective of this research was to perform the lactic acid fermentative processes using different probiotic bacteria species unspecific to epiphytic microbiota of vegetables, with a view to achieve new knowledge concerning the possibility of developing and preservation their viability in vegetable juices. The carrots and the red beet were evaluated as potential substrat for the production of probiotic juices by some species of lactic acid bacteria and bifidobacteria. Different concentrations of inoculum were tested and the effects of the initial cells number of starter culture on fermentation ability of vegetable juices, respectively the growth of them and lactic acid production were analyzed. All the tested strains were found capable of rapidly utilizing vegetables for cell synthesis and lactic acid production. They are produced a greater amount of lactic acid and are reduced the pH of fermented juices from an initial value of 6.4 to below 4.4 after 48 h of fermentation. The lactic cultures in fermented juices gradually lost their viability during cold storage.

Keywords: bifidobacteria, *Lactobacillus acidophilus*, vegetable juices, survival

1. Introduction

Recent social and economic developments may lead to serious health problems. Stress and the busy lifestyle of humans induce the so-called diseases of civilization such as heart attack, high-blood pressure, intestinal disorders, and various types of cancer. Improper nutrition may cause colon problems and negatively influence the immune system, but a balanced nutrient intake ensures a long and healthy life [1]. Discoveries in several areas of bioscience support the hypothesis that, beyond nutrition, diet may modulate various bodily functions [2]. These new concepts have led to the introduction of functional foods that encompass a wide range of ingredients and functional aspects [3].

One promising solution for the prevention or elimination of the mentioned diseases is the design and consumption of probiotic foods which contain living micro-organisms [4].

The beneficial effects of probiotics on the gut microbiota comprise antagonist and imunitar effects. The use of probiotic bacterial cultures stimulates the growth of preferred microorganisms, crowds out potentially harmful bacteria and reinforces the body's natural defence mechanisms (Dunne, 2001, quoted by [5]).

Most commonly probiotic supplements contain Lactobacillus acidophilus and Bifidobacterium, both of which are part of the normal intestinal microbiota.

Foods containing these microorganisms are sold in many countries, although their survival in foods is doubtful, since some of the strains are extremely sensitive to a series of factors [6].

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Also, methods for counting these organisms have not yet been well established, which is an essential requirement to determine their survival in commercial products [7].

The extreme sensitivity or thermal tolerance of Bifidobacterium to spray-drying and storage temperature has proven to be a major impediment to the effective application of these bacteria in functional foods. Some papers concluded that more attention should be directed to the identity, safety and functionality of these strains. This is also of interest to both industry and consumers [8]. The higher resistance to freezing and freeze-drying processes is one of the main choices of the probiotic strains. The results of Modesto [9] points out that the resistance to storage processes is a typical characteristic of the strain and not of the species.

Since most of the probiotic foods are dairy products, they cannot be consumed by humans who are allergic to milk proteins or have severe lactose intolerance [1]. Adding probiotics to juices is more complex than formulating in dairy products where the bacteria can be easily added to other cultures.

The standard for some foods with healthy claims for the probiotics addition is a miniumum content by 106 – 107 CFU alive probiotic bacteria/g [10].

2. Materials and Method

2.1. Vegetable juices

Fresh vegetables (carrots and red beet) were purchased from a local store of the Dambovita County (Romania). After being washed thoroughly, they were specifically processed by scrubbing and removing non-edible pieces. The prepared vegetables were transformed into juices with a domestic juice extractor. They're not supplemented with nutrients, and no water was added.

All the juices were thermal treated at 800C/10min with a view to destroy the undesirable microorganisms. The samples were rapidly cooled at 400C.

2.2. Microorganisms

Two Christian Hansen single strain cultures containing Bifidobacterium BB12, respectively Lactobacillus acidophilus LA-5 were used for juice's fermentation. Both lyophilized pure cultures were characterized as thermophilic lactic culture. Another thermophilic multiple culture, containing Bifidobacterium infantis, Lactobacillus acidophilus, Streptococcus termophilus and Lactobacillus bulgaricus (DI-PROX YBA 986) provided by Enzymes & Derivates, was used.

The fermentation temperature was 370C (the optimum temperature for all these strains).

2.3.Fermentation process

The lyophilized single pure cultures were aseptically added in proportion of 0.2g/l to the carrot juice, respectively to the red beet juice, and vigorous homogenized for 15 min. The DI-PROX culture was added in proportion of 0,3g/l to the carrot juice and also vigorous homogenized. 100ml juice from each experimental batch was distributed in sterile tubes. The anaerobiosis was created by covering the cotton stopper of the tube by metal folia. Each tube was represented a single sample and the experiments were performed in double. The lactic acid fermentation was performed in a thermostat at 370C. The samples were investigated during the lactic acid fermentation through chemical and microbiological analysis. Samples were taken at regular intervals and the colony-forming unit (CFU) or the cells number was determined. In addition, the pH was measured and the lactic acid content of the samples was determined.

2.4. Determination of microbial population

The count of L. acidophilus and DI-PROX were determined by plate count method using Man–Rogosa–Sharpe agar, enriched with cysteine, and incubation at 37 °C for 48h. Generally, all samples were prepared in serial tenfold dilution before culturing. The results were expressed as CFU/ml juice.

The bifidobacteria amount was directly counted on the microscope using a Burker-Turk counting chamber and expressed as cells/ml juice.

2.5. Assays

The pH was measured with a HACH pH-meter. Titrable acidity, expressed as g lactic acid/100mL, was determined by titration with NaOH 0.1N in the presence of phenolphthalein (in the case of the carrot juice), respectively in the presence of bromthymol blue (in the case of the red beet juice).

3. Results and Discussion

The thermal treatment of vegetable juices at the above mentioned temperature was reduced the contaminant microbiota under the detection limit, while remaining relevant from the food safety standpoint.

During lactic acid fermentation with the multiple culture containing bifidobacteria and *Lb. acidophilus* of the carrot juice the useful microorganisms counts reached 10^8 CFU/ml after 7 h and remained viable up to 24 h. Meanwhile, the pH values dropped from above 6.24 to 4.06. The correlation between these two parameters, important for the probiotic quality on the one hand, respectively for the stability of the final product on the other hand, it is showed in Figure1.

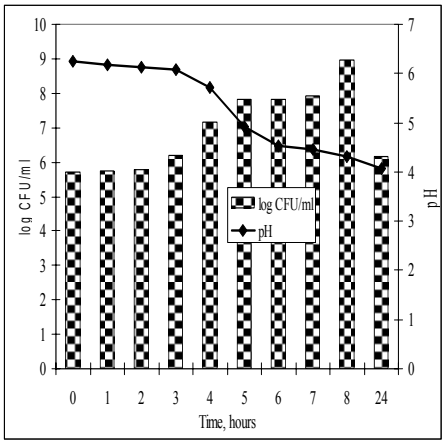

Figure 1 The dynamics of the useful bacteria and the pH values during lactic acid fermentation of carrot juice with DI-PROX

After a short lag phase, a significant increase of the count of useful microorganisms (about 30 times) was determined after 4h from the beginning of the process, this interval being important for the accommodation of the culture to a new environment. The pH decrease was also slowly in the same period of time.

After 5h of fermentation the pH value was declined below 5, due to intense metabolism of the bacteria strains. The tested culture (used as a rule for obtaining the probiotic yoghurt) was found to be capable of growing well on pure carrot juice without nutrients added. In the interval 7-8 h it increase 10 times, reaching a maximum cell number by $9 \cdot 10^8$ CFU/ml. Until 24h the bacteria number was declined, due to the inhibitory effect of the acids resulted as metabolites. The lactic acid production in the carrot juice with DI-PROX YBA 986 culture after 24 hours was about 6.2g/l.

The correlation between the biomass amount and the production of lactic acid (Figure 2) was described using the Luedeking & Piret model [11]. 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 fermentation at a given pH, by the expression:

$$dP/dt = \alpha\, dN/dt + \beta\, N$$

where the constants α and β are determined by the pH of the fermentation.
A simplified presentation of the above model relates to the linear part of the equation which is presented as:

$$(p - p_0) = \alpha\,(x - x_0)$$

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.

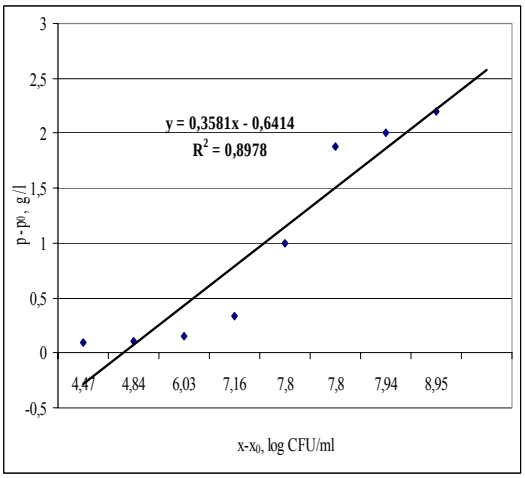

Figure 2 The lactic acid production by DI-PROX culture in the carrot juice

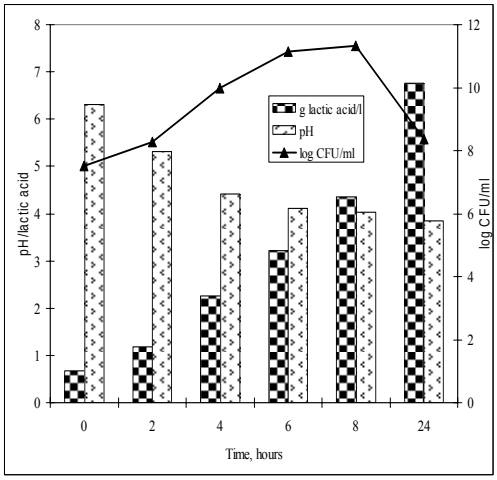

Figure 3 The dependence of the useful bacteria concentration on some chemical parameters in the case of the lactic acid fermentation of carrot juice with LA-5

The R squared coefficient about 0.9 shows a better linear correlation, respectively a strong connection between the lactic acid production and the lactic acid bacteria growth in the case of the tested culture. According [12], the deviations from the linear dependence are mostly caused by nutritive limitations of the substrates, and are related to the specific bacterial species. The volumetric productivity of DI-PROX by $1.12 \cdot 10^8$ CFU/L·h can be considered as moderate in comparison with the data from references.

The lactic acid fermentation of the carrot juice, respectively of the red beet juice with a single strain culture containing *Lactobacillus acidophilus* LA-5 was also performed.

It seems that this strain was well adapted in the carrot juice than in the beet juice, probably due to the presence of some inhibitors in the last raw material.

After 4h of lactic acid fermentation of carrot juice the growth of *Lb. acidophilus* was explosive, until 24h the maximum count by $2.1 \cdot 10^{11}$ CFU/ml being reached. In this interval 0-4h the pH was decreased from 6.31 to 4.41, this being an important proof of the performance of the strain to be able to grow in a vegetable juice. After 8h the pH value of the environment by 4.03 was become inhibitory for bacteria, but it was remained viable in an important count.

The production of lactic acid until 24h was also vigorous (Figure 3), this being essential for the shelf life of the final product. The maximum value of this parameter was by 6.75g/L. From the sensorial point of view the fermented carrot juice can't be qualified as excessive sourly by some consumers, due to the residual content of glucose that mask partially the higher value of the total acidity.

At the end of the incubation, after 24h, the cell concentration decrease to $2.3 \cdot 10^8$ CFU/mL, the volumetric productivity of *Lb. acidophilus* being $2.71 \cdot 10^{10}$ CFU/l·h. In this time the carrot juice medium was acidified to a pH level of less than 3.9.

Until 4 weeks parallel batches were introduced at the refrigeration temperature, with a view to monitories the cells survival. The useful microbial population was drastically reduced to $8.6 \cdot 10^3$ CFU/mL in only one week (Figure 4).

After 3 weeks of keeping at the refrigeration temperature the viable cells of *Lb. acidophilus* were no detected.

In the case of the lactic acid fermentation of the red beet juice with the same strain LA-5, the maximum count by $5 \cdot 10^{10}$ CFU/mL was reached after 8h of fermentation. This time the pH was ranged from 6.29 to 4.62,

while the lactic acid was increased to 2.65g/L (Figure 5).

No important amounts of lactic acid were resulted from the metabolism of lactic acid bacteria than in the case of the carrot juice fermentation.

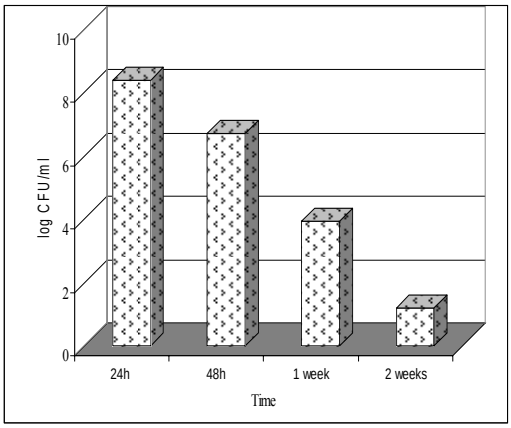

Figure 4 The dynamics of *Lactobacillus acidophilus* during preservation of lactic acid fermented carrot juice at refrigeration temperature (4^0C)

After 24h the fermented red beet juice was characterized by 3.69g/l lactic acid, corresponding to the pH value by 4.28, the lowest value that can be reached in the experimental conditions (24h later the pH value increase to 4.31 at 37^0C). The cell concentration was decreased also to $1.68 \cdot 10^8$ CFU/ml, while the volumetric productivity of *Lb. acidophilus* in red beet juice was only by $6.2 \cdot 10^6$ CFU/ L·h.

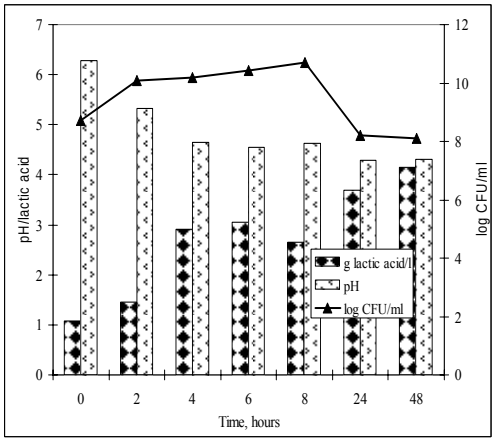

Figure 5 The dynamics of the useful bacteria concentration during lactic acid fermentation of red beet juice with LA-5

Some studies are reported on the growth and productivity of bifidobacteria in various laboratory media and food matrices such as milk, soymilk, vegetable juice, etc. ([13], [14]). According [15] most strains of bifidobacteria are unable to grow in an artificial medium and require complex nitrogenous substrates such as milk whey or yeast extract. A vegetable medium containing 6% of carrot juice, 12% of cabbage juice, and 3% of onion was reported to be adequate for the propagation of *B. breve* and *B. bifidum* [16].

The changes of cell numbers of bifidobacteria strain BB-12 during fermentation of carrot juice, respectively of red beet juice are presented in Figures 6 and 7.

The maximum concentration was reached after 4h in the case of the carrot juice ($8.28 \cdot 10^8$ cells/mL), respectively after 24h in the case of the red beet juice ($8.96 \cdot 10^8$ cells/mL). In both processes the bacteria kept their viability throughout the 48-h fermentation (higher than $4.5 \cdot 10^8$ cells/mL).

Volumetric productivities of BB-12 in carrot juice and red beet juice were $9.4 \cdot 10^7$ cells/ L·h, respectively $3.2 \cdot 10^7$ cells/ L·h.

Due of the production of acidic metabolites the pH values of the carrot juice dropped from the initial 6.17 to 4.28 in 48h, while in the case of the red beet juice it ranged from 6.03 to 4.41 in the same interval of time.

Acid production of the tested bifidobacteria in vegetable juices was intensive after the first 6 h of fermentation. Likewise *Lb. acidophilus*, the *Bifidobacterium* strain BB-12 was needed a lot of time for accommodation in the red beet juice as against the carrot juice. If 8.1g lactic acid/L was achieved through 24-h fermentation of the carrot juice, a closed value by 7.9g lactic acid/L was determined in the case of lactic acid fermentation of the red beet juice 24 h afterwards

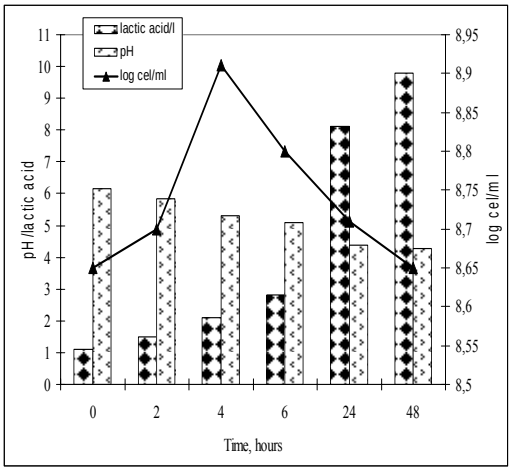

Figure 6 The dynamics of cells number of bifidobacteria during lactic acid fermentation of carrot juice with probiotic strain BB-12

According [17] and [18], the low pH of fermented products is harmful to some species/strains of *Bifidobacterium*. However, the present study was shown that after 48h the cells concentration of the carrot juice was about $4.5 \cdot 10^8$/mL, the substratum being characterized through a pH value by 4.28, respectively a lactic acid content about 9.8g/L.

Heenan et al. (quoted by [1]) studied the growth media for culturing probiotic bacteria and reported that the maximum cell number of *B. lactis* Bb-12 was 2.6×10^8 CFU/mL using MRS as a fermentation medium. Investigating the growth behaviour of *B. lactis* Bb-12 strain on carrot juice, RCM and TPY, no lag phase was observed during the fermentation of carrot juice while about 6-h of lag phase was noted, when it was cultivated on laboratory media such as RCM and TPY (data are not shown). Based on these results, carrot juice appeared to be very promising as a growth medium for bifidobacteria.

One of the basic requirements demanded from probiotic products is a proper number of probiotic bacteria at the level of at least 6 log jtk/cm^3 ([19]). The survival rate of *Bifidobacterium strain* at the level of about 8.6 log CFU/mL of both fermented carrot juice and red beet juice after 48h from inoculation represent a reason for the possibility of using the fermentation technology for the production of juice of probiotic properties.

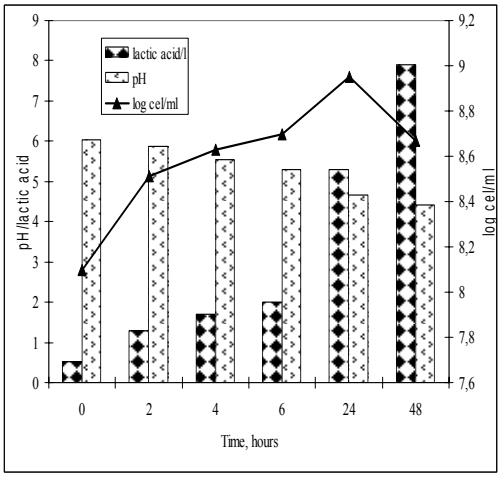

Figure 7 The evolution of the cells number of bifidobacteria during lactic acid fermentation of red beet juice with probiotic strain BB-12

Concerning the survival rate of *Lactobacillus acidophilus*, the mentioned value by 6 log CFU/mL was also reached regardless of the vegetable juice used as raw material, but in the case of the carrot juice is necessary to stop the fermentation after 24h, because 24h later the CFU/mL decrease about 45 times.

From the data obtained through the analysis of all the batches was resulted that the lactic acid fermentation of red beet juice with different probiotic bacteria need, as against the fermentation of the carrot juice, the enrichment of the substratum with prebiotics or/and growth factors. Our previous experiments were showed that the inulin addition to the red beet juice can improve the increase and the maintenance of the number of living bacteria.

4. Conclusion

The carrot and red beet juices represent suitable and alternative food matrices for the production of probiotic products with *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12 strain.

All tested strains were proved a good growth capacity in vegetable juices without

nutrients added, this being a guarantee on the one hand for the normal evolution of the fermentation and on the other hand for the stability of the final product. Thus it seems that the nutrients are available in acceptable forms and in optimal concentrations in the tested vegetable juices.

Further, the viability of the probiotics is essential for the quality of the fermented juices. In most analyzed cases the minimum of 10^7 viable bacteria per gram that represent the international standard claiming health benefits for the fermented products was reached in 24-48h. It be necessary that this minimum to be respected at the time of purchase.

Acknowledgements

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