

Biological Active Compounds Used as Probiotics in Yoghurt

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Abstract: The aims of our work were to study the effect of some prebiotics such molasses, amino acids on the growing rate of some probiotics lactic bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, *Bifidobacterium breve*, *Bifidobacterium infantis* and a mix of these four strains) in cow and goat yoghurt. We have been studied comparatively yoghurt without and with different concentrations of prebiotics mentioned above, their chemical composition (fat and lactose), survival rate of probiotics bacteria and the production of lactic acid as a result of lactose and other saccharides fermentation.

Regarding the growth rate of lactic bacteria it can be concluded that the number was increased significantly in goat yoghurt at the concentration of 1% and 2% molasses added.

The concentrations of lactic acid were also increased in the samples treated with molasses, the best results have been obtained for goat yoghurt, especially after 7 days of incubation.

The lactose amount was lower in the samples with molasses concluding that lactic bacteria fermented more efficient lactose in the presence of prebiotics.

There is a direct correlation between presence of prebiotics and probiotics bacteria activity.

Keywords: prebiotics, probiotics, yoghurt.

INTRODUCTION

Probiotics have a very long history of use in humans and animals, with the first recorded intakes dating back to several hundred years ago .

The emergence of antibiotic-resistant bacteria and natural ways of suppressing the growth of pathogens has contributed to the concept of 'probiotics'. Probiotic bacteria not only compete and suppress 'unhealthy fermentation' in human intestine, but also produce a number of beneficial health effects of their own. Fuller have defined probiotics as 'a live microbial feed supplement, which has beneficial effects on the host by improving its intestinal microbial balance'. Probiotics can influence the structure and functions of the gastrointestinal tract, there are opportunities for using diet as a "management tool" to affect the resident microbiota. Fermentable milk increase the densities of beneficial bacteria and stimulate growth and functions of the healthy intestine. Probiotic bacteria could be applied to balance disturbed intestinal microflora and related dysfunction of the gastrointestinal tract (Fuller 1989). Although yet to be verified decisively, it is understood that probiotic organisms fight and hold back the growth of undesirable microorganisms in the colon and small intestine, and thus help to make digestive system stable. Other effects include prevention of intestinal infections, expression of antitumor activities, and improvement of lactose utilization in the human gut (Kirjavainen and Gibson, 1999; Glodin 1998).

Microbes that are frequently isolated from human and animal intestines and selected as probiotics, include species of the genera *Bifidobacterium*, Research performed with germfree animals and the introduction of improved anaerobic culture techniques have been particularly useful in clarifying the significance of the interrelationships of diet and intestinal

microbiota in health and disease (Kirjavainen and Gibson, 1999; Friedrich, 2000; Lu 2001). Over the last few decades, some strains of lactic acid bacteria belonging to Bifidobacteria and Lactobacilli have been introduced in food products for human consumption, with the aim to improve human health (Guarner and Schaasama 1998).

Another approach to increasing the numbers of Bifidobacteria in the GI tract is the incorporation of prebiotics in the diet. A prebiotic is a non-digestible dietary supplement that modifies the balance of the intestinal microflora stimulating the growth and/or activity of the beneficial organisms and suppressing potentially deleterious bacteria. Molasses are by-products of the sugar cane industry; they have been widely used as a cereal substitute in livestock feeds (FAO 1992). Molasses referred specifically to the final effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane and from sugar beets (Fermin *et al.* 1994).

Teeming with minerals and vitamins, it contains more calcium than milk, more iron than eggs, and more potassium than any other food. Molasses is that the body seems to assimilate all the minerals quite readily.

MATERIALS AND METHODS

Strains and Cultivation: The species of *Lactobacillus casei*, *Lactobacillus platarum* *Bifidobacterium breve*, *Bifidobacterium infantis* and a mix of these bacteria were provided by Christian Hansen Company.

Treatment of Molasses (Sugar Cane Honey). Molasses was supplied by the Sugar Factory Ludus, Romania. Molasses were prepared by dilution with water. The diluted molasses (one part of molasses to 3 parts water) was repeatedly centrifuged (3 times) at 4000 rpm for 20 min each. The muddy precipitate was then discarded.

Microorganisms count. For each milk and yoghurt sample, the dilutions 10^{-2} , 10^{-3} , and 10^{-4} were prepared and the number of bacteria were counted by Thoma camera using the microscope.

Fat analysis of raw milk and yoghurt samples were determined by Gerber method.

Lactose evaluation was performed by polarimetric method for raw milk and yoghurt samples.

Lactic acid bacteria activity evaluation by HPLC and IR spectroscopy. To determine culture activity, cow and goat yoghurt containing 0,5; 1% and 2% percent (w/v Molasses) fermented with the five strains of probiotic bacteria were prepared for High Performance Liquid Chromatography (HPLC Agilent HP 1200) analysis using sodium sulphate 100mM, pH=2,65 adjusted with MSA as mobile phase and the detection was made at 210nm. Culture activity was determined by measuring the end products of fermentation (lactic acid) using HPLC.

For IR spectroscopy analysis it was used an Shimadzu IRPrestige21 device and the domain recorded for lactic acid was $600-4000\text{ cm}^{-1}$ with an maximum at 1700 cm^{-1} .

RESULTS AND DISCUSSIONS

In this study, we found that Molasses are growth promoters for *Lactobacillus casei*, *Lactobacillus platarum* *Bifidobacterium breve*, *Bifidobacterium infantis* .The fermentation process was monitored by measuring growth rate, fat and lactose quantification, and organic acid (lactic acid), both of four strains demonstrated a relation between growth rate (CFU/ml) and acid production that was measured as lactic acid after 24 h and 7 days of incubation.

Influence of molasses on Microorganisms rate growth: The samples of cow and goat yoghurt inoculated with different concentration of molasses increase semnificatively the total number of probiotic bacteria, even in the refrigerated condition (4-6⁰C) after 24h of inoculation.

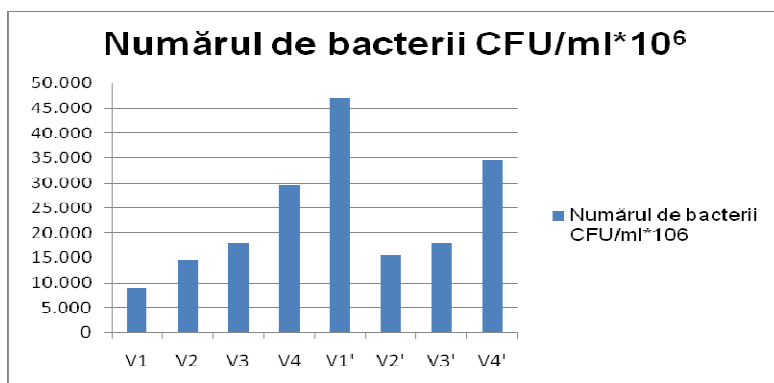


Fig.1. Molasses influence on probiotic bacteria number inoculated in cow yoghurt

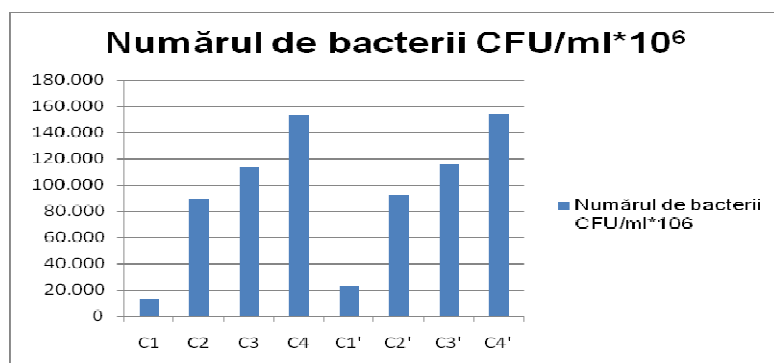


Fig.2. Molasses influence on probiotic bacteria number inoculated in goat yoghurt

The best results in both cases were obtained at 2% molasses concentration in yoghurt after 24h of inoculation.

Lactose evaluation: The lactose amount in all types of yoghurt was determinated based on optic active properties of it. After the removing of proteins by precipitation lactose was quantified by polarimetric method. Results revealed that that lactose decrease in samples inoculated with molasses which is in accordance with the increasing growth rate of bacteria.

$$\text{g lactose/100ml} = \alpha * 100 * 2 / 54,5 * 1$$

Tab. 1

The amount of lactose in some yoghurt samples

Sample	Lactose (g/100ml)
V1	5,68
V3	5,10
C1	5,94
C3	5,57

V1 = cow yoghurt without molasses; V2 = cow yoghurt with 1% molasses;
C1 = goat yoghurt without molasses; C3 = goat yoghurt with 1% molasses

Fat analysis: These concentrations decrease in yoghurt comparatively with raw milk during the technological process with an average of 20%, but the resulted values were framed in the normal limits. Milk with an higher concentration of fat provide a better yoghurt. Experimental concluded that molasses don't have influence regarding the fat concentration.

Lactic acid bacteria activity evaluation by HPLC and IR spectroscopy: Enhanced organic acid production by Bifidobacteria, in the presence of molasses was unexpected. Molasses is considered Bifidogenic factor as like all oligosaccharides have been shown to increase growth and activity. Fructooligosaccharides (FOS) and Galactooligosaccharides (GOS) having lower degree of polymerization (DP), were best in supporting growth of Bifidobacteria. These low substrates with low DP oligosaccharides may be the favored substrates for Bifidobacterial support, thereby enhancing lactic acid production as observed in the present study. In contrast, carbohydrates with high DP were poor Bifidobacteria substrates. Very little is known about the mechanism of carbohydrate uptake by Bifidobacteria (Kleessen *et al.*, 1997).

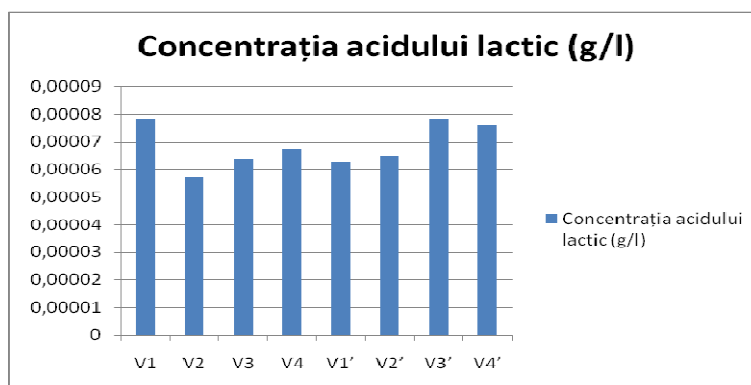


Fig.3. Concentration of lactic acid in cow yoghurt determined by HPLC

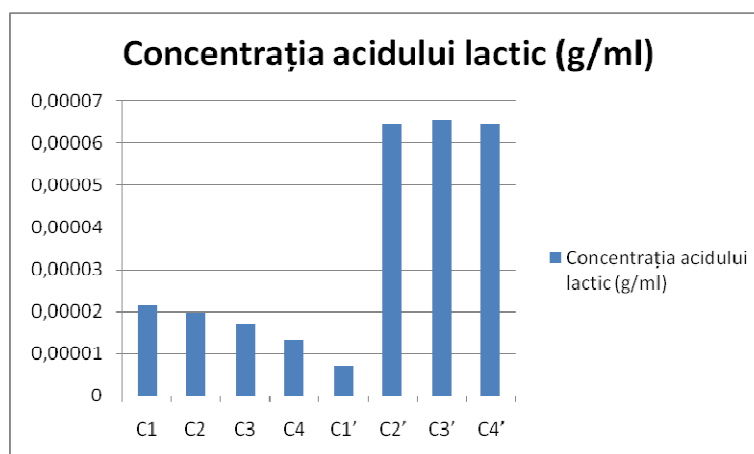


Fig.4. Concentration of lactic acid in goat yoghurt determined by HPLC

Fast growing bacteria generally produced more lactic acids after 7 days of incubation as it was expected because molasses provided saccharides which are the suport of lactic fermentation. A great part of the total lactic acid is produced by bacteria after growth,

especially during the stationary phase. thus indicating that molasses support acid production and were not inhibitory.

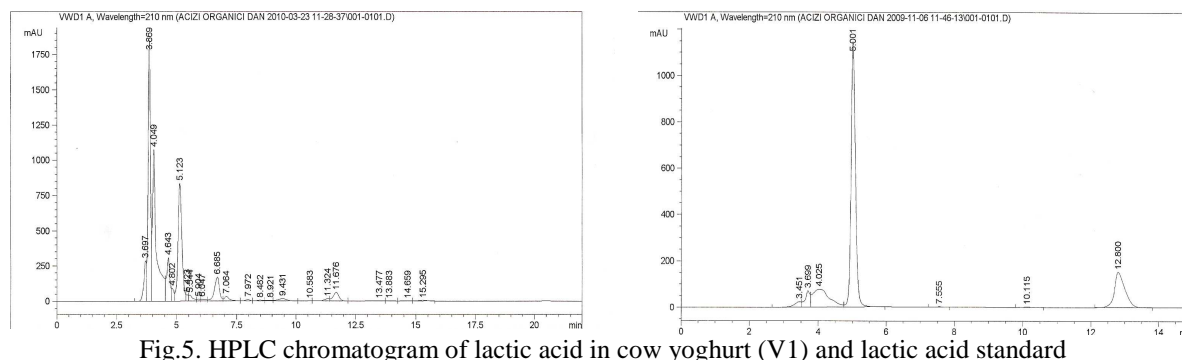


Fig.5. HPLC chromatogram of lactic acid in cow yoghurt (V1) and lactic acid standard

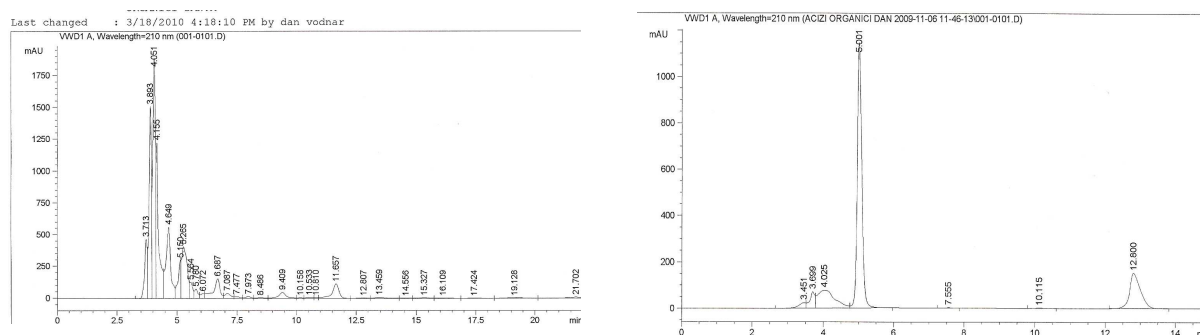


Fig.6. HPLC chromatogram of lactic acid in goat yoghurt (C1) and lactic acid standard

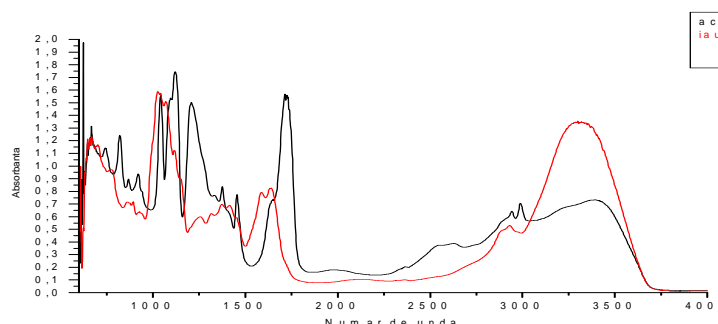


Fig. 7. FT-IR spectra of lactic acid (black line) comparative with cow yoghurt (red line)

CONCLUSIONS

Growth conditions could affect the metabolism and explain the differences among growth of bifidobacteria and lactic acid bacteria. In practical terms, the in vitro properties of new prebiotics will probably relate reasonably well to their physiologic function and analytic results, and these can be used to screen potential prebiotics. These analytic results should be taken in hand because the nature of the carbohydrate determines its fermentability is a question that has barely been addressed and include 1) a detailed description of the chemical structure, 2) measurement of resistance to gastric juice, 3) measurement of resistance to pancreatic enzymes, and possibly 4) measurement of resistance to brush border enzymes.

Results from this study has shown that Molasses contains highly active growth promoters for probiotic bacteria. Molasses is rich in nutrients such as copper, calcium, iron,

potassium ,magnesium, chromium, manganese, molybdenum, zinc, phosphorous, pantothenic acid, vitamin E and Inositol, also contains high concentrations of C6 sugars and other fermentable carbohydrates as well as significant concentrations of B vitamins, especially biotin, which enhance fermentation rates. Molasses were not inhibitory to lactic acid and bifidobacteria when added to milk at a level 2 %. Furthermore, understanding the substrate preference of bifidobacteria will facilitate development of probiotics, perbiotics, and synbiotics (Beerens, *et al.* 1980; Poch, and Bezkorovainy. 1989; Bezkorovainy and Topouzian. 1981) In conclusion, we have shown that molasses can be used effectively as a prebiotic because of the increase in probiotic bacteria numbers and lactic acid, giving an added possible health benefit.

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