Dynamics of Some Chemical Parameters During Lactic Acid Fermentation of Carrot Juices in the Presence of Inulin

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The probiotic products represent an important sector of the market in continuous expansion - the functional foods. The probiotics were defined in different modalities, depending on the degree of understanding their mechanism in correlation with the human healthy effects. In this context the aim of this study was to obtain information about the dynamics of titrable acidity and volatile acidity during the lactic acid fermentation of carrot juice (with and without inulin) by a strain of bifidobacteria. These data were correlated with the reducing sugar consumption and the evolution of pH as result of the microorganisms increase and growth. The particularity of this research consists in the fact that the bifidobacteria are not constitutive from the epiphytic microbiota of vegetable juices. The lactic acid content of the juices was ranged between 0.113g/100ml to 0.978g/100ml, while the decreasing of pH was about 2 unities during 48 h of fermentation. The maximal acetic acid value reached 0.455g/100ml, the influence of the prebiotic inulin on its accumulation being obvious. The addition of inulin influenced more the viability of the microbiota and not their metabolic activity. The obtained results can be an argument in the development of functional foods, but from this point of view it is necessary a completely understanding of the changes that occur in the chemical composition of juices, respectively how can this composition be favourable influenced by using different bifidogenic and/or growth factors.

Keywords: lactic acid fermentation, inulin, pH, short chain fatty acids, bifidobacteria

The systematic investigation of the interactions between food components or food ingredients and genomic, biochemical, cellular, or physiological functions is a unique way to improve both our knowledge and the role of nutrition in maintaining good health and preventing disease. The presence of bifidobacteria is regarded as a marker of the stability of the human intestinal microbiota, also for prevention of many diseases and for maintaining good health [1]. The benefit of bifidobacteria concerning the host health imposes that these ones to be metabolically active in the lower gastrointestinal tract. As a prerequisite to their survivability, these bacteria require a carbohydrate source to use for fermentation that has not been metabolized by the human digestive system before reaching the colon [2].

Also, the probiotics efficiency can be improved by the selection of new strains, by testing their biotechnological potential, by genetic handling in the sense of potentiate the useful characteristics and the combination of as many strains [3].

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 [4-5]. The basic idea of prebiotics is that these are naturally occurring sugars that are bound in such a way that animals, including humans, cannot digest them [1]. There is consistent evidence from in vitro and in vivo studies that these are not digested by normal human enzymes, but are readily fermented by anaerobic bacteria in the large intestine [6].

At the present time, all prebiotics described are short-chain carbohydrates with a degree of polymerisation between two and about sixty, and are thought to be non-digestible by human, or animal digestive enzymes. However, the defining property of prebiotics is their effect on the microbiota of the large bowel, so neither a specific chain length nor non-digestibility are essential to the definition, although such properties may be essential to their effect on health [4].

Whether the nature of the carbohydrate determines its fermentability is a question that has barely been addressed. A range of different shortchain carbohydrates with widely different sugar compositions and molecular sizes were produced and their breakdown by several strains of bifidobacteria, clostridia, bacteroides, lactobacilli, etc. it was tested [7]. Fermentability differed with oligosaccharide structure. So bifidobacteria utilised low degree of polymerisation (DP) carbohydrate first, and bacteroides those with a high DP. Both the structure of the carbohydrate and the bacterial species present in the ecosystem are probably important factors in controlling fermentation of prebiotic carbohydrates.

Inulin occurs as a reserve carbohydrate found in the roots or tubers of plants like Jerusalem artichoke, chicory, and dahlia. It is isolated from these by water extraction, that induces a relative easy solubilisation and after some times it crystallises [8]. 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 [9]. Because of their unique chemical structure, inulin and oligofructose are not absorbed in the small intestine but they are fermented in the colon to combustible gases, lactate, and short chain fatty acids [10].

Breakdown of polysaccharides in the large intestine is a complex process involving various enzymes (poly- and oligosaccharidases) from many different species and cross-feeding by the microbiota. Three species of *Bifidobacterium* were examined for the ability to grow with fructo-oligosaccharides (FOS) as carbohydrate source [11]. *B. thermophilum* inulinase was induced by inulin, while the enzymes from the other two strains were constitutive. Production of inulinase by all three strains was

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regulated by catabolite repression. Strains which grew best on inulin required the presence of this FOS for maximal induction of the exo-inulinase [12].

Bifidobacteria are nutritionally less fastidious than lactobacilli [13]. Carbohydrates are degraded exclusively and characteristically by the fructose-6-phosphate shunt, and acetic acid and lactic acid are major metabolites [14-15]. Some researchers have proposed a theoretical molar ratio of acetic acid to lactic acid of 1.5, although other scientists have proven that this ratio is not always obtained [16-18]. The variation is explained by the production of other sugar metabolites, such as formic acid and ethanol. This limits the production of lactic acid, which results in an increase in the theoretical ratio of acetic acid to lactic acid. Moreover, degradation of inulin-type fructans increases acetic acid production at the cost of lactic acid [19-21]. Bifidobacteria are also able to produce small amounts of succinic acid, although this has never been studied in detail [21]. Increased amounts of formic acid and ethanol were produced when oligofructose was used as an energy source at the cost of lactic acid [20].

Acetic acid has been observed to exert a higher antimicrobial effect than lactic acid, most likely due to a higher amount of undissociated acid at intestinal *p*H values (5.8) common to bifidobacteria and lactobacilli [22].

Detailed knowledge about the metabolism of bifidobacteria is very important because of the widespread use of these microorganisms as probiotics or as target organisms for prebiotic substrates.

Because bifidobacteria are not constitutive in the epiphytic microbiota of vegetable juices the aim of this study was to obtain informations about the dynamics of titrimetric acidity and volatile acidity during the lactic acid fermentation of carrot juice by a strain of bifidobacteria and about how inulin can favourable influence this processes in order to develop functional foods. The amounts and the ratio between these acids are directly involved on the *p*H evolution, respectively on the microbiological stability of lactic acid fermented juices. The dynamics of the reducing sugars as substratum represent another independent parameter which causes the metabolic pathways of bifidobacteria.

The fermentation of hexose occurs in the genus *Bifidobacterium* through the following sequence of reactions (bifid shout) ([13], [23 - 24]):

Bifidobacteria are glucidolitics and they produce acetic acid and lactic acid (3/2, without CO_2), according rather to the same reaction sequences [25].

The fermentation of two moles of glucose leads globally to three moles of lactic acid and 2 moles of acetic acid [26]. In reality piruvic acid can be broken down along two pathways: the first is the reduction of the pyruvate to form L(+)-lactate by L(+)-dehydrogenase (E.C. 1.1.1.27), an enzyme controlled by fructose-1,6-diphosphate. The second pathway involves the splitting of pyruvate by the phosphoroclastic enzyme to form formic acid and acetyl phosphate, a portion of which is subsequently reduced to form ethyl alcohol and so regenerate NAD. However, tests carried out to detect phosphoroclastic enzyme have been unsuccessful.

The proportion of the final fermentation products vary considerably from one strain to another and even within the same species. 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.

The schematic diagram of bifido-bacterial sugar metabolism is the following (fig. 1) [21]:

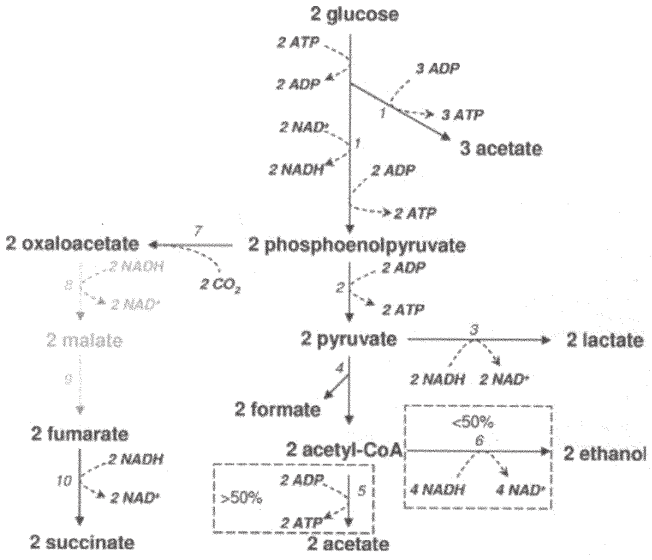

Fig. 1. Schematic diagram of bifidobacterial sugar metabolism (after [21])

$$\text{fructose-6-P} + H_3PO_4 \xrightarrow{F6PPK} \text{erythrose-4-P} + \text{acetyl-P} + H_2O$$

$$\text{erythrose-4-P} + \text{fructose-6-P} \xrightarrow{\text{transaldolase}} \text{sedoheptulose-7-P} + \text{glyceraldehyde-3-P}$$

$$\text{sedoheptulose-7-P} + \text{glyceraldehyde-3-P} \xrightarrow{\text{transketolase}} \text{ribose-5-P} + \text{xylulose-5P}$$

$$\text{ribose-5-P} \xrightarrow{\text{isomerase}} \text{xylulose-5-P}$$

$$2\ \text{xylulose-5-P} + H_3PO_4 \xrightarrow{\text{xylulose phosphoketolase}} 2\ \text{glyceraldehide-3-P} + 2\ \text{acetyl-P} + 2\ H_2O$$

$$3\ \text{acetyl-P} \longrightarrow 3\ \text{acetate}$$

$$2\ \text{glyceraldehide-3-P} \xrightarrow{\text{EMP enzymes}} 2\ \text{lactate}$$

Concluding: 2 *hexose* $\longrightarrow$ 3 *acetate* + 2 *lactate*

F6PPK - Fructose-6-phosphate phosphoketolase
EMP - Embden-Meyerhof Pathway

Experimental part

Fermentation substrate

Fresh carrots were bought from the local free vegetable market of the Dambovita County (Romania) in January. The vegetables were specifically processed by washing, scrubbing and removing non-edible pieces and then transformed into juices with a domestic juice maker. The juices were thermal treated at 80^{0}C/10min and cooled to 40^{0}C.

Microorganisms and prebiotics

A Christian Hansen lyophilized pure culture of *Bifidobacterium* BB12 was used for juice fermentation. The fermentation temperature was 37^{0}C (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.

Process performing

The lyophilized pure culture was aseptically added, in proportion of 0.2g/L to the carrot juice and vigorous homogenized. In the same manner the commercial inulin was added too. There were three experimental batches of juices: M – the sample without inulin (the control batch); P1 – the sample with 3% (w/v) inulin added; P2 – the sample with 6% (w/v) inulin added.

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 represented a single sample and the experiments were performed in double.

The lactic acid fermentation was performed in a thermostat at 37^{0}C during 48 h. The samples were investigated during the lactic acid fermentation through chemical analysis at 2, 4, 6, 8, 24, and 48 h. These analyses were secondary accomplished by microbiological investigations.

Assays

The *p*H was measured with a HACH pH-meter. Reducing sugars were analyzed using a spectrophotometrical method with 3,5-dinitrosalicilic acid (DNS) after defecation with basic lead acetate. The results are expressed as g glucose/100mL.

Titrable acidity, expressed as g lactic acid/100mL, was determined by titration with NaOH 0,1N in presence of phenolphthalein.

The values of the volatile acidity, expressed as g acetic acid/100mL, were established by steam distillation. The aliquot was titrated with NaOH 0,1N in presence of phenolphthalein.

Table 1
CHEMICAL PARAMETERS OF THE FRESH CARROT JUICE

Parameters	Experimental batches		
	M	P1	P2
pH	6.17	6.16	6.12
Titrable acidity (g lactic acid/100ml)	0.113	0.118	0.127
Reducing sugars (g glucose/100ml)	4.49	5.3	6.19
Volatile acidity (g acetic acid/100ml)	0.001 (tracks)	0.0012 (tracks)	0.0015 (tracks)

Data processing

The data were processed using the computer application Microsoft Excel. In order to evaluate the dynamics of the lactic acid fermentation process at first there were performed column plots to point out the differences of the analysed chemical parameters between the three experimental batches. Next, time depending line plots were used to point out the evolution of each analysed parameter during the investigation period. Finally the best regression equations with the highest R squared coefficient were calculated on scatter plots to more accurately evaluate the dependence between the final products of lactic acid fermentation and the *p*H value.

Results and discussions

Experimental results

Before the beginning of lactic acid fermentation the fresh juices as raw material for the lactic acid fermentation process had the following analytical parameters (table 1).

For a better understanding of the dynamics of the chemical parameters during the lactic acid fermentation, it was pointed out with the help of titrable acidity expressed in g lactic acid/100mL juice, that in all experimental batches there was no lactic acid at the initial moment.

From the viewpoint of optimal lactic acid fermentation course, the content of sugars in raw materials must be sufficient i.e. at least 40g/dm^3 [27]. This condition was accomplished even in the sample without inulin, so that it wasn't necessary the fortification of the substratum.

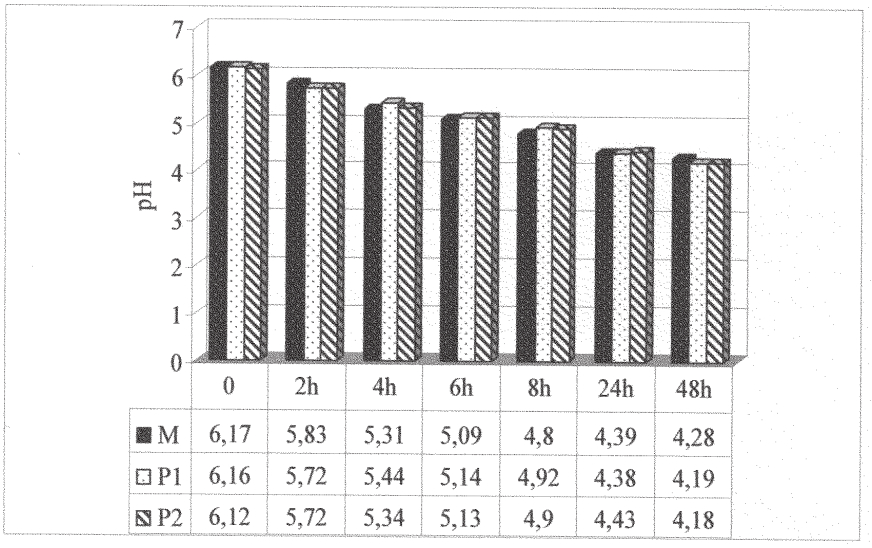

Fig. 2 Dynamics of the pH value during lactic acid fermentation of carrot juice

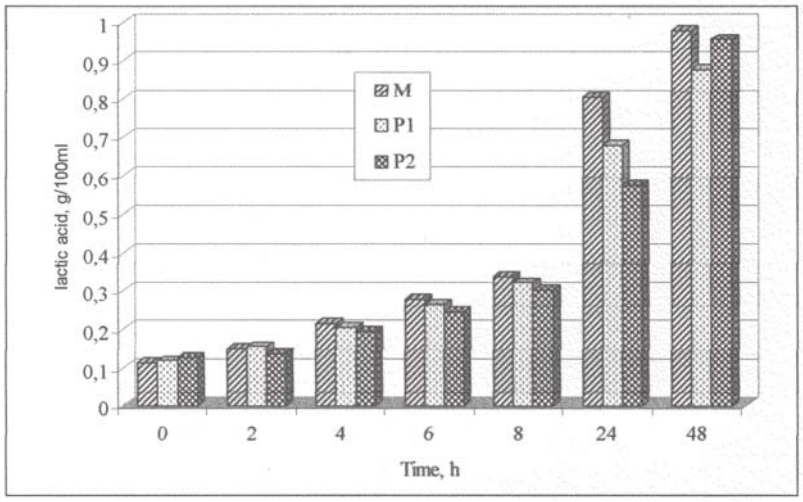

Fig. 3. Evolution of lactic acid during lactic acid fermentation of carrot juice

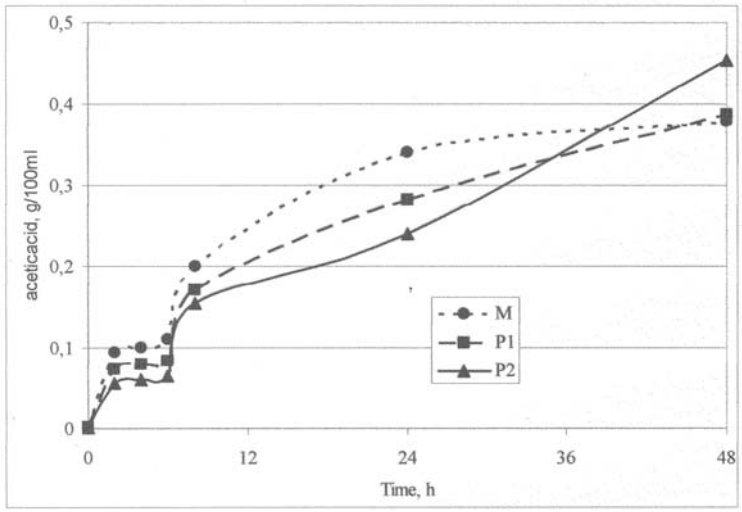

Fig.4. Accumulation of acetic acid during the lactic acid fermentation of carrot juice

The variation of the most important parameters which define the lactic acid fermentation process in the three experimental batches is presented in figure 2-5.

The *p*H of the carrot juices decreased during the analyzed period of time to values between 4.18 (P2) to 4.28 (M), as results from figure 2. The highest pH decrease was achieved nevertheless in the carrot juice with 3% inulin (P1), being by 1.97 unities in the interval 0 – 48 h. However, in absolute value it isn't as significant as against the other samples: 1.94 unities for P2, respectively 1.89 unities for M.

At the end of the fermentation the titrable acidity of the juices (expressed as lactic acid) varied from 0.878g/100mL (P1) to 0.978g/100mL (M), as it is shown in figure 3.

Virtually, the most important decrease of the *p*H values (about 1.7 – 1.8 unities) was determined, in all samples, during the first 24 h of fermentation. It's so obvious that the *Bifidobacterium* strain has the ability to accommodate at a new type of substratum that is different from the medium of origin. On the other hand, the rapidly decrease of the juices *p*H is a guarantee of the inhibition of the spoilage bacteria.

While not very significant quantitatively, in the interval between 24-48 h the decrease of *p*H is more than double in P2 comparatively with M. This evolution was correlated with an important decrease of the reducing sugars content in the sample with inulin (0.6g/100mL), respectively with a gain of lactic acid about 0.383g/100mL and a gain of acetic acid by 0.215g/100mL. In this period of time the theoretical molar ratio of acetate 1.5: lactate 1.0 was found.

Our previous investigations showed that after 48 h of carrot juice fermentation with the same number of *Bifidobacterium* viable cells, it was reached the minimal pH value. This is due not to the substrate depletion, but to the inhibitory action of the higher acidity upon the useful microorganisms. The inulin addition (in both variants) seems to have a smaller influence on this parameter, very important from the point of view of the final products preservation. So, in this moment the fermentation was stopped by keeping the samples in the range of refrigeration temperatures. The soluble dry matter increase, due of the biomass production, had as consequence the slowly increase of the *p*H values.

Undissociated molecules of acetic acid and the acid feature of inulin were contributed to the lower *p*H of P2 comparatively with the control juice. The subsequent studies have to analyse the role of the amino acids that acts as buffer during the lactic acid fermentation of vegetable juices.

In the interval 0 – 48 h the increase of the lactic acid content (fig.3) ranged between 6.44 times (P1) and 7.65 times (M). In the sample with a higher amount of added inulin, the same parameter had a closer value toward the other with a half content of polysaccharide – 6.52 times in 48 h. The differences between the final values of the titrable acidities of the samples were rather close. So, between the lactic acid content of M, respectively P1 was registered a difference about 0.1g/100mL, while concerning the juices M and P2 the difference was only 0.022g/100mL. The sequence isn't the same analysing the *p*H values, due of the different amount of acetic acid achieved during lactic acid fermentation of the carrot juices (fig.4).

Statistical data correlation

Analysing the correlation between the titrable acidity and the *p*H values (fig.5) of the carrot juices during lactic

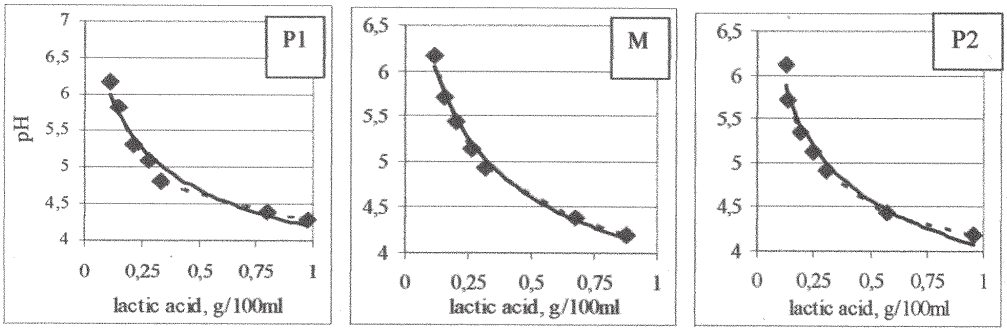

Fig. 5. Correlation between the titrable acidity and the *p*H values of the carrot juice during lactic acid fermentation

acid fermentation using the regression analyse of the concentration of lactic acid and the pH values, the power regression was found the best describing this correlation because it had the highest R squared coefficient value. The equations and determination coefficients in the case of the three experimental batches are the following:

(M) $y = 4{,}1797x^{-0{,}1665}$; $R^2 = 0{,}964$
(P1) $y = 4{,}0499x^{-0{,}1871}$; $R^2 = 0{,}9917$
(P2) $y = 4{,}0475x^{-0{,}1781}$; $R^2 = 0{,}9654$

So, using these regression equations it is possible to evaluate the contribution of the value of lactic acid content of juice (x) in the determination of a suitable *p*H value (y). The deviations from the ideal value of the R squared coefficient (1) are caused mainly by the influence of the acetic acid content on the pH values.

In absolute values, after 48 h the increase of the titrable acidity was maximal in the sample without inulin (0.865g/100mL) and minimal in the sample P1, with 3% inulin (0.76g/100mL). The same minimal increase of the volatile acidity was registered in the sample M, which confirm the tendency of the metabolic pathway to the reduction of the pyruvate to form L(+)-lactate by L(+)-dehydrogenase. The ratio between the accumulated lactic and acetic acid in the juice M after 48 h of fermentation was about 2.31, while not a large difference was observed in the case of the juice P1, this parameter being about 2.05.

If the dependence between the decrease of the *p*H values and the increase of the lactic acid content of the fermented carrot juices with a strain of *Bifidobacterium* were better described by a power regression, a polynomial function correlates at higher R squared the values the *p*H and volatile acidity from the analysed samples (fig.6). For certain momentary value of acetic acid (x) can be determined the resulted value of *p*H (y) on the basis of the following regression equations:

(M) $y = 10{,}804x^2 - 9{,}1435x + 6{,}2134$; $R^2 = 0{,}9265$
(P1) $y = 11{,}908x^2 - 9{,}6539x + 6{,}1512$; $R^2 = 0{,}9555$
(P2) $y = 12{,}732x^2 - 9{,}7954x + 6{,}0159$; $R^2 = 0{,}9344$

The differences between ''1'' and the R squared coefficient values are larger than in the case of the analysis of dependence *p*H – lactic acid. It's so obvious the preponderant influence of the lactic acid content on the pH values of lactic acid fermented juices in the mentioned experimental conditions.

During 48 hours of fermentation, the acetic acid content was ranged between 0.001 to 0.378g/100mL in the sample without inulin (M), from 0.0012 to 0.386g/100mL in the sample P1, respectively from 0.0015 to 0.455g/100ml in the sample with a higher amount of added inulin (P2), as it is shown in figure 4. The most important variation of this compound was in the interval 0 - 24 h in the juices M (increasing by 339 times) and P1 (increasing by 233 times). A total different dynamics pattern was established in the sample P2, in the sense that the increase of the acetic acid content was only about 159 times in the first 24 h and about 143 times in the next 24 h. So, it seems that the higher amount of the reducing sugars in the samples P1 and P2 at the initial moment, due to the presence of glucose residue at the end of the linear chains of the inulin, induces a slowly starting of the lactic and acetic acids accumulation in the first 24 h. In this period of time the balance between the growth of bifidobacteria and the acidity production was inclined towards the microorganisms development in the juices with inulin (P1 and P2). However, the increase of the volatile acidity in the sample P2 during the interval 24 - 48 h proves the favourable effect of the inulin addition on the growth of bifidobacteria, which produce short chain fatty acids (SCFA).

After 48 h the acetic acid production is direct proportional with the quantity of added inulin. If the differences aren't so significant between the juices P1 and M (only 0.0072g/100mL), it seems that a higher amount of polysaccharides in the juice P2 induces preponderant the second pathway mentioned by [26], that involves the splitting of pyruvate by the phosphoroclastic enzyme to form formic acid and acetyl phosphate, a portion of which is subsequently reduced to form ethyl alcohol and acetic acid. Thus can be

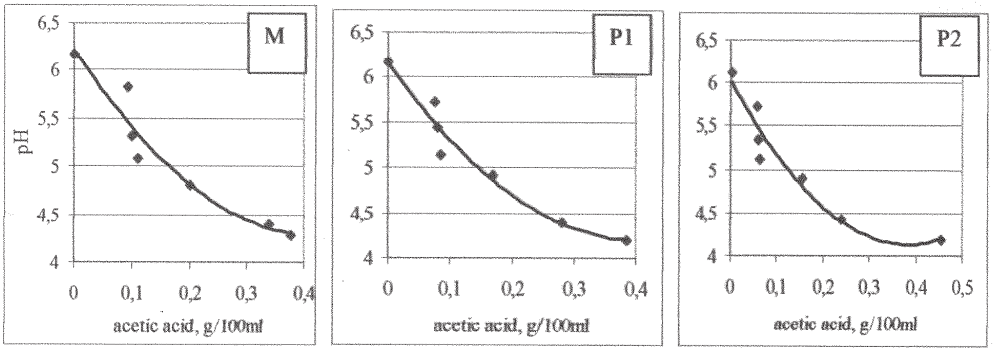

Fig. 6. Correlation between the volatile acidity and the *p*H values of the carrot juices during lactic acid fermentation

explained the regeneration of NAD^+ and ATP, important for the microorganisms development.

Assessment on the substrate break down by lactic acid bacteria during lactic acid fermentation of the carrot juice

During the lactic acid fermentation of carrot juices the sugars were utilised in the metabolic processes by the *Bifidobacterium* strain (fig.7).

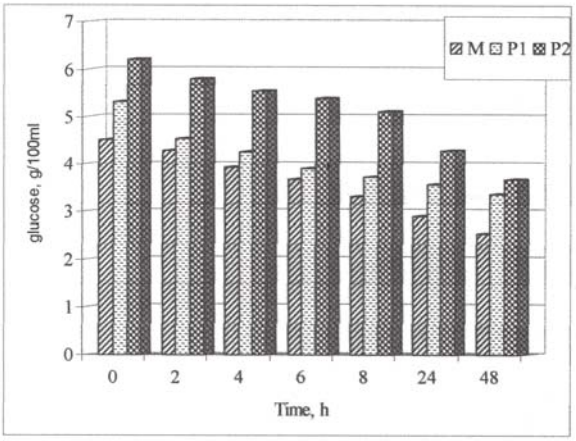

Fig.7. Decrease of the reducing sugars of carrot juices during lactic acid fermentation

After 48 h all juices have a residual sugar content of 56.35% (M) to 63.4% (P1) from the original concentration of the reducing sugars present in the raw materials. The highest consumption speed of these compounds was in the first 24 h in all samples, situation correlated with the lactic and acetic acids accumulation.

The various responses of bifidobacteria to fructans of different lengths were described not only in terms of their fermentation capability but also in terms of their ability to induce differently located enzymes [28[.

The initial contribution of the polysaccharide addition in the juice P1 concerning the reducing sugars amount toward the control juice (M) was about 0.81g/100mL. After 48 h of fermentation the difference between the two samples remained at the value of 0.83g/100mL. On the other hand, at the same moment the values of *p*H, lactic acid content, acetic acid content and reducing sugars consumed were much closer. All these data denote that the added quantity of inulin was used for *Bifidobacterium* growth (which it was also proved through microbiological analysis) and it seems that the strain hadn't the ability to produce extracellular inulinase. The smaller differences between the chemical parameters of the juices M and P1 proved the contribution of inulin only to the development of bacteria.

The production of inulinases was mentioned in literature [28], but the experimental *Bifidobacterium* strains from ATCC and DSMZ cultures were tested on a semisynthetic medium concerning the growth on inulin as single carbon source.

Analyzing the differences between the initial and the final content of reducing sugars of the control juice and the juice P2 resulted that it was decreased from 1.7 to 1.14g/100ml. So, 0.56g/100mL represent the amount of substratum that was consumed both from lactic and acetic acids production. This situation was reported especially for the interval 24 – 48 h of fermentation, which showed that the surplus of inulin was especially used for the volatile compounds (acetic acid, ethanol) production, as final products of bifidobacteria metabolism. In the last 24 h of fermentation the reducing sugars consumption in the juice P2 was about 1.7 times higher than those from juice M, respectively about 2.8 times comparatively with P1. These dynamics was also caused by the increase of the bifidobacteria cells number in the sample P2.

In the period 0-24 h of lactic acid fermentation the amounts of lactic and acetic acid formed were represented about 63% from the reducing sugars consumed in the control juice (M), 48.5% in the juice P1, respectively 35.6% in the juice P2. The differences between the samples with and without inulin prove not only the influence of this prebiotic on the bacteria development (which is clear demonstrated through microbiological analysis), but the possibility that the metabolic pathway was directed to non-volatile compounds production, as succinate.

Analyzing all the data, it seems that the long chain of inulin inhibits the production of lactic and acetic acids as metabolites in the first hours of fermentation. In specialty literature it was mentioned that bifidobacteria preferentially use the short fractions of oligofructose rapidly [19 – 20].

The influence of the inulin used as prebiotic can be observed by representing the dynamics of the principal physico-chemical parameters involved on the one hand in the lactic acid fermentation evolution on the other hand in the shelf life of the final products.

From the juice stability point of view the time needed for a rapidly decrease of *p*H is the most important factor. From the sensorial point of view the accumulation of short chain fatty acids is also important [29]. For the global quality of the fermented juices the equilibrium between the *p*H values and the lactic and acetic acids content after a period of time is essential.

So, the dynamics of the *p*H values and the increase of titrable and volatile acidity during fermentation, for all the experimental batches, can be observed in figure 8.

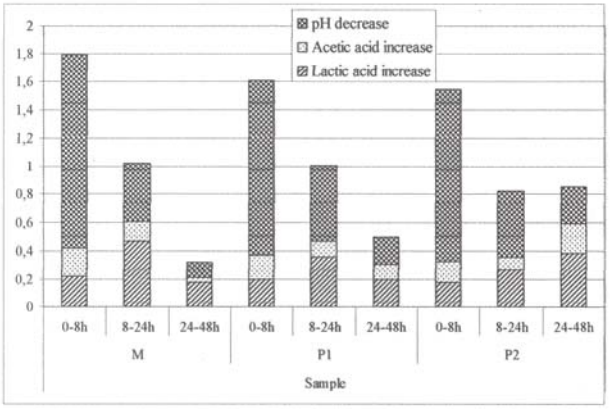

Fig. 8. Dynamics of *p*H, lactic and acetic acids during the three intervals of lactic acid fermentation of juice

Analysing the changes of the chemical parameters that contributes to the juices preservation, during three established periods of time (0-8 , 24-48 and 24-48 h), their tendency of evolution was the same in the control batch (M) and in the batch with 3% inulin.

In the first 8 h of fermentation the presence of a higher amount of inulin inhibits in some proportion the lactic and acetic acids production. Also, the presence of this polysaccharide in juice determined a delay in the their accumulation in the interval 0 – 24 h, situation that was changed in the next period of time in the sense of directly adequacy between the amount of added inulin and the increase of lactic and acetic acids content.

The influence of the organic acid type resulted as metabolit on the *p*H values of juices isn't obvious due to the smaller difference between the pK_a values of acetic acid and lactic acid [30] (about 0.05 unifies). After 48 h of

fermentation the control batch was characterized by a lower amount of acetic acid and a higher pH value, while the situation was opposite concerning the juice with 6% added inulin.

The efficiency of reducing sugars conversion in lactic and acetic acids after 48 h of fermentation was very strongly influenced by the amount of added inulin (fig. 9). If this parameter had the same value in the case of the control juice (M), as that one determined after 24 h (63%), the situation is different in the others cases. So, due to the increase of the acids content in the juices with inulin (P1 and P2) during the interval 24 – 48 h, it can be concluded that the efficiency was enlarged to 58% in the case of juice P1, respectively to 50% in the case of the juice P2 at the end of the fermentation.

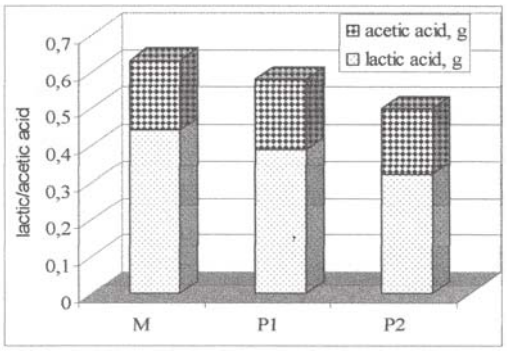

Fig.9. Lactic and acetic acids production accordingly to 1g reducing sugar consumed after 48 h of fermentation

These data emphasize once more that the prebiotic inulin was used preponderant as carbon source for the bifidobacteria multiplication and their cellular compounds synthesis and not in the energy producing metabolic pathways. In the literature it was mentioned that other products of the bifidobacteria metabolism, like succinat or carbon dioxide, represent smaller amounts [26]. It was demonstrated also that the theoretical ratio of acetate 1.5: lactate 1.0 is scarcely ever found in growing cultures of bifidobacteria [24].

Also, several factors related to the specific sugar consumption rate, such as the sugar uptake systems and the conversions necessary to incorporate a specific sugar into the fructose-6-phosphate pathway, can have a high influence on which metabolic route is used [31].

Conclusions

The rapidly decrease of the *p*H values of juices during the first 24 h of fermentation, associated with a higher consumption of the reducing sugars, shows that the substratum is favourable for bifidobacteria development. The low specific rate of sugar conversion in lactic and acetic acids during the growth of the used *Bifidobacterium* strain in the presence of prebiotic inulin indicates also that the polysaccharide addition influences mainly the bacteria development, not the lactic and acetic acids production. However, the *p*H values and the values of the volatile acidities of the carrot juices were favourable influenced by the polysaccharide addition after 48 h of fermentation, so their preservation.

Results indicated that the ability of a strain to growth on carrot juices with added inulin isn't related to the production of extracellular enzymes that hydrolyze long-chain fructans.

The carrot juices fermented with bifidobacteria represent not only drinks for persons with lactose intolerance, but symbiotics that promote persistence of living bacterial cells and selectively stimulates the metabolism of bifidobacteria.

This study suggests that a better understanding of *Bifidobacterium* metabolic activity is possible through the knowledge of the chemical changes during the lactic acid fermentation that will help to manage these biochemical processes with a view to obtain wholesome foods.

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