Romanian Biotechnological Letters *Vol.17, No.4, 2012*

ORIGINAL PAPER

Evaluation of the cabbage and cucumber juices as substrate for *Lactobacillus acidophilus* LA-5

Received for publication, March 29 2011
Accepted, November 22, 2011

BURULEANU CLAUDIA LAVINIA, IULIANA MANEA,
MAGDA GABRIELA BRATU, DANIELA AVRAM, CARMEN LEANE NICOLESCU
Valahia University of Targoviste, Faculty of Environmental Engineering and Biotechnologies, Department of Food Engineering, 18-24 Unirii Bd., 130082 Targoviste, Romania
Corresponding author: Tel.: +40245206108, Fax: +40245206108,
E-mail address: laviniaburuleanu@yahoo.com

Abstract

The capacity of the juices obtained from cucumber, white and red cabbage respectively to represent a substrate for the growth of Lactobacillus (Lb.) acidophilus, in order to obtain probiotic products, was investigated. Lb. acidophilus was very well adapted to the cabbage as new substrate, because in the first 2h the pH has declined by one unit, while the microbial population has increased almost 100 times. After 48h the decrease of the reducing sugars ranged from 1.16 to 1.54 g/100ml and the titratable acidity was increased about 13 times. Lb. acidophilus was faster accommodated in cucumber juice, the maximum rate of acidification v_{max} (dpH/dt) being achieved in 2h ($9.33x10^{-3}$ units/min). If some differences were established referring to time to reach pH 5.0 (more than 2h in the case of the red cabbage), the time to complete the fermentation ($t_{pH\ 4.2}$, hours) was for all the batches about 6h. Applying the multivariate statistical methods, the variables were classified into three clusters and the tree chart was described as well. The Principal Component Analysis (PCA) reduced the five original analytical variables to one independent component, which accounted for 80.43% of the total variance.

Keywords: lactic acid fermentation, vegetable juice, probiotic*,* modelling

Introduction

In the recent years, based on *in vitro* and *in vivo* studies, many probiotic products have been developed in order to improve the organism's health (W.A. WALKER, cited by D. PELINESCU [1]). The nutritional potential of vegetables is remarkable, so the production of lactic acid fermented juices seems to be an alternative for the lactose-intolerant people or vegetarians, and in the same time for the ones concerned by the own healthy.

An optimal balance of microbial organisms in the intestine is suggested to be an important aspect of maintaining good health. Certain bacteria, such as lactobacilli and bifidobacteria that help maintain such a favorable balance are considered to be probiotics (S. HEKMAT and D.J. McMAHON [2]). The probiotic arsenal includes multiple mechanisms for preventing infection, one of them being represented by the interaction with the host immune system in a strain-dependent manner (D. PELINESCU & al. [1]).

The genus *Lactobacillus* (*Lb.*) is widely distributed in nature. Several species, including *Lb. acidophilus*, are components of the normal intestinal flora of healthy humans. The taxonomy is extremely complex, and complete agreement on the designation of specific strains cannot be found (M. SILVA & al. [3]).

Lactobacilli are important inhabitants of the gastrointestinal (GI) tract and some species are considered to have probiotic properties, offering a number of benefits to health and well being (G. REID & al. [4]). The most well known members of this group are classified as the ''acidophilus complex,'' composed of six species of closely related lactobacilli that have historically been isolated from the GI tract of humans and animals (HEILIG & al. [5]). For such microbes, survival and residence in the GIT relies partially on their ability to survive gastric passage and use available nutrients (R. BARRANGOU & al. [6]).

Brassica vegetables, including all cabbage-like ones, are consumed in enormous quantities throughout the world and are important in human nutrition (F. FERRERES & al. [7]). They are reported to reduce the risks of some cancers especially due to its content of glucosinolates and their derived products (O.K. CHUN & al. [8]), although phenolic compounds are also considered to contribute to this capacity (G.GALATI and P.J. O'BRIEN [9]).

The composition of cucumbers varies with fruit size and cultivar (R.F. McFEETERS and L.A. LOVDAL [10]). As fruit size increased, malic acid content (wet basis) decreased slightly from 0.28 to 0.21%, while glucose and fructose contents (wet basis) increased from 0.80 to 1.16% and 0.95 to 1.25%, respectively. The total sugar content (wet basis) in cucumbers was in the range of 1.7 to 2.4 %, depending on fruit size. Smaller fruit, however, contain less sugar and a higher natural buffering capacity than the larger ones (Z. LU & al. [11]).

Naturally occurring microbial inhibitors have been recovered from a wide variety of foods including onions, garlic, fruits, vegetables, cereals and spices. Many of these antimicrobials contribute to the food stuffs natural resistance to deteriorations. The flavor components consist of such compounds as alcohols, aldehydes, esters, terpens, phenols, organic acids and others, some of which have not yet been identified (L.A. SHELEF & al. [12]). Onion bulbs contain tannin, pectin, quercetin and glycosides. Onion extracts shows antibacterial properties (S.K. ADESINA & al. [13]). The antimicrobial activity of *Allium* is due to volatile sulfur compounds derived from S-allyl-L-cysteine sulfoxide, a non-protein amino acid found in vegetables, by the action of an enzyme, cysteine sulfoxide lyase (J.W. KIM & al. [14], [15]).

The essential step in the use of statistical experimental design methods is to select the suitable ranges of the selected control factors in the initial experiments (G. BAHRIM & al. [16]). The multivariate statistical methods are used in all scientific branches. In the analysis of the food quality, the Principal Component Analysis (PCA) is advantageously applied for the evaluation of the experimental data. Thus, processing a great number of variables it can be obtained a smaller set, the losing of information being minimized. The purpose of Cluster Analysis (CA) was to join data into clusters in order to increase their within group homogeneity as well as the differences among the clusters and the individual groups (Z. KOHAJDOVÁ and J. KAROVIČOVÁ [17]).

The aim of this work was the evaluation of the growth of *Lb. acidophilus* into different vegetable juices through kinetic analysis and applying PCA and CA to the experimental data as well.

Materials and methods

Microorganisms

LA-5 is a single strain culture containing *Lb. acidophilus* that was obtained from Christian Hansen (Romania). It was defined by the producer as thermophilic lactic culture.

Lactic acid fermentation performing

Fresh white cabbage (*Brassica oleracea* L.), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), cucumbers (*Cucumis sativus*) and red onion (*Allium cepa* var. *ascalonicum*) were purchased from a local store and specifically processed by removing the non-edible pieces. Using a home-made extractor, the vegetables were turned into juice.

Four experimental batches were performed: Cb - cabbage juice, RCb - red cabbage juice, Cc - cucumber juice, CcO - cucumber juice with 0.1% onion juice added after the heating and cooling of the batches.

The heating treatment of the juices, applied at 80 degrees C with a view to destroy the undesirable microorganisms under the limit of detection, was followed by cooling at 40 degrees C. The lyophilized culture was aseptically added in proportion of 0.2g/L to the juices and vigorous homogenized for 15 min.

50mL 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 ± 0.2^0C. The samples were analyzed during the process dynamic through chemical and microbiological analysis.

Analytical methods

The count of *Lb. acidophilus* was determined by plate count method using Man-Rogosa-Sharpe agar, enriched with L-cysteine HCl, after serial tenfold dilutions in peptone water. The Petri dishes were incubated for 48h at 37 °C and the results were expressed as CFU/ml juice.

The optical density of biomass was measured with the UV-Visible spectrophotometer at 610nm. In the preparation of the calibration curve for optical density vs. dry cell weight several dilutions of the samples were made. According D. ALTIOK ([18]), for each dilution, 2 ml of sample was used to obtain optical densities at 610 nm wavelength and 15 ml of sample was filtered with a pre-weighed cellulose acetate membrane filter having a pore size of 0.45 µm using a vacuum pump. The biomass collected on the filters was washed with 15 ml of water and the filters were dried at 100^0C for approximately 24 h until constant weight was observed. The results were expressed in g/l.

The pH values were measured with a HACH pH-meter. From the chemical point of view, the titratable acidity, expressed in g lactic acid/l, was determined by titration with NaOH 0.1N in the presence of phenolphthalein (in the case of the white cabbage and cucumber juices), respectively in the presence of bromthymol blue (in the case of the red cabbage juice). The reducing sugars were analyzed applying the spectrophotometric method with 3.5-dinitrosalicilic acid (DNS) after the removing of the substances with reducing character using basic lead acetate. The results were expressed in g glucose/l.

Mathematical modeling

The mathematical models were applied for the product formation and substrate utilization.

Concerning the *product formation*, lactic acid fermentation was described by R. LUEDEKING and E.L. PIRET ([19]). 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):

$$\frac{dP}{dt} = \alpha \frac{dN}{dt} + \beta N$$

where the constants α (the growth associated product formation) and β (the non growth associated product formation) are determined by the pH of the fermentation.

A simplified presentation of the above model relates to the linear part of the equation was presented by A. AMRANE and Y. PRIGENT ([20]):

$$(P - P_0) = \alpha\,(X - X_0)$$

where P_0 and P are the concentrations of lactic acid (g/l) initially and at time t, respectively, and X_0 and X are the increases of the biomass (g/l) initially and at time t, respectively.

The *kinetic of the substrate utilization* for the lactic acid fermentation of the juices with *Lactobacillus acidophilus* was underlined by the biomass and product yield coefficients on substrate (Y_{XS}, respectively Y_{PS}), defined as stoichiometric coefficients:

$$Y_{XS} = \frac{X_f - X_0}{S_0 - S_f}$$

$$Y_{PS} = \frac{P_f - P_0}{S_0 - S_f}$$

where S_0 and S_f are the concentrations of reducing sugars (g/l) initially and at time t, respectively.

Statistical analysis

For the evaluation of the analytical results, the multivariate statistical methods (Principal Component Analysis and K-Means Cluster) were applied. The prediction of the linear models using the regression and the matrix correlation between all the experimental data was realized with SPSS software package for MS Windows version 19 (trial).

Results and discussions

The pH evolution and the titratable acidity dynamics during the lactic acid fermentation of different vegetable juices with *Lb. acidophilus* are shown in Fig.1 and Fig.2 respectively.

The pH values ranged from 6.21 to 3.7, no significant differences between the batches being observed. After 24 hours, the highest decrease of pH was observed in the case of the cucumber juice (2.51 units), correlated with the increasing of the titratable acidity by 12.69 times. In the same time, the pH of the white cabbage juice was declined with 2.27 units, corresponding to the lactic acid accumulation until a value by 8g/l. Although the pH values of the above mentioned samples (Cb and Cc) were close during the process development and also the initial and the final values of the titratable acidity were almost the same, few different aspects were determined in time. In this sense, the maximum rate of acidification v_{max} which registered better values in the case of the cucumber juice can explain the fermentation slowness in the batch Cb the interval 6 - 8 hours. Correlated with the results of the microbiological analysis, it seems that the process was directed towards the growth of the useful bacteria. Until 24 hours, the tendency of pH and titratable acidity in the cabbage and cucumber juices has become similar.

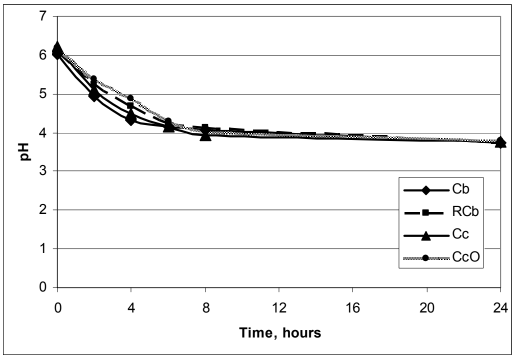

Fig. 1. The pH dynamics during the lactic acid fermentation of juices with *Lb. acidophilus* LA-5

A relative different behavior was observed in the case of both red cabbage juice and cucumber juice with onion juice added, in the sense of the slowdown of the metabolism materialized in the dynamics of the parameters that describe the process unfolding. This way, the time to reach $pH_{5.0}$, kinetic parameter important from the stability of the fermented products point of view, was increased from almost 2 hours in the case of the samples Cb and

Cc to 2.87 hours in the case of RCb, respectively 3.5 hours in the case of CcO. The above mentioned differences could be explained through the presence of some chemical constituents which has inhibitory action on useful bacteria, as the anthocyanins in the red cabbage, respectively the constituent sulfides in the onion juice. According J.W. KIM & al. ([15]) sulfides, especially those with three or more sulfur atoms, apparently possess potent antimicrobial activity. However, concerning the batch with onion juice added the initial trend was attenuated after 6 hours of fermentation, the oils and their sulfides constituent showing weak antimicrobial activity (J.W. KIM & al. [14]). Referring to the red cabbage juice, although after 24 hours of fermentation the pH values were similar, the titratable acidity was lesser with about 2g/l compared with the lactic acid fermented juice from white cabbage. This could be due to the amphoteric nature of the anthocyanins.

The initial titratable acidity of the unfermented juices, expressed as lactic acid, ranged from 0.51g/l (sample RCb) to 0.78g/l (sample Cb). As it can be seen in Fig.2, the dynamics of this parameter in the case of the red cabbage juice was invariable behind those registered for the other batches, although the kinetic parameters (Table 1) pointed out that the fermented product was characterized through microbiological stability on the background of some moderate characteristics from the sensorial point of view. In absolute values, the accumulation of the lactic acid after 24 hours of the vegetable juices fermentation were determined as follows: 7.23g/l (Cb), 5.83g/l (RCb), 9.36g/l (Cc) and 8.95g/l (CcO).

Y. KOHAJDOVÁ & al. ([21]) studied the fermentation of cucumber juices with a 0.5%, 1% and 2% additions of the onion juices by *Lb. plantarum* CCM 7039. It was found that in the initial stages of fermentation, the presence of onion in the juices positively influenced lactic and acetic acid production. However, in further course of fermentation, slight inhibition effects of onion in the fermented juices were observed, especially at elevated onion/cucumber ratio.

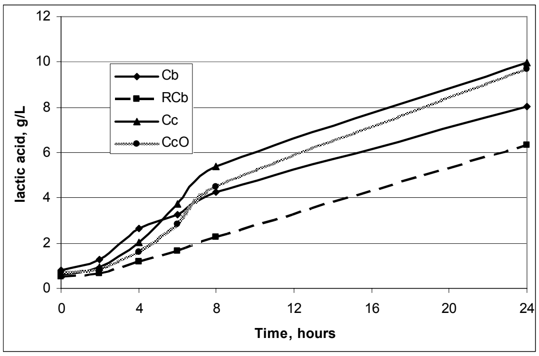

Fig. 2 The titratable acidity evolution during the lactic acid fermentation of different vegetable juices with *Lb. acidophilus* LA-5

The initial content of sugars in cucumber juice was situated at the maximum limit determined by Z. LU & al. ([11]), while in the case of the white cabbage juice was close to that one determined by K.Y. YOON & al. ([22]). The metabolization of the reducing sugars after 24 hours of lactic acid fermentation of vegetable juices with *Lb. acidophilus* LA-5 (Fig. 3) ranged between 29.29% (RCb) to 54.09% (Cc).

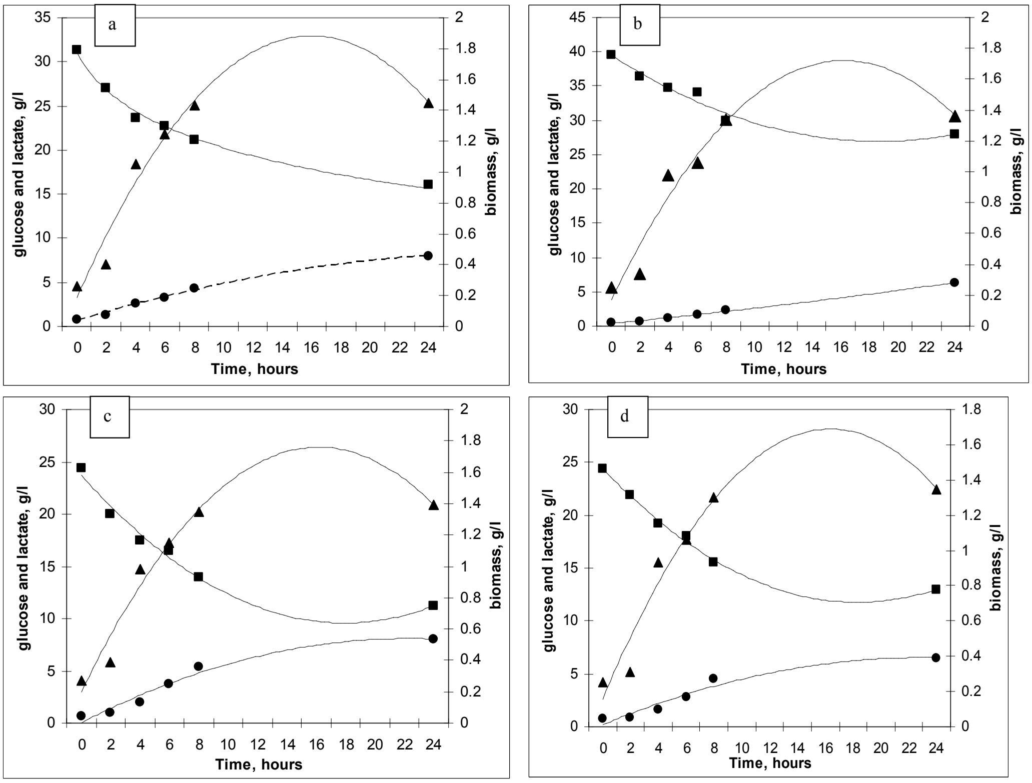

Fig. 3 Correlation between the substrate consumption, lactate production and biomass formation for the samples Cb (a), RCb (b), Cc (c) and CcO (d)
■ - glucose, ● – lactate, ▲ - biomass (points - experimental data, smooth lines - predicted values)

A direct proportionality was determined between the sugar utilization and the lactic acid production in the fermentation of all the vegetable juices, the rapport between the lactic acid production and the glucose consumption varying from 0.47 to 0.56.

As it is known, the strains of the group *Lb. acidophilus* were characterized as lactic acid bacteria with strictly homofermentative metabolism (>85% lactic acid) (G. BAHRIM & al. [23]). The comparative evaluation of the experimental data with the theoretic ratio of lactate production from glucose must take into account that *Lb. acidophilus* is not present in the epiphytic microbiota of vegetables, the chemical composition of the environment needing to be analyzed from the available carbohydrates, respectively from the feasible metabolic pathway point of view. In this sense, diammonium citrate was found to be degraded to succinic acid by the *Lactobacillus* strains (C. KANEUCHI & al. [24]). Nevertheless, according to F. DELLAGLIO & al. ([25]), the fermentation of citrate itself is a fairly general property within the strains in the genus *Lactobacillus*. The presence of fermentable carbohydrates such as glucose and lactose may influence the extent of citric acid utilization.

Close values were obtained by other authors in lactic acid fermentation of vegetable juices. Thus, the utilization of sugar during fermentation in a mixture of beetroot juice and carrot juice and different content of brewer's yeast autolysate with *Lb. plantarum* A112 and with *Lb. acidophilus* NCDO 1748 varied from 19.4 to 24.1% (M.B. RAKIN & al. [26]).

Except the dynamics of the reducing sugars in the lactic acid fermentation of the cabbage juice, which registered a logarithmic tendency, the prediction functions of the values of the analyzed parameters in all the samples (Fig. 3) were defined as polynomial, the R squared being very close to unit.

Expressing the viable cells count in log CFU/ml, the evolution of this parameter during the fermentation of the samples is shown in Fig. 4. Correlated with the increasing of the titratable acidity, the values were lower until 6 hours in the red cabbage juice and cucumber with onion juice added respectively. The difference was diminished in the next run of the process.

The tested culture, routinely used for dairy products, was found to be capable of growing on pure vegetable juices without nutrients added. In the experimental conditions mentioned above, for an initial concentration of *Lb. acidophilus* LA-5 by $8x10^5$CFU/ml, the maximum volumetric productivity was determined after 8 hours in all the samples as follows: $19.25x10^{14}$ CFU/(L·h) for Cb, $11.9x10^{14}$ CFU/(L·h) for RCb, $18.6x10^{14}$ CFU/(L·h) for Cc and $10.25x10^{14}$ CFU/(L·h) for CcO respectively.

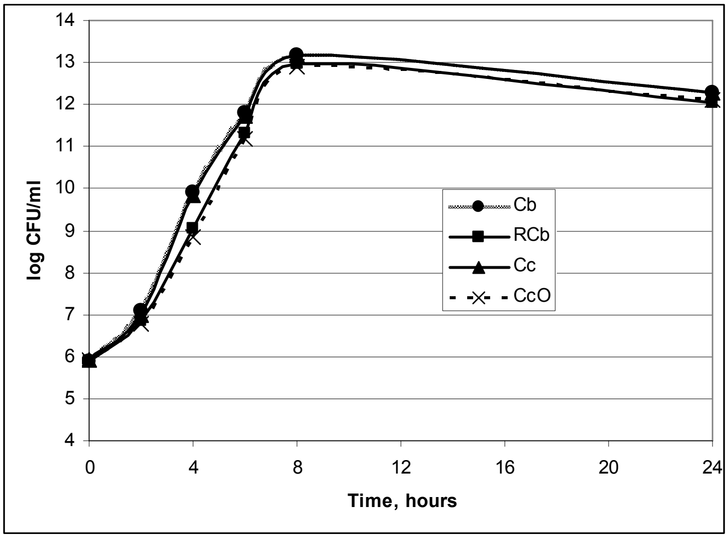

Fig. 4. The evolution of viable cells during the lactic acid fermentation of different vegetable juices with *Lb. acidophilus* LA-5

Until 24 hours the lower pH values become inhibitory in all the fermented juices, the number of viable cells decreasing by about one log. However, the storage of the final products at refrigeration temperatures beneficial influenced the survival rate of bacteria, a count by 10^6/ml being viable after one month. The above mentioned aspect is important for the evaluation of the probiotic character of the vegetable juices.

The maximum rate of acidification v_{max} was calculated as the time variation of pH (dpH/dt) and expressed as pH units/min (Table 1). The highest maximum rate of acidification was determined in the case of the cucumber juice, no significant differences between the red cabbage juice and the cucumber juice with onion juice added being noticed. The time to reach v_{max} was close to 2 hours for all the samples, while the time to reach pH 5.0 ($t_{pH\ 5.0}$, hours), important indicator referring to the stability of the final products, varied between 1.9 hours (Cb) and 3.5 hours (CcO).

According D. ALTIOK ([18]), the parameters could be divided into two groups: kinetic parameters and stoichiometric parameters. The kinetic parameters were the Monod parameters (μ_{max} and K_s) and Luedeking-Piret equation parameters (α and β). The stoichiometric coefficients are the yield coefficients for biomass and product on substrate (Y_{XS} and Y_{PS}).

Table 1. Kinetic parameters of the lactic acid fermentation of vegetable juices with *Lb. acidophilus* LA-5

Sample	v_{max}, units/min	$t_{pH\ 5.0}$, hours	Y_{XS}, g/g	Y_{PS}, g/g	α	β
Cb	8.83	1.9	0.077	0.469	4.102	0.626
RCb	6.83	2.87	0.072	0.502	3.158	0.709
Cc	9.33	2.3	0.084	0.558	5.24	1.373
CcO	6.66	3.5	0.083	0.437	4.175	0.778

Using SPSS 19, the values of the growth associated product formation (α) and non growth associated product formation (β) for all the samples are summarized in Table 1. From previous studies the data for the same experimental are not available. For other design, a lot of data are quoted in literature for different strains of *Lactobacillus* and *Lactococcus*. In this sense, the above mentioned values were close to those determined by others in the lactic acid fermentation of different media. For example, with *Lb. helveticus* J. BIAZAR & al. ([27]) calculated values by 4.6 for the growth associated product formation and 0.23 for the non-growth associated product formation, while using *Lb. rhamnosus* S. KWON & al. ([28]) established values by 6.6 for α and 0.33 for β respectively.

Both values for product yield coefficient ($Y_{P/S}$) and substrate yield coefficient ($Y_{X/S}$) were higher in the case of the cucumber juice. In fermentation of different media with *Bifidobacterium animalis* subsp. *lactis* Bb12, H. JALILI & al. ([29]) obtained for Y_{XS} values from 0.05 to 0.13, while for Y_{PS} values from 0.47 to 0.57. J. BIAZAR & al. ([27]) determined 0.064 and 0.61 as values for the substrate and product yield coefficient respectively.

Table 2. The Pearson coefficients for the experimental batches

Parameter	pH	lactic acid	glucose	viable cells	biomass
pH	1	-0.801**	0.513*	-0.942**	-0.946**
lactic acid		1	-0.643**	0.781**	0.810**
glucose			1	-0.508*	-0.507*
viable cells				1	0.979**
biomass					1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

The correlation between the most important parameters of the lactic acid fermentation of vegetable juices with *Lb. acidophilus* LA-5 were evaluated using Pearson correlation analysis ($p < 0.01$), summarized in Table 2. The correlations were moderate between glucose - pH (0.513), respectively glucose - biomass (-0.507) and glucose - viable cells (-0.508), while a strong relationship for pH - viable cells (-0.942) and pH - biomass (-0.946) can be considered (Table 2). Thus, the pH decrease during fermentation correlates with the viable cells and biomass increasing. The coefficient by -0.801 showed also a strong correlation between pH and lactic acid, the higher indirect linear dependence among these variables being a consequence of the activity of lactic acid bacteria, which produced mainly lactic acid.

The PCA was used for reduction of information of a large number of variables into a smaller set while losing only a small amount of information (Z. KOHAJDOVÁ and J. KAROVIČOVÁ [17]). Applying PCA to the experimental data, the analytical variables were reduced to one principal component, which accounted for 80.43% from the total variance. According to the component matrix (Table 3), the most notable variables were: pH (loading 0.954), lactic acid (loading 0.902), viable cells (loading 0.956) and biomass (loading 0.963).

Table 3 Component loadings expressed by the first principal component for the analytical variables

Analytical variable	Component 1
pH	-0.954
lactic_acid	0.902
viable_cells	0.956
glucose	-0.676
biomass	0.963

According PCA method, each eigenvalue represent the part of the explained variance, attracted by the respective component. The dependence between the number of principal components and the eigenvalues for the analytical parameters of lactic acid fermented juices is shown in Fig.5.

For the results evaluation, the cluster method of centroid was used. The distances between objects were measured as squared Euclidean distance. CA divided the experimental data into three groups, characterized by similar analytical properties, as follows:

- **cluster 1**: cabbage juices inoculated with *Lb. acidophilus* LA-5 from 2th to 8th h of fermentation (Cb2 - Cb8); red cabbage juices inoculated with *Lb. acidophilus* LA-5 from 8th to 24th h of fermentation (RCb8 - RCb 24); cucumber juices inoculated with *Lb. acidophilus* LA-5 from 0th to 2th h of fermentation (Cc0 - Cc2); cucumber juices with onion juice added inoculated with *Lb. acidophilus* LA-5 from 0th to 4th h of fermentation (CcO0 - CcO4);

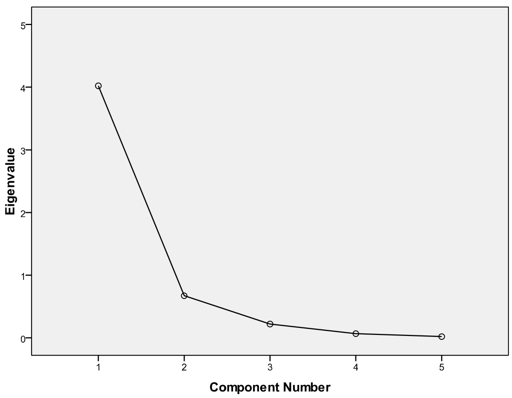

Fig. 5 Screen plot of the eigenvalues for the analytical parameters of lactic acid fermented vegetable juices

- **cluster 2**: cabbage juices inoculated with *Lb. acidophilus* LA-5 in 24th h of fermentation (Cb24); cucumber juices inoculated with *Lb. acidophilus* LA-5 from 4th to 24th h of fermentation (Cc4 - Cc24); cucumber juices with onion juice added inoculated with *Lb. acidophilus* LA-5 from 6th to 24th h of fermentation (CcO6 - CcO24);

- **cluster 3**: cabbage juices inoculated with *Lb. acidophilus* LA-5 in 0^{th} of fermentation (Cb0); red cabbage juices inoculated with *Lb. acidophilus* LA-5 from 0^{th} to 6^{th} h of fermentation (RCb0 - RCb 6).

None of the cucumber juice samples were included in the cluster 3, due to the general trend of both the chemical and microbiological parameters, which underlined a vigorous development of the lactic acid fermentation. The white cabbage juice at the initial moment of time, respectively the red cabbage juices until the first 6 hours of fermentation are present in the cluster 3, due to the low or moderate values of the experimental data into the general dynamics of the analytical parameters.

Cluster 2, characterized through a higher titratable acidity, a lower pH values, a lower content in reducing sugars and also a higher content of viable cells consists of the cabbage juice in 24 hours of fermentation, cucumber juices (in both experimental) from 6 to 24 hours and also the cucumber juice without onion juice added in 4 hours of fermentation.

Finally, cluster 1 was made by samples with average values of the experimental data. Illustrating the above mentioned aspects, in Fig. 6 are shown the clusters in axes of two selected variables.

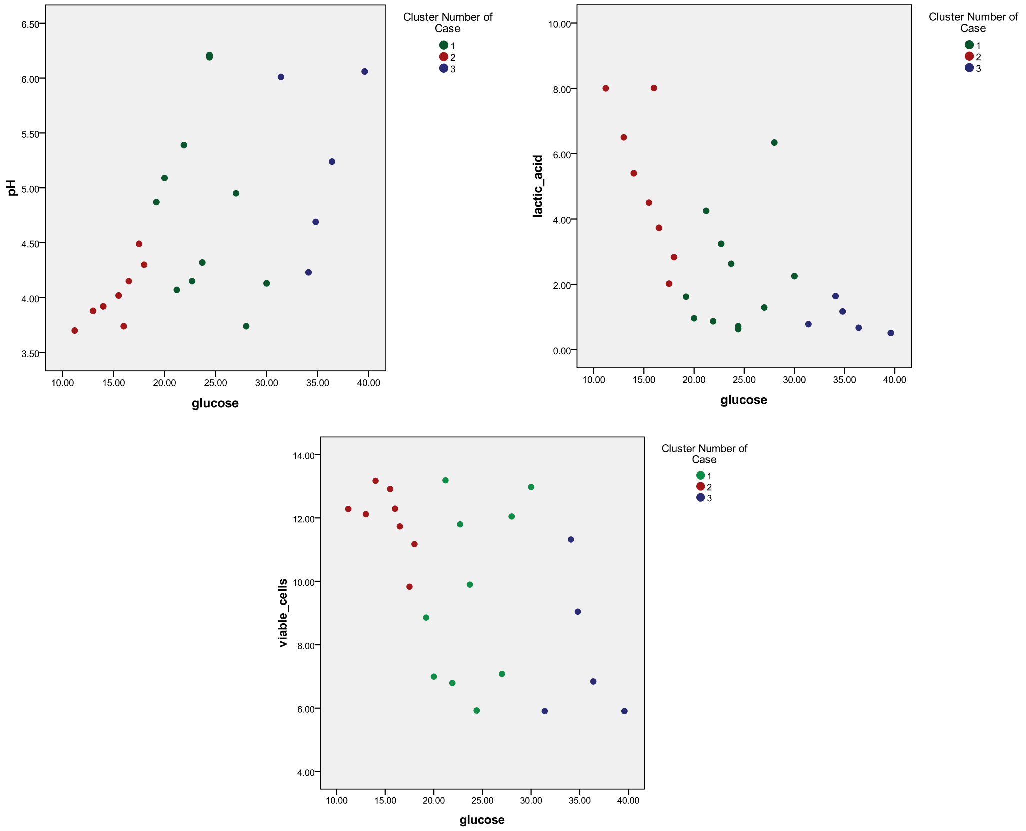

Fig. 6 Clusters plotting in coordinate of two selected variables: glucose - pH; glucose - lactic acid; glucose - viable cells.

J. KAROVIČOVÁ and Z. KOHAJDOVÁ ([30]) used PCA, FA and CA for the relationships evaluation between analytical and sensory parameters measured during the fermentation of cabbage-carrot juices inoculated with *Lb. plantarum*. PCA reduced the 7 analytical variables to one independent component that accounted for 96.92% of the total variance, while CA divided the samples into two groups, in function of the lactic and acetic acids content. If *Lb. plantarum* was used for the fermentation of the cabbage juices with various additions of onion juices, the PCA reduced seven original analytical parameters to one principal component that accounted for 81.45% from the total variance. CA divided samples into five groups that were characterized by similar analytical properties (Z. KOHAJDOVÁ and J. KAROVIČOVÁ [17]).

Applying PCA to the lactic acid fermentation of cabbage juices with various microorganisms, Z. KOHAJDOVÁ and J. KAROVIČOVÁ ([31]) established that the original 7 analytical variables were reduced to 2 independent components that explained 88.2% from total variance of input data (PC1 66.9% and PC2 21.3%). Two PC which explain 70.3% of the total variance were determined by R. Di CAGNO & al. [32] using PCA to viscosity, tonalities, ascorbic acid, glutathione, total antioxidant activity, glutamic acid and total free amino acids of tomato juices fermented with ten autochthonous strains and stored for 40 days.

Conclusions

Although some differences between the growths trends of *Lb. acidophilus* LA-5 were determined, all the analyzed vegetables are suitable for obtaining lactic acid fermented juices with a higher self-life. With a view to underline the probiotic characteristics of these ones, the number of viable cells during the storage must be monitored.

Application of Principal Component Analysis selected the most important parameters from analytical point of view: pH, lactic acid, biomass and viable cells, while the Cluster Analysis divided the experimental variables into three groups.

Acknowledgement

The researches were performed in the frame of the Project PN-II-ID-PCE-2008-2 (ID_1359) *Researches concerning the preparation of lactofermented juices with specific probiotic and evaluation through methods specific for intelligent modelation.*
The microorganisms were kindly provided by Chr. Hansen, Romania

References

1. D. PELINESCU, M.C. CHIFIRIUC, L.M. DITU, I. SARBU, C. BLEOTU, T. VASSU, I. STOICA, V. LAZAR, N. CORCIONIVOSCHI, E. SASARMAN, Selection and characterization of the probiotic potential of some lactic acid bacteria isolated from infant feces, *Rom. Biotechnol. Lett.*, **16**(3), 6178-6189 (2011).
2. S. HEKMAT, D.J. McMAHON, Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in ice cream for use as a probiotic food, *J. Dairy Sci.,* **75**(6),1415-1422 (1992).
3. M. SILVA, N.V. JACOBUS, C. DENEKE, S.L. GORBACH, Antimicrobial substance from a human Lactobacillus strain, *Antimicrob. Agents Chemother.*, **3**1(8), 1231-1233 (1987).
4. G. REID, M.E. SANDERS, H.R. GASKINS, G.R. GIBSON, A. MERCENIER, R. RASTALL, M. ROBERFROID, I. ROWLAND, C. CHERBUT, T.R. KLAENHAMMER, New scientific paradigms for probiotics and prebiotics, *J. Clin. Gastroenterol.,* **37**(2), 105-118 (2003).
5. H.G. HEILIG, E.G. ZOETENDAL, E.E. VAUGHAN, P. MARTEAU, A.D. AKKERMANS, W.M. de VOS, Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA, *Appl. Environ. Microbiol.*, **68**(1), 114-123 (2002).
6. R. BARRANGOU, M.A. AZCARATE-PERIL, T. DUONG, S.B. CONNERS, R.M. KELLY, T.R. KLAENHAMMER, Global analysis of carbohydrate utilization by *Lactobacillus acidophilus* using cDNA microarrays, *PNAS*, **103**(10) (2006).
7. F. FERRERES, C. SOUSA, V. VRCHOVSKÁ, P. VALENTÃO, J.A. PEREIRA, R.M. SEABRA, P.B. ANDRADE, Chemical composition and antioxidant activity of tronchuda cabbage internal leaves, *Eur. Food Res. Technol.*, **222**(1-2), 88-98 (2006).

8. O.K. CHUN, N. SMITH, A. SAKAGAWA, C.Y. LEE, Antioxidant properties of raw and processed cabbages, *Int. J. Food Sci. Nutr.*, **55**, 191-199 (2004).
9. G. GALATI, P.J. O'BRIEN, Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties, *Free Radic. Biol. Med.*, **37**, 287-30 (2004).
10. R.F. McFEETERS, L.A. LOVDAL, Sugar composition of cucumber cell walls during fruit development, *J. Food Sci.*, **52**(4), 996-1001 (1987).
11. Z. LU, H.P. FLEMING, R.F. McFEETERS, Effects of fruit size on fresh cucumber composition and the chemical and physical consequences of fermentation, *J. Food Sci.*, **67**(8), 2934-2939 (2002).
12. L.A. SHELEF, O.A. NAGLIK, D.W. BOGEN, Sensitivity of some common food-borne bacteria to the spices sage, rosemary and allspice, *J. Food Sci.*, **45**(4), 1042-1044 (1980).
13. S.K. ADESINA, Studies on some plants used as anticonvulsants in Amerindian and African traditional medicine, *Fitoterapia*, **5-6**,147-162 (1982).
14. J.W. KIM, Y.S. KIM, K.H. KYUNG, Inhibitory activity of essential oils of garlic and onion against bacteria and yeasts, *J. Food Prot.*, **67**(3), 499-504 (2004).
15. J.W. KIM, J.E. HUH1, S.H. KYUNG, K.H. KYUNG, Antimicrobial activity of alk(en)yl sulfides found in essential oils of garlic and onion, *Food Sci. Biotechnol.*, **13**(2), 235-239 (2004).
16. G. BAHRIM, C. IANCU, N. BUȚU, T. G. NEGOIȚĂ**,** Production of a novel microbial transglutaminase using Streptomyces sp. polar strains, *Rom. Biotechnol. Lett.*, **15**(2), 5197-5203 (2010).
17. Z. KOHAJDOVÁ, J. KAROVIČOVÁ, Sensory and chemical evaluation of lactic acid-fermented cabbage-onion juices, *Chem. Pap.*, **59**(1), 55-61 (2005).
18. D. ALTIOK, Kinetic modelling of lactic acid production from whey, Disseration Thesis, Izmir Institute of Technology, Turkey, 26 (2004).
19. R. LUEDEKING, E.L. PIRET, A kinetic study of the lactic acid fermentation. Batch process at controlled pH. *J. Biochem. Microbiol. Technol. Eng.*, **I**(4), 393-412 (1959).
20. A. AMRANE, Y. PRIGENT, Analysis of growth and production coupling for batch cultures of *Lactobacillus helveticus* with the help of an unstructured model, *Process Biochem.,* **34**(1), 1-10 (1999).
21. Y. KOHAJDOVÁ, J., KAROVIČOVÁ, M. GREIFOVÁ, Analytical and organoleptic profiles of lactic acid-fermented cucumber juice with addition of onion juice, *J. Food Nutr. Res.,* **46**(3), 105-111 (2007).
22. K.Y. YOON, E.E. WOODAMS, Y.D. HANG, Production of probiotic cabbage juice by lactic acid bacteria, *Bioresour. Technol.,* **97**(12), 1427-1430 (2005).
23. G. BAHRIM, D. BORDA, G.M. COSTIN, *Produse lactate fermentate*, Ed. Academica, Galati, 2005, pp. 12-85.
24. C. KANEUCHI, M. SEKI, K. KOMAGATA, Production of succinic acid from citric acid and related acids by Lactobacillus strains, *Appl. Environ. Microbiol.*, **54**(12), 3053-3056 (1988).
25. F. DELLAGLIO, S. ARRIZZA, A. LEDDA, Classification of citrate fermenting lactobacilli isolated from lamb stomach, sheep milk, and Pecorino Romano cheese, *Zentralbl. Bakteriol.,* **2**(4), 349-356 (1981).
26. M.B. RAKIN, J.K. BARAS, M.S. VUKAŠINOVIĆ, Lactic acid fermentation in vegetable juices supplemented with different content of brewer's yeast autolysate*, Acta Periodica Technol.*, **36**, 71-80 (2005).
27. J. BIAZAR, M. TANGO, E. BABOLIAN, R. ISLAM, Solution of the kinetic modelling of lactic acid fermentation using Adomian decomposition method, *Appl. Math. Comput.*, **144**, 433-439 (2003).
28. S. KWON, I. YOO, W.G. LEE, H.N. CHANG, Y.K. CHANG, High-rate continuous production of lactic acid by *Lactobacillus rhamnosus* in a two-stage membrane cell-recycle bioreactor, *Biotechnol. bioeng.*, **73**, 25-34 (2001).
29. H. JALILI, H. RAZAVI, M. SAFARI, A. AMRANE, Kinetic analysis and effect of culture medium and coating materials during free and immobilized cell cultures of Bifidobacterium animalis subsp. lactis Bb12, *Electron. J. Biotechnol.*, **13**(3), http://www.ejbiotechnology.info/content/vol13/issue3/full/4/ (2010).
30. J. KAROVIČOVÁ, Z. KOHAJDOVÁ, The use of PCA, FA, Ca for the evaluation of vegetable juices processed by lactic acid fermentation, *Czech J. Food Sci.*, **20**(4), 135-143 (2002).
31. Z. KOHAJDOVÁ, J. KAROVIČOVÁ, Optimisation of method of fermentation of cabbage juice, *Czech J. Food Sci.,* **22**(2), 39-50 (2004).
32. R. Di CAGNO, R.F. SURICO, A. PARADISO, M. De ANGELIS, J.C. SALMON, S. BUCHIN, L. De GARA, M. GOBBETTI, Effect of autochthonous lactic acid bacteria starters on health-promoting and sensory properties of tomato juices, *Int. J. Food Microbiol.*, **128**(3), 473-483 (2009).