# STUDY OF THE EFFECTS SHOWN BY THE ACTION OF VARIOUS MICROORGANISMS ON THE LACTIC FERMENTATION OF JUICES

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***Abstract***
*Research has established the most effective lactic acid bacteria used for fermentation of fruit and vegetables juices. The cultures were used for Bifidobacteria, Lactobacillus acidophilus and culture of micro flora obtained spontaneous shoots. Lactic fermented juice was obtained from cabbage, carrots and beetroot. Working in these variants were determined lactic fermentation parameters and some of the bioactive compounds from this process: pH, reducing sugars, viable cell counts. Determination of reducing sugars calculated as glucose was conducted using the 3.5 dinitrosalicilic acid method (DNS method), pH was determined with a pH meter electronics. Chemical parameters listed were determined every two hours during the first 24 hours, then 48 and 72 hours in thermostatic conditions at 37 degrees C and 7 days storage at refrigerator temperature. At the end of the lactic fermentation, the lowest pH 4.31, which also provide juice conservation was obtained using a pure culture of Lactobacillus acidophilus. Reducing sugar is metabolized significantly when the juice of beetroot, fermented with Lactobacillus acidophilus culture, 75.19%. The results may be an argument for obtaining juice fermented lactic acid, which are popular functional foods, to obtain a chemical composition and sensory their optimum.*

Keywords: *Bifidbacteria*, *Lactobacillus acidophilus,* lactic fermentation juice, reducing sugars, biomas

## 1. INTRODUCTION

Fundamental reasons whey product development and acceptance may be related to conservation, nutrition and flavorings to improve properties and substrate transformation into products with high validity, human health benefits. Lactic acid bacteria fermented substrate is a sugar, most commonly glucose, and primary product is lactic acid plus carbon dioxide and other organic acids - without the need for oxygen. The formation of lactic acid is obtained by acidification below pH 4.2 which is a safety factor, but gives nice taste and whey products. This allows high fruit and vegetable juice fermented lactic without using preservatives [1,2,3,4,5].

Lactic bacteria are part of the largest group of bacteria that are in close contact with humans. They are found naturally on the mucosal surface, especially the gastrointestinal tract, but are found on the surface of fruit, cereals and vegetables, dairy products, wine [6]. Most probiotic microorganisms are lactic acid bacteria such as *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus,* and *Streptococcus lactis* [7,8].

Association of lactic acid bacteria with human environment and their beneficial interactions, both in nutrition and digestive tract leading to the general conclusion that this group can be considered safe [4].

Effect of probiotic fermented vegetables can not occur unless the bacteria remain alive to colonize the digestive tract, which excludes heat treatment or addition of preservatives.

Lactic bacterium have many positive physiologic effects, as the antimicrobial action, the improvement of immunitary potential [9], to prevent cancer [10] and to reduce the level of cholesterol [11, 12].

The hypothesis regarding the nutritional benefits and in that what concerns human health, dates of the *Lactobacillus* species refer to production of enzymes (lactase), the colonization and maintenance of a normal microflora, the competitive exclusion of unwanted microorganisms [13,14], the microbial interference and antimicrobial action,

the elimination of pathogens agents, stimulation and modeling of immunity, reduction or elimination of cholesterol, disconjugation of biliary acids, the anticarcinogenetic and antimutagenic action, reduction of antitoxin in the case of liver diseases by the alcohol consumption [1,2,3,4,5].

## 2. MATERIAL AND METHODS

### 2.1. Vegetable juices

For the experiment fruits and vegetables were taken from southern Romania, especially in Dambovita. Juice was obtained after conditionings are species by extraction. The heat treatment was carried out in 80 degree Celsius for 10 minutes. After cooling to 37-40 degrees Celsius, the temperature at which the inoculation is done with the rennet of lactic bacteria, juice was strewn with *Lb. Acidophilus*, *Bifidobacteria* and culture of micro flora obtained spontaneous shoots.

### 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 rennet has been obtained as a powder, from 2 separate pure cultures of bacteria *Lb. Acidophilus* and *Bifidobacteria*, powder which has been solubilized in a physiological serum, then introduced in the carrot juice.
The juices were then supplied with brine inoculums with $10^5$ cells/mL lactic bacteria mixture.
Then analyzes the thermostat to 37 degrees Celsius were made 2-2 in the first 10 hours after seeding, then 24 and 48 hours.

### 2.3. Process performing

Changes in pH, sugar content and viable cell counts during fermentation under controlled conditions were monitored. The pH of probiotic juice was measured with an electronic pH meter.
The dosing of the reducing sugar calculated as the glucose has been effectuated using the DNS method, which is a colorimetrical method, described by Segal. Viable cell counts (CFU/mL) were determined by the standard plate method with Lactobacilli MRS medium after 48 h of incubation at 30 degrees Celsius.
Concerning on the appreciation of the obtained lacticfermentated juices, there has been kept at the temperature of refrigeration another 3 weeks.
There were made the following notes:

- LB - juices with *Lb. Acidophilus*
- BB – juices with *Bifidobacteria*

The mathematical analysis of the analytical data has been realized using Microsoft Excel XP – Trend lines. The evolution of the processes has been analyzed using the mathematical simulation [$MEX_P$] (http://office.microsoft.com, 2004).

## 3. RESULTS AND DISCUSSION

The three species of vegetables have a different content of reducing sugars expressed as glucose. Carbohydrates are reducing substrate fermentescible in the context of lactic fermentation and their content is a criteria for selecting materials for lactofermented juice.
Reducing sugar is a downward trend during fermentation. If cabbage juice reduction is 50.92% for LB sample and 25.15% for sample BB (Fig.1.). The same trend was maintained in case of carrot juice, but the intensity of fermentation was lower: 30% for LB culture and 15% for culture BB (Fig.2.). To sample BB carrot juice shows an increase in reducing sugar content after 48 hours 0.12g/100g greater than 24 hours. This is due *Bifidobacteria* ability to secrete the polysaccharide polymer that can be hydrolyzed partially to fermentescibile carbohydrates.

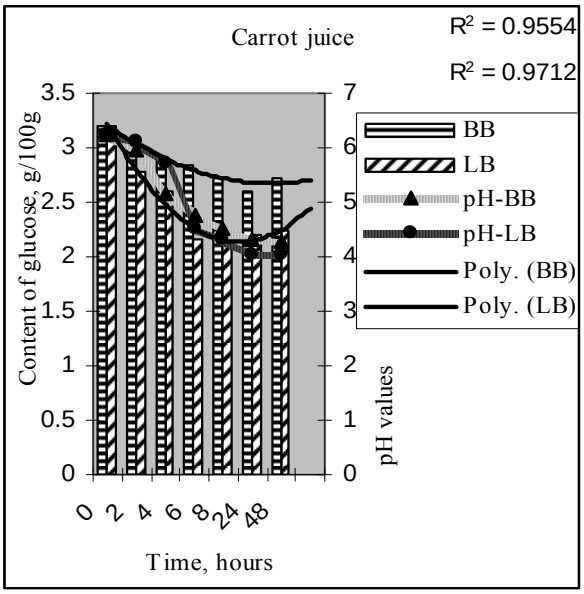


**Fig. 1 The variation of reducing sugar content and pH during the lactic fermentation with BB starter culture**

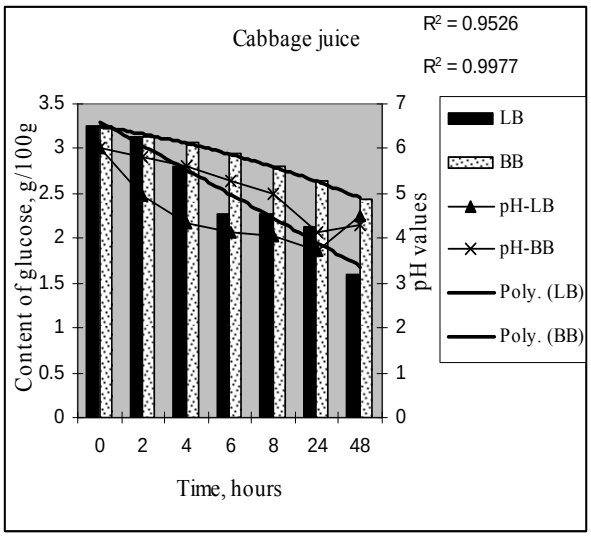


**Fig. 2 The variation of reducing sugar content and pH during the lactic fermentation with LB starter culture**

The polysaccharide polymer can impart functional effects to foods and confer beneficial health effects. The formation of lactic acid, while the metabolism of reducing sugars, pH values are decreasing first 24 hours, then recorded a small rise to 48 hours. This trend is correlated with the accumulation of reducing sugars also recorded at 48 hours and is 0.76 for LB sample and 0.13 for sample BB.

Beetroot juice has initial content of reducing sugars lowest (2.62g/100g), compared with other species analyzed, but at higher pH (6.29). Sugar metabolism is achieved after 48 hours at a rate of 75.19% for the LB sample. Samples seeded with BB shows a low intensity of fermentation after 24 hours, being metabolized 11.8% of reducing sugars. After 48 hours there is a noticeable accumulation of 0.11 g/100 g glucose, similar to that juice shown in fig. 1. Lactic fermented red beet juices have the highest pH compared to other juices analyzed, thus combining the beet juice with other juices with higher acidity, so to obtain a pH of finished product to ensure conservation and optimum sensory qualities (fig.3).

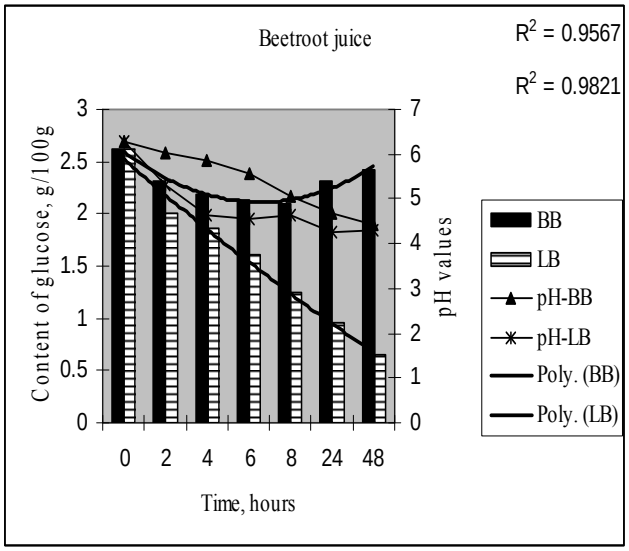


**Fig. 3 The variation of reducing sugar content and pH during the lactic fermentation of beetroot juice with BB and LB starter culture**

All the mathematical models use trend line. They are used to represent graphically the data trends and to analyze prediction issues referred to as regression analyses. The trend lines are the most efficient if the value of R squared is 1 or close to 1.

The polynomial trend model is a curved line which is used to illustrate the data fluctuation. Calculates the least squares fit through points by using the following equation:

$$y = b + c_1 x + c_2 x^2 + c_3 x^3 + \ldots + c_6 x^6$$

where $b$ and $c_1$-$c_6$ are constants.
The polynomial trend model hasn't rendered in the best way the evolution of the lactic fermentation. The curved line of the model, its equation and the determination coefficient are present in figure 1 (for the carrot and cabbage juices) and figure 2 (for the beetroot juice). In all the analyzed cases the calculated coefficient $R^2$ ($R$ squared) ranged from 0,95 and 0,99. The lacto fermentation conditions offer clear conditions for a polynomial evolution: Poly. (BB) and Poly (LB).
On the other hand, a number of viable cells in the fermented cabbage juice after 24h by $31{,}5x10^8$ CFU/ml represent an argument to characterize the product as functional, because it is known that the standard for some foods with healthy claims for the probiotics addition is a miniumum content by $10^6$ – $10^7$ CFU alive probiotic bacteria/g (www.fao.org.). The number of viable cells when lactic fermentation of cabbage juice was after 24 hours$79{,}2x10^{11}$.

## 4. CONCLUSIONS

Lactic fermentation takes place with greater intensity when using culture of *Lactobacillus acidophilus*, obtaining juices with low pH and residual sugar content lower than the fermentation of *Bifidobacteria*. Content of biomass is higher in the case of fermentation with *Lactobacillus acidophilus* than with *Bifidobacteria*.
Lactic fermentation is well approximate, in terms of sugar metabolism fermentescibile, by the polynomial model. According the quoted authors, the deviations from the linear dependence are mostly caused by nutritive limitations of the substrates, and are related to the specific bacterial species.

## 5. ACKNOWLEDGMENTS

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