Probiotic vegetable juices

Dana MORARU*, Iulia BLEOANCĂ** and **Rodica SEGAL***

* Biochemistry – Technology Dept., Faculty of Food Science and Engineering Galati *Dunarea de Jos* University
111 Domneasca Street, 800201 Galati, Tel./Fax: +40 236 460165, e-mail: Dana.Moraru@ugal.ro
** Food Bioengineering Dept., Faculty of Food Science and Engineering, *Dunarea de Jos* Galati University
111, Domneasca St., 800201, Galati, Romania

Abstract
Physico-chemical characteristics of celery and beetroot juices, with and without pulp, fermented with a bifidobacteria culture. Therefore, *p*H, acidity (as lactic acid), fermentative and reducing sugars were analyzed during 48 hours of fermentation. Also, the evolution of bacteria number was assayed in order to achieve a level appropriate for a probiotic product. During fermentation, the sugars from the celery juice are rapidly consumed and the acidity is 4.2 higher, while in beetroot juice, the process of fermentation is slower (smaller slope) and lactic acid accumulation is only 2.7 times. It worth be mentioned that although the level of acidity is different, the *p*H values are similar. This could be explained by the different type of acids present in the two juice types or by the existence in the beetroot juice of a higher level of nitrogen compounds, which act as buffers. The bacteria counting demonstrated that only after 36 – 48 hours of fermentation a level of 10^7 – 10^8 cells is attained, values which are characteristic to a probiotic product. The fermented beetroot juice has a pleasant taste, while the celery juice has a pronounced sour taste and needs to be corrected.

Keywords: Probiotics, vegetables, bifidobacteria, fermented juices.

1. Introduction

The nutritional and biological potential of fruit and vegetable juices makes them food products with multiple implications in maintaining the organism's equilibrium. The beneficial effects of the juices, high rated by nutritionists, have determined an extension of their use as remedies for the most diverse illnesses.

Due to their content of potassium salt, bioflavones, vitamins and alkalines miliequivalents, fruit and vegetable juices have good results in prophylaxis and even in treating cardiovascular diseases; the absence of fats offer to these food products special virtues for the mentioned treatment.

The consumption of fruit and vegetable juices is of most importance for liver and gallbladder affections, as they have a purifying effect (Segal, 1999).

The beneficial effects of fruit and vegetable juices can be enhanced through a biotechnological treatment- the lactic fermentation.

Researchers consider nowadays the vegetables' lactic fermentation a more than natural conservation method. Vegetable juices from the new generation are obtained by controlled fermentation, with selected strains of *Lactobacillus*. The majority of the lactic bacteria used for fermentation of vegetable juices are isolated from the human digestive tract and have probiotic characteristics.

Traditionally, probiotics are used for yoghurts and other dairy products, but lactose intolerance and their cholesterol content are two major drawbacks for part of the consumers.

The use of probiotics in the fruit and vegetable juice industry offers to consumers with special needs (vegetarians, people with allergic reactions to milk proteins) the possibility to experience the positive

University *Dunarea de Jos* of Galati
Faculty of Food Science and Engineering
The paper has been presented at the International Symposium **Euro - aliment 2007** 20 – 21 September 2007

effects of probiotic bacterias (Luckow and Delahunty, 2004).

This paper presents the physico-chemical features of celery and beetroot juices fermented with bifidobacterias.

2. Materials and methods

2.1 Fermentation substrate

Fresh vegetables bought from the market were transformed into juices with a laboratory juice maker. The juices were sterilized at 121°C, 15 minutes.

There were four types of juices: Bp- beetroot juice with pulp; Cp- celery juice with pulp; Bc- clear* beetroot juice; Cc- clear* celery juice.

2.2 Bacteria culture

A Christian Hansen lyophilized pure culture of *Bifidobacterium* strain, BB12, was used for juices' fermentation. The initial number of bacteria was 10^5-10^6 CFU/mL. For inoculation 0.02g lyophilized pure culture/100mL juice were used. The fermentation temperature was 37°C and the period of time- 48 hours.

2.3 Assays

During the fermentation process the following characteristics were analyzed:

- *the growth of bacteria number*, using Breed method with single dying;
- *the p*H - measured with Hanna Instruments *p*H-meter;
- *total acidity,* expressed as g lactic acid/100g d.w. and determined by titration with 0.1N NaOH and phenolphthalein for celery juices and tymolphtaleine for beetroot juices.
- *sugars:*
  - reducing sugars – were determined using a spectrophotometrical method, with 3, 5-dinitro-salicilic acid (DNS) reactive, after a process of defecation with basic lead acetate; the results are expressed as g glucose / 100g d.w.
  - fermentative sugars – were analysed in the defecated tests, in acid hydrolysis with HCl d=1.125, at 70° C , for 5 minutes; the results are expressed in g glucose/100 g d.w.
- *total soluble substances* (TSS) were assayed with the laboratory refractometer Zeiss, after a filtration of the tests through filter paper. The results were expressed as % soluble substance, at 20°C.

3. Results and discussion

The management of the fermentative process is important from several points of view, among which obtaining drinks with desirable sensory characteristics (for example sweet- sour taste, pleasant aroma) and the existence of at least 10^7 CFU lactic bacteria /mL, which makes the drink a probiotic product (de Vuyst,, 2000).

Taking this into consideration, the fermentation was maintained for 48 hours and the best moment for stopping the process was due to be established afterwards.

As consequences of the fermentation process a variation of the fermentable sugars and an accumulation of acidity are to be expected.

The results presented in figures 1 and 2 demonstrate the existence of a correlation between the dynamics of these two phenomena: the decrease of the fermentative sugars and the increase of the titratable acidity. The same evolution is in the case of clear and cloudy celery juices, but with increased slopes.

The shape of the curves suggests that the rate of sugars transformation into lactic acid is different from one juice to another.

During 48 hours of fermentation in the cloudy beetroot juice the acidity increased 2.7 times, while in the case of the cloudy celery juice the increase was of 4.2, indicating a rate of formation almost two times higher, although the content of fermentative sugars was 1.5 higher in the beetroot juice compared to the celery one. It is possible that the rate of beetroot juice fermentation is influenced by inhibitors in the soil, which can be found in the beetroot itself and can inhibit the activity of the lactic bacteria.

* The juices were clarified by centrifugation at 6000 rot/min. for 10 minutes

University *Dunarea de Jos* of Galati
Faculty of Food Science and Engineering
The paper has been presented at the International Symposium **Euro - aliment 2007** 20 – 21 September 2007

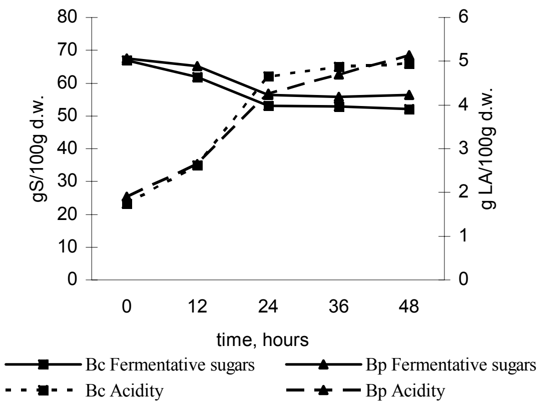

Figure 1. Timeline variation of fermentative sugars and acidity in clear and cloudy beetroot juice

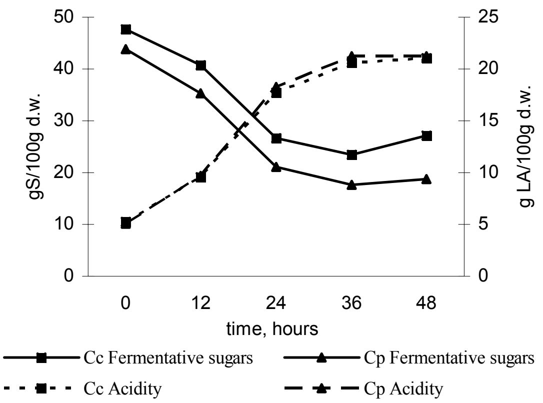

Figure 2. Timeline variation of fermentative sugars and acidity in clear and cloudy celery juice

Although there are small differences between the acidities of the two types of juices (5.14 g LA/100g d.w. for cloudy beetroot juice and 21.26 g LA/100g d.w. for the cloudy celery juice), the values for the *p*Hs are identical. This could be explained by the different type of the original and final acids from the two juices or by the existence in the beetroot of a high quantity of nitrogen compounds that can act as buffer.

The lack of correlation between the values of the *p*H and acidity is demonstrated also by the statistical evaluation of these physicochemical characteristics (figures 3 and 4).

The production of lactic acid is determined by the number of bacterias in the broth. This explains the study of evolution for the colony formation units throughout the fermentation period.

For a better representation of the correlation between the increase of cell number and the production of lactic acid the *Luedeking and Piret model* was used (Luedeking and Piret, 1959).

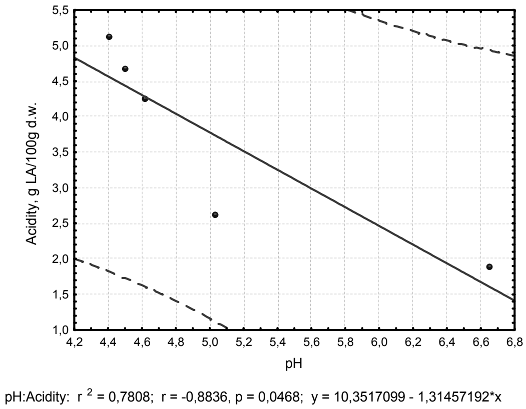

Figure 3. The correlation between the pH and acidity for the cloudy beetroot juice

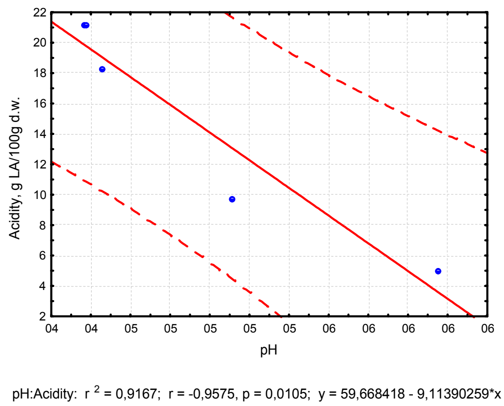

Figure 4. The correlation between the pH and acidity for the cloudy celery juice

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 the fermentation process at a given *p*H, by the equation (1):

$$dP/dt = \alpha \, dN/dt + \beta N \qquad (1)$$

where the constants α and β are determined by the *p*H of the fermentation.

In a simplified form the equation (1) can be written in form known as as the *Amarane and Prigent equation* (2), (Amarane and Prigent, 1999):

University *Dunarea de Jos* of Galati
Faculty of Food Science and Engineering
The paper has been presented at the International Symposium **Euro - aliment 2007** 20 – 21 September 2007

$$(p - p_0) = \alpha\,(x - x_0) \qquad (2)$$

where p_0 and p are the concentrations of lactic acid (g lactic acid/100g d.w.) at the initial time and at time t, x_0 and x reprezents the increase of biomass (log CFU/mL) at the initial moment and at time t.

The graphical representation of these data shows the correlation between the production of lactic acid and the increased number of bacteria. As shown in figures 5 and 6, there is a better linear correlation between the increse in the number of cells and the production of lactic acid.

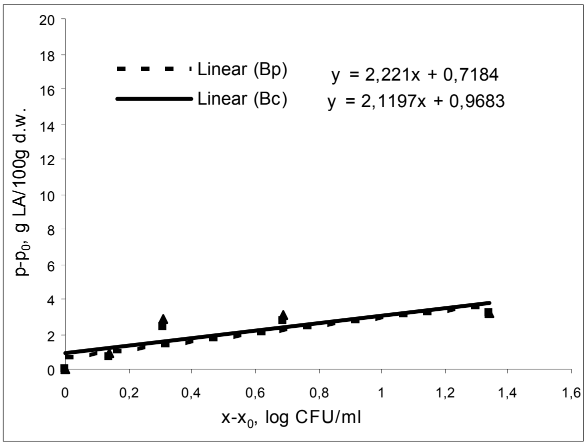

Figure 5. Correlation between the accumulation of lactic acid and the bacteria number in the beetroot juices

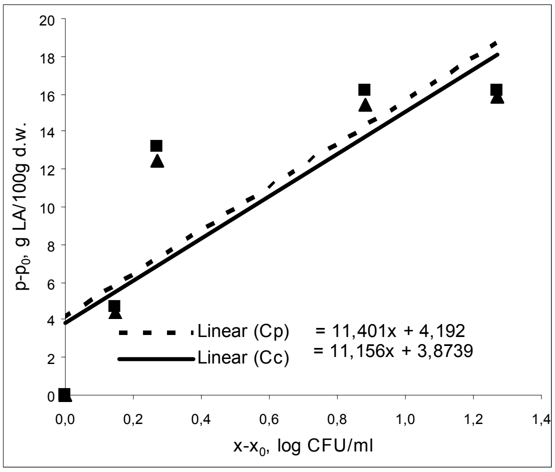

Figure 6. Correlation between the accumulation of lactic acid and the bacteria number in the celery juices

The above mentioned graphics demonstrate a more powerful dynamic in the case of clear or cloudy celery juices compared to the beetroot ones.

The increased slope for correlation between the acidity and the number of cells demonstrates that in the celery juice the beginning of the fermentative process is stronger than in the beetroot juice. This evolution correlates with the rate of conversion of the fermentative sugars into acids, presented in figures 1 and 2.

The evolution is similar no matter the type of juice, clear or cloudy.

Taking into consideration the high nutritional potential of the cloudy juices compared to the clear ones, the biochemical processes were examined for this type of juice, considering also the number of bacteria (CFU/mL).

Figures 7 and 8 present the variation of the fermentative and reducing sugar content, total soluble substances, in correlation to the number of bacteria from the analysed juices.

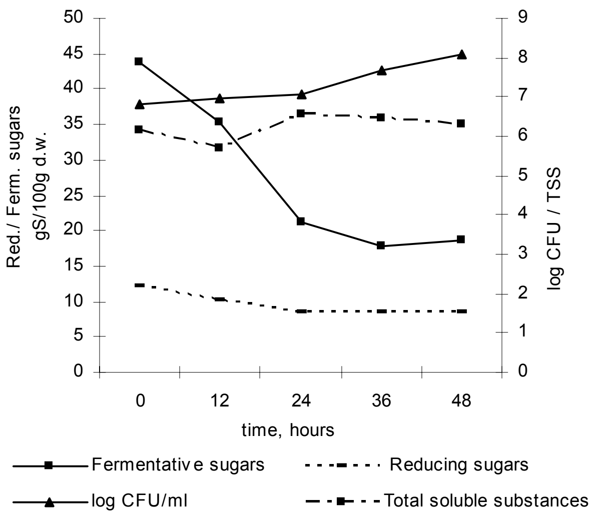

Figure 7. Variation of reducing and fermentative sugars content, of total soluble substances and bacteria number for the cloudy beetroot juice

The celery juice fermentation relies mostly on the existence of sugars known as *fermentative* and very little on the reducing sugars (reduced slope).

After 48 hours of fermentation the fermentative sugar content in the cloudy celery juice dropped at 57.11% and the number of forming units increased with 18.65%, to the value of 1.2×10^{8} CFU/mL, which corresponds to the characteristics of a probiotic product.

University *Dunarea de Jos* of Galati
Faculty of Food Science and Engineering
The paper has been presented at the International Symposium **Euro - aliment 2007** 20 – 21 September 2007

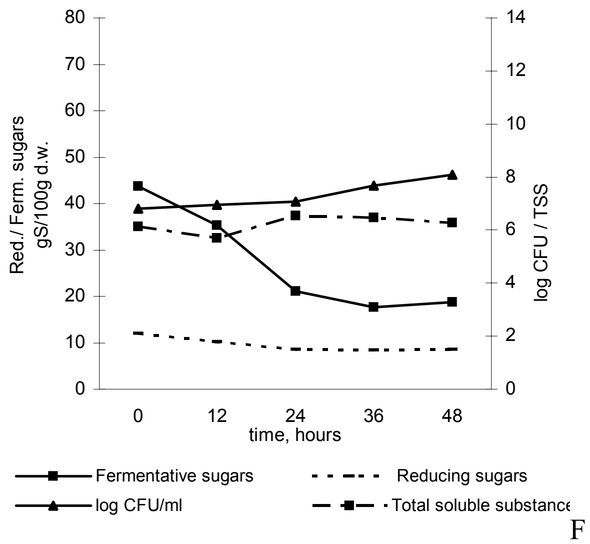

Figure 8. Variation of reducing and fermentative sugars, of total soluble substances and bacteria number for the cloudy celery juice

For the cloudy beetroot juice, the decrease of the fermentative sugars' slope is smaller, but the increase of the bacteria number is made in the same rhythm as in the celery juice. In this case, the evolution of the reducing sugars is constant, at a level of approximately 21% d.w. This is also reflected on the final sensory characteristics of the juice: pleasant sweet- sour taste, without any severe acidity, as in the case of celery juice.

After 48 hours of fermentation the beetroot juice has 1.4×10^8 bacteria cells, corresponding thus to the criteria of probiotic product.

The results of this study demonstrate that by pitching beetroot and celery juice with 6.4×10^6 CFU/mL and fermenting it for 48 hours at 37°C, biochemical processes occur, *i.e.* the decrease of the fermentative sugar content and accumulation of acids, through a different dynamic for each type of juice. For obtaining the probiotic character, in both cases the period of fermentation had to be prelonged to 48 hours, but the sensory characteristics were different in this case. The beetroot juice fermented in these conditions has a pleasant taste, but the celery juice has to be corrected in terms of sweetness or improved by blending. These observations will be further investigated.

4. Conclusions

By pitching celery and beetroot juices with 10^6 BB12 cells/ mL and fermenting for 48 hours, vegetable beverages with 10^8 CFU/mL were attained, values which correspond to probiotic products.

During the fermentation process there is an accumulation of acids with higher rate in the celery than in the beetroot juice. This makes the celery juice much pleasant in terms of sensory traits.

The dynamic of the fermentation process is stronger for the celery juice, which leads to accumulation of a higher quantity of acids and imposes a correction of taste.

5. References

Amarane, A. and Prigent, Y., 1999. Analysis of growth and production coupling for batch cultures of Lactobacillus helveticus with help of anunstructurated model, *Process Biochemistry*, **34,** 1-10;

De Vuyst, L., 2000. Technology aspects related to the application of functional starter cultures, Food Technol. Biotech, **38 (2)** 105-112;

Luckow, T., and Delahunty, C., 2004. Which juice is healtier? A consumer study of probiotic non-dairy juice drinks, Food Quality and Preference, **15 (7-8)**, 751-759;

Luedeking, R., and Piret, E.L. 1959. A kinetic study of the lactic acid fermentation. Batch process at controlled pH, *J. Biochem. Microbiol. Tech. Engr.* **1,** 393;

Rakin, M., Vukasinovic, M., and Maksimovic, M., 2004. The examination of parameters for lactic acid fermentation and nutritive value of fermented juice of beetrrot, carrot and brewer's yest autolysate. *J. Serb. Chem. Soc*., **69 (8-9)**, 625-634;

Segal, B. and Segal, R. 1991. *Tehnologia produselor alimentare de protectie,* chapter no.7, Ceres, Bucuresti;

Segal, R. 1999. Alimente de protectie. In: *Alimente functionale,* (Editors Costin G.M. and Segal R.), 255-294, Academica, Galati.

University *Dunarea de Jos* of Galati
Faculty of Food Science and Engineering
The paper has been presented at the International Symposium **Euro - aliment 2007** 20 – 21 September 2007