Effects of prebiotics on the quality of lactic acid fermented vegetable juices

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Abstract The comparative evaluation of lactic acid fermentation of carrots and red beet juices inoculated with bifidobacteria and supplemented of commercial prebiotics containing inulin was performed. Some recommendations referring to the interval of time for interruption the fermentation with the aim to obtain the desirable characteristics of products were made. The inoculation of juices was accomplished after the epiphytic microbiota inactivation, in the optimum conditions of temperature. According the results obtained through phisico-chemical analysis (pH, titrable acidity, volatile acidity, reducing sugars content), the objective of this paper was to prepare suitable substrates regarding the consumer's perception and the practical possibilities of application the probiotic cultures. In each experiment was established the final point of the fermentation, with the aim to avoid first the undesirable action of the environment acidity on the strain viability.

Keywords: bifidobacteria, vegetable juices, prebiotics, lactic acid fermentation

1. Introduction

The probiotics were defined in different modalities, depending on the degree of understanding their action mechanism in correlation with the human healthy effects.

According to the currently adopted definition by Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics are ''Live microorganisms which when administered in adequate amounts confer a health benefit on the host'' [1]. The capacity of probiotic microorganisms to survive and to multiply in the body is conditioned by their positive influence regarding healthy.

Consumers are becoming increasingly aware of the importance of the human gut microbiota in well-being and health. The modulation of the intestinal microbiota by diet, toward a composition with increased proportions of beneficial bacteria such as bifidobacteria and lactobacilli, has recently gained a lot of interest [2].

The probiotic juices represent an alternative to dairy products that suits consumers who don't want to eat dairy foods or are lactose intolerant. Adding probiotics to juices is more complex than formulating in dairy products.

A prebiotic can be defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health [3], [4].

Inulin is a well-known fructose-based prebiotic which has been shown to stimulate the growth of bifidobacteria, a bacterial group generally considered beneficial for intestinal health [5].

Fructo-oligosaccharides (GFn) and inulo-oligosaccharides (Fm) act as a type of soluble dietary fiber with a reduced caloric value [6]. Further, ingestion of these oligosaccharides exerts a bifidogenetic effect in the large intestine, where the increased number of bifidobacteria eliminate other unfavorable bacteria such as *Escherichia coli* and *Clostridium perfringens* that produce toxic substances and can cause enteritis [7].

Furthermore it has also showed that humans consuming 15 g inulin/day (DP 2 to 60; avg. 10 units) significantly increased the bifidobacteria: from lg 9.2 to lg 10.1 per gram in a two week period, rendering them the dominant population [8].

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2. Experimental

2.1. Fermentation substrate

Fresh vegetables (carrots and red beet) were bought from the local free vegetable market of the Dambovita County (Romania) at the end of January and specifically processed by washing, scrubbing and removing non-edible pieces. The conditioned vegetables were transformed into juices with a domestic juice maker. The juices were thermal treated at 80^0C/10min with the aim to destroy the undesirable microorganisms. All the samples were rapidly cooled at 40^0C. This way the substratum was prepared for inoculation with pure culture.

2.2. Microorganisms and prebiotics

A Christian Hansen lyophilized pure culture of *Bifidobacterium* BB12 was used for juice's fermentation. The fermentation temperature was 37^0C (the optimum temperature for this strain).

Raftiline HP (inulin) is long-chain inulin (average DP, 25; FOS with DP of <5, absent) that was provided by Enzymes & Derivates.

2.3. Process performing

The lyophilized pure culture was aseptically added, in proportion of 0.2g/L to the carrot juice, respectively to the red beet juice, and vigorous homogenized. In the same manner the commercial inulin was added too. There were the following experimental batches of juices: CC - the carrot juice without inulin (control batch); C1 - the carrot juice with 3% (w/v) inulin; C2 - the carrot juice with 6% (w/v) inulin; CRB - the red beet juice without inulin (control batch); RB1- the red beet juice with 1.5% (w/v) inulin; RB2 - the red beet juice with 3% (w/v) inulin.

Identical samples by 100ml juice from each experimental variant were distributed in sterile tubes. Thus it was possible, at each interval of time, to analyse one sterile batch, obtained in the same conditions with the others.

The anaerobiosis was created by covering the cotton stopper of the tube by metal folia. Each tube represented a single sample and the experiments were performed in double.

The lactic acid fermentation was performed in a thermostat at 37^0C during 48 hours. The samples were investigated during the lactic acid fermentation through chemical analysis at 2, 4, 6, 8, 24 and 48 hours.

2.3. Assays

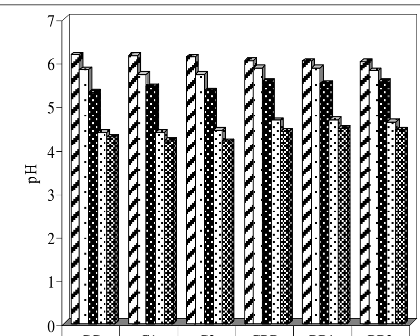
The pH was measured with a HACH pH-meter.

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).

The values of the volatile acidity, expressed as g acetic acid/100mL, were established by steam distillation. The distilled was titrated with NaOH 0.1N in presence of phenolphthalein. Reducing sugars were analyzed using a spectrophotometrical method with 3,5-dinitrosalicilic acid (DNS) after defecation with basic lead acetate. The results were expressed as g glucose/100mL.

3. Results and Discussions

The rapid decrease of the pH values of vegetable juices during lactic acid fermentation is essential for their microbiological stability.



	CC	C1	C2	CRB	RB1	RB2
0	6,17	6,16	6,12	6,03	6,02	6,01
2h	5,83	5,72	5,72	5,87	5,86	5,8
4h	5,31	5,44	5,34	5,55	5,51	5,55
24h	4,39	4,38	4,43	4,66	4,68	4,63
48h	4,28	4,19	4,18	4,41	4,48	4,44

Fig.1. The evolution of the pH values during lactic acid fermentation of vegetable juices

The influence of inulin on the dynamics of this parameter can be observed in Fig. 1. In the case of carrot juices the pH values of batches C1 and C2 were only about 0.1 unities less than in the case of the sample without inulin after 48 hours from the starting of fermentation. No very significant differences were also observed on running of the fermentative process.

A similar situation can be described concerning the lactic acid fermentation of the red beet juices with and without inulin. However, between the pH values of the juices from different vegetables can be observed that the red beet exert an inhibitory action on the bifidobacteria development. This was distinguished through higher pH values, respectively lower values of titrable and volatile acidity in comparison with the carrot juices.

At the initial moment of fermentation the red beet juices were characterized through pH values closed to 6.0. If in the interval 0-48 hours the decrease of the analyzed parameter was about 1.5-1.6 units, in the other batches (CC, C1 and C2) it was about 1.9 units.

The difference concerning the dynamics of the pH values of carrot juices and red beet juices it is also obvious analyzing the data from table 1. The maximum rate of acidification v_{max} was calculated as the time variation of pH (dpH/dt) and expressed as pH units/min. Other kinetic parameters were also calculated: time to reach v_{max} (t_{max}, hours), time to reach pH 5.0 ($t_{pH\ 5,0}$, hours), time to complete the fermentation ($t_{pH\ 4,2}$, hours).

Table 1 Acidification kinetic parameters of fermentation of vegetable juices by *Bifidobacterium* sp.

Sample	$v_{max}\cdot 10^{-3}$ (units/min.)	t_{max} (h)	$t_{pH\ 5,0}$ (h)	$t_{pH\ 4,2}$ (h)
CC	3.58	4	6.62	≈ 48
C1	3.66	2	7.27	46.73
C2	3.33	2	7.13	46.08
CRB	2	4	8.28	> 48
RB1	2.12	4	8.81	> 48
RB2	1.91	4	8.91	> 48

In the absence of prebiotics (control sample CC) time to reach v_{max} was greatest with two hours, the values v_{max} for all the carrot juices being closed. In this case the prebiotic inulin has accelerated the acidification, but not the time to reach pH 5.0. Numerous authors were indicated the pH value 4.2 as being a parameter that guarantee a shelf life of the fermented juices. It seems that the addition of polysaccharide was exerted a positive influence on the time to reach pH 4.2 in comparison with the control CC (directly proportional with the quantities of inulin added).

The time to reach v_{max} in the case of lactic acid fermentation of red beet juices with *Bifidobacterium* sp. was about 4 hours. The others kinetic parameters were also large, from the viewpoint of juices preservation it being unfavourable.

The fermentation of hexoses occurs in the genus *Bifidobacterium* through bifid shout [9]. Bifidobacteria are saccharolitic species [10] and they produce acetic acid and lactic acid (3/2, without CO_2). The evolution of the titrable acidity of juices during lactic acid fermentation is showed in Fig. 2.

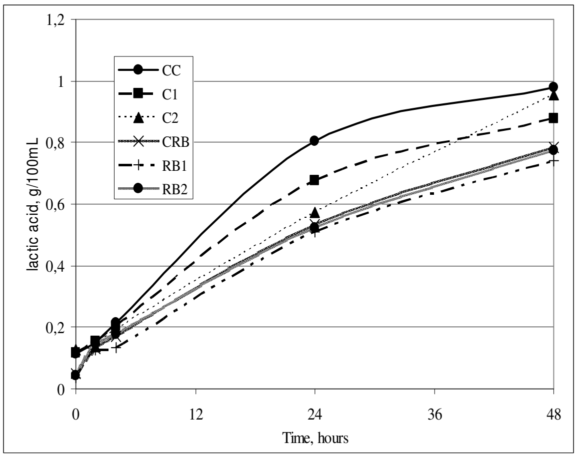


Fig.2. The dynamics of the titrable acidity during Bifidobacterium sp. growing on vegetable juices

It can be observed that in both experiments the titrable acidity of control remains upper than in the samples with inulin. Just after 48 hours of fermentation in the batches C2, respectively RB2, the values are very closed than those of the controls.

In the interval 0 - 48 hours differences between samples were determined only in the case of the carrot juices, in the sense of delay of lactic acid production by bifidobacteria in the presence of inulin. For the others batches (red beet juices with and without prebiotic) the curves tendency of titrable acidity was the same.

At the end of the analyzed period of time it seems that a supplement by 6% inulin exerts a favourable effect on the dynamics of this parameter.

The titrable acidity of juices was expressed in lactic acid, although at the initial moment of fermentation in all experimental batches there were others organic acids. The fermented vegetable juices were registered an increase of titrable acidity that ranged between 0.704g/100mL (sample RB1) and 0.865g/100mL (sample CC). Nevertheless, in absolute value the increase of this parameter in the red beet juice with 1.5% inulin (RB1) was about 20 times.

Values of the titrable acidity of fermented carrot juices about 0,9g/100mL and values of the pH about 4,2 represent, from the sensorial point of view on the one hand, respectively from the preservation point of view on the other hand, long-expected parameters from the global quality of the final products. Consequently, the lactic acid fermentation of the carrot juices with and without inulin can be stopped after 48 hours through the change of the temperature. A sufficient shelf-life of juices correlated with a sufficient viability of bifidobacteria can be obtained at values of temperatures about 20^0C [11].

Another final product of lactic acid fermentation is the acetic acid, the evolution of the volatile acidity of vegetable juices being showed in Fig. 3.

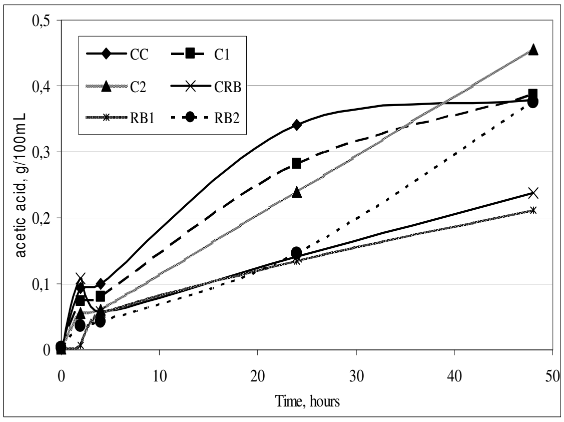


Fig.3. The dynamics of the volatile acidity during lactic acid fermentation of juices with and without inulin

A significant increase of the volatile acidity after 48 hours of fermentation was determined in the case of the juices with a great amount of inulin added. So, in the carrot juice C2 the difference between the final and the initial value of this parameter was about 0.453g/100mL, while in the red beet juice RB2 it was about 0.372g/100mL. The influence of prebiotic on the production of acetic acid, which has a favourable effect on the flavour of the final product, it's so obvious.

If the increase of the titrable acidity of the juices was relative slowly, not the same was happened concerning the dynamics of the volatile acidity. For example, after 4 hours from the beginning of fermentation the determined value of this parameter was about 100 times higher in the control obtained from carrots, respectively about 36 times higher in the control obtained from red beet.

The proportion of the final fermentation products vary considerably from one strain to another and even within the same species [12]. Small quantities of succinic acid are produced by some strains, and a small amount of CO_2 may be produced during the degradation of gluconate.

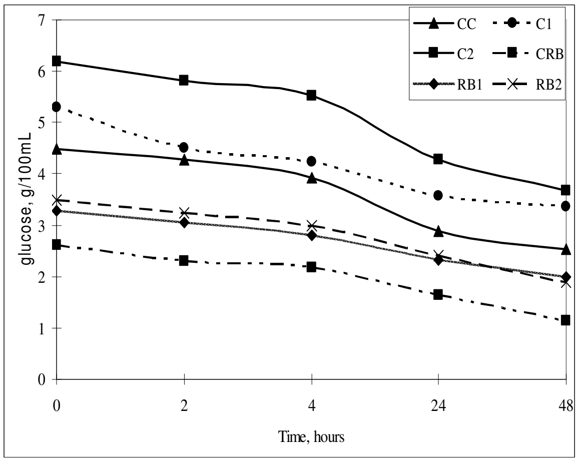


Fig.4. The decrease of the reducing sugars content during lactic acid fermentation of vegetable juices

The ratio between the titrable acidity and the volatile acidity ranged after 48 hours from 1.83 (C2) to 2.29 (CC) in the case of the fermented carrot juices, respectively from 1.96 (RB2) to 3.38 (RB1) in the case of red beet juices. It seems that the metabolism of *Bifidobacterium* sp. in these two types of vegetable juices was directed preponderant to the increase of the titrable acidity, the inulin exerting positive influence on the volatile acidity.

The decrease of the reducing sugars of raw materials, expressed as glucose, it is shown in Fig. 4. Between all the analyzed batches, the control red beet juice was remarked through a small amount of substratum for lactic acid fermentation.

Because inulin consists of linear chains of β-2,1-linked D-fructofuranose molecules terminated at the reducing end by a glucose residue attached through a sucrose-type linkage, the addition of this prebiotic on juices was influenced the amount of their reducing sugars at the initial moment of fermentation.

For a vigorous fermentation and a rapidly decrease of pH, the reducing sugars of raw materials must be sufficient (about $40 g/dm^3$) [13]. It can be observed that this condition it was not accomplished in the case of the juices obtained from red beet. Nevertheless, after 48 hours the titrable acidity (expressed as lactic acid) was at least 0.7g/100mL, while the volatile acidity (expressed as acetic acid) was about 0.2g /100mL, as result of sugar metabolism. These values has corresponded at a pH value about 4.4.

In percentages the highest diminution of the reducing sugars content was determined in the case of the control batches (43.65% for CC, respectively 56.48% for CRB). In the interval 24-48 hours the increase of volatile and titrable acidity of the juices with important amounts of inulin added (C2 and CRB2) was correlated with an important consumption of the reducing sugars.

As result of the metabolization of 1g substratum in 48 hours in the case of the carrot juices, the titrable acidity ranged between 0.32g/100mL (C2) to 0.39g/100mL (C1), while the volatile acidity was varied between 0.16g/100mL (CC) to 0.19g/100mL (C1). The efficiency of the conversion was approximately the same concerning the increase of the volatile acidity during the fermentation of the red beet juices. Instead the titrable acidity was with about 0,1g/100mL higher in lactic acid fermentation of the red beet juices than in the carrot juices (0.45g/100mL in the case of the batch RB2, respectively 0.54g/100mL in the case of the batch.RB1). The explanation of these different efficiencies for the lactic acid fermentation of different vegetable juices consists in the extensive development of the useful microorganisms in the carrot juices.

4. Conclusions

The influence of the inulin addition on the evolution of lactic acid fermentation of vegetable juices by *Bifidobacterium* sp. was exercised especially referring to the increase of the volatile acidity. From the sensorial point of view this aspect is important.

The increase of the titrable acidity was stumbled, more obvious in the case of the carrot juices than in the red beet juices. After 48 hours of fermentation the differences between the batches with and without inulin are not so significant concerning this parameter, involved in the preservation of the final products.

Between the analyzed vegetable juices, those obtained from red beet were represented a substratum with inhibitory action on the bifidobacteria development, aspect which was reverberated on the pH decrease, respectively on the increase of the titrable acidity after 24 hours of fermentation. As consequence, the lactic acid fermented red beet juices were characterized through a lesser stability than the carrot juices, especially due to the relative highest values of pH. The inulin addition wasn't important from this point of view.

The added quantities of inulin were used for bifidobacteria growth (which it was also proved through microbiological analysis) and it seems that the strain hadn't the ability to produce extracellular inulinase.

The residual content of the reducing sugars in the fermented juices, thanks to the presence of glucose at the end of the inulin chains, was conferred to them a sweet taste agreed by some consumers, for the red beet juices this aspect being obviously.

5. Acknowledgments

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6. References

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