The Use of PCA, FA, CA for the Evaluation of Vegetable Juices Processed by Lactic Acid Fermentation

JOLANA KAROVIČOVÁ and ZLATICA KOHAJDOVÁ

Department of Food Technology – Faculty of Chemical and Food Technology, Slovak Technical University, Bratislava, Slovak Republic

Abstract

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The cabbage-carrot juices were inoculated with *Lactobacillus plantarum* 92H at the concentration of 8×10^6 CFU/ml and fermented in a thermostat at 24°C for 150 hours. During the fermentation, both analytical (pH, total acidity, reducing sugars, L-ascorbic acid, lactic, acetic and citric acids, biogenic amines) and sensory (appearance, colour, turbidity, sediment and taste) parameters were followed. For the evaluation of the results of the chemical and sensory (taste) analyses of these juices the multivariate statistical methods were applied. PCA and FA reduced the 7 original analytical variables to 1 independent component (factor) that accounted for 96.92% of the total variance, and CA divided samples into 2 groups according to the contents of lactic and acetic acids. PCA and FA reduced the 8 taste and mixture taste descriptors to 2 components (factors) that accounted for 97.28% of the total variance.

Keywords: fermentation; vegetable juices; chemical analysis; sensory analysis; multivariate statistical methods

The vegetable juices processed by lactic acid fermentation introduce a change in the beverage assortment for their high nutritive value and high contents of vitamins and mineral compounds. For the juices processed by lactic acid fermentation, the content of lactic acid is important from the nutritive point of view. This acid shows disinfecting effects that are caused mainly by its acidity (KYZLINK 1980).

For the achievement of a fast and controlled fermentation of vegetable products, a pure culture of lactic acid bacteria is used. The strains of *Lactobacillus* genera are studied (DRDÁK *et al*. 1994) that improve the aroma of juices, allow a rapid decrease of pH, produce mainly lactic acid, allow the utilization of nitrates and nitrites, and decrease the contents of biogenic amines.

The producers require cultures of micro-organisms that improve aroma and allow to reach a rapid decrease of pH in the juices (KAROVIČOVÁ *et al*. 1999).

DRDÁK *et al.* (1994) tested 16 strains of the *Lactobacillus* genera in samples of white cabbage and carrot juices. After 7 days of fermentation at 27°C or 30°C, total acidity, pH value, and the contents of reducing sugars, organic acids, amonnia, biogenic amines, nitrates and nitrites were determined. On the basis of the results and of the sensory acceptance, 3 strains were selected.

KUCHTA *et al.* (1994) fermented vegetables (gourd, cabbage, celery) using *Lactobacillus plantarum*, *Lb. pentosus* and *Lb. brevis*. The gourd was fermented for 4 days, cabbage for 7 days, and celery for 9 days, respectively. Sauerkraut manufactured in this way had a pleasant acid taste, elastic texture and fresh light colour.

Principal component analysis (PCA) is used in all scientific branches. This method is advantageously applied for the evaluation in the food analysis (POKORNÝ *et al*. 1995; VELÍŠEK *et al*. 1995; FRAU *et al*. 1999). PCA is used for the reduction of information on a large number of variables into a smaller set while losing only a small amount of information (KOSCHIN *et al*. 1992). The major feature of this method is a reduction of the dimensionality in a set of variables by constructing an uncorrelated linear combination of them. The combinations are computed in such a way that the first component accounts for the major part of variance that is the major axis of the points in the *p*-dimensional space (LAMOŠ & POTOCKÝ 1989).

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The factor analysis (FA) is a multidimensional statistic method whose purpose is the analysis of the structure of mutual dependences of variables. This method is based on the assumption that these dependences are the consequence of a lower number of variables that can not be measured. These variables are designated as specific factors. The purpose of FA is to determine the structure of the specific factors, based on mutual dependences of observed variables (HEBÁK & HUSTOPECKÝ 1987). The procedure of FA involves the selection of principal components of variables using original variables or correlation, and covariation matrix from these variables, respectively. This method is similar to the principal components with the exception of the factor weights that are scaled. The sum of square of factor weight is equal to eigenvalue. The eigenvalue expresses the total variance defined by this factor. The procedure calculates the estimations of communalities for each variable using squares multiplying correlation between the given variable and all other variables. For the selected mathematical models, the communalities show the variability proportions of each variable with other variables in a set of data (CHANDIAK 1992).

Cluster analysis (CA) ranks among methods that study the similarity of multidimensional objects and classify samples into clusters. This method is used for such objects that have the natural tendency to group (KOSCHIN *et al*. 1992). The purpose of CA is to joint data into the clusters in order that their withingroup homogeneity as well as the differences between the clusters and the individual groups may be the greatest. The clustering goes out from the concrete observed values of atributes (CHANDIAK *et al*. 1999).

DESTEFANIS *et al*. (2000) used PCA for the study of relationships between chemical, physical and sensory variables (18 variables) measured on various beef meat specimens. The first three components accounted for 63% of the total variance (PC1 34%, PC2 20.6% and PC3 38%).

SORIA *et al*. (1999) applied PCA for the evaluation of apples that were cleaned-up after collection by different methods. The authors also evaluated the qualitative attributes of apples as well as ethylene production.

POKORNÝ *et al*. (1995) followed the time dependence in the determination of the intensity of bitterness in bitter liqueurs. The results were evaluated by 3 methods: averaged, multiplying regression and PCA. PCA was shown to be the most suitable method. The first principal component accounted for 85.4% of the total variance of data, the second component for 6.9%, and the third for 3.7%, respectively.

MATERIAL AND METHODS

Preparation of vegetable juices

Fresh vegetables (cabbage, carrot, celery and beet) were purchased in a local fruit vegetable market in Slovakia. From the cabbage, the outer leaves were removed and the cabbage was chopped to small slices. The carrot was chopped to smaller pieces.

The juices were obtained by pressing crushed vegetables. The juices were filtered and mixed in the ratio of 2:1 (v/v) (2 parts of cabbage juice and 1 part of carrot juice). After mixing, 2% D-glucose and 0.5% salt were added and the juices were placed into 250 ml sterile flasks. Each flask was inoculated by *Lactobacilllus plantarum* 92H at the concentration of 8×10^6 CFU per ml and sealed with sterile rubber plugs. The juices were fermented in a thermostat at 24°C for 150 h.

pH determination

The measurement of pH was performed using a LABOR-pH-meter CG-843 SCHOTT, Germany.

Determination of total acidity

The total acidity was determined by the visual titration with a 0.1 M solution of NaOH using phenolphtalein indicator and expressed as lactic acid.

Determination of reducing sugars according to Schoorl

The non-reacted Cu^{2+} was determined after the formation of Cu_2O. The KI was oxidized by $CuSO_4$ to I_2 that was determined by titration with $Na_2S_2O_3$ (DAVÍDEK & VELÍŠEK 1992).

Determination of L-ascorbic acid

L-ascorbic acid was determined spectrophotometrically with 2,6-dichlorophenolindophenol.

Determination of organic acids (lactic, acetic, and citric acids) and biogenic amines (histamine, cadaverine, putrescine) by capillary isotachophoresis

The measurement was carried out with isotachophoretic analyser ZKI 01 Vill Labeco Spišská N. Ves using a conductivity detector. For the identification and determination, electrolytic systems of the following compositions were applied.

Organic acid

The concentration of the leading electrolyte10^{-2} mol per dm^3, counter-ion 6-aminocapronic acid, pH 4,25, the additive 0.1% methylhydroxyethylcelulose. The terminating electrolyte 5×10^{-3} mol/dm^3 capronic acid. The samples were analysed under the driving current of 300 μA (KAROVIČOVÁ *et al*. 1990).

Biogenic amines

The concentration of the leading electrolyte 10^{-2} mol per dm^3 KOH, counter-ion valine, pH 9.9. The terminating electrolyte 2×10^{-2} mol/dm^3 TRIS, counter-ion HCl, pH 8.3. The samples were analysed under the driving current of 200 μA (KOHAJDOVÁ *et al*. 2001).

The quantitative analysis was performed by calibration.

Determination of biogenic amines by HPLC

The biogenic amines (histamine, tyramine and putrescine) were determined as danzylderivates according to GREIF *et al.* (1997, 1999).

Sensory evaluation of the cabbage-carrot juices

The samples were evaluated by 10–14 assessors. Before the sensory analysis, frozen samples of juices were defrosted and warmed to laboratory temperature and evaluated. The temperature of the evaluated samples was 15–18°C.

The sensory evaluation of the appearance, colour, turbidity, sediment, and taste was made.

Turbidity and colour were evaluated by a 5-point intensity scale (1 – nonturbid, 5 – very strong turbid). The appearance was evaluated by a 5-point hedonic scale (1 – not suitable, 5 – very good). For the evaluation of taste, 100 mm graphical non-structured abscissas with the description of extreme points were applied (maximal or minimal intensity of descriptors). By this method, mixture tastes and aroma: sweet-acid, cabbage-carrot and harmonic (the optimal harmonization of individual taste and mixture tastes) was also evaluated.

Statistical methods

For the evaluation of the sensory and analytical results, the multivariate statistical methods: correlation analysis, PCA, FA, and CA were applied. The data matrix of the analytical and sensory results by SGWIN (Statgraphic for Windows), Version 1.4. was analysed.

RESULTS AND DISCUSSION

In the previous work (KAROVIČOVÁ *et al.* 2001) we were engaged in the evaluation of cabbage juices processed by lactic acid fermentation using inoculation with *Lactobacillus plantarum* 92H in various concentrations. Based on the results of the chemical analysis, sensory evaluation, and using of PCA, we continued in the solution of the following problem.

The purpose of this work was the sensory and analytical evaluation of different vegetable juices (cabbage, cabbage-carrot, cabbage-carrot-celery, cabbage-celery and cabbage-beet) processed by lactic acid fermentation. The juices were prepared in various ratios using *Lactobacillus plantarum* 92H. In this publication are shown only the results of the evaluation of cabbage-carrot juice (2:1) that was the most recommended by the assessors.

For the evaluation of the results of the chemical (pH, total acidity, reducing sugars, organic acids and biogenic amines) and the sensory (taste) analyses of these juices, the multivariate statistical methods (correlation analysis, PCA, FA, CA) were applied.

For the fermentation of non-sterile cabbage-carrot juice, the lactic acid bacteria strain *Lactobacillus plantarum* 92H was selected. During the fermentation, the following analytical parameters were pursued: pH, total acidity, reducing sugars, L-ascorbic acid, lactic-, acetic-, and citric acids, and biogenic amines (histamine, cadaverine, tyramine, putrescine). The changes of pH value, total acidity, and in the contents of reducing sugars, and L-ascorbic acid and organic acids in the course of fermentation are shown in Table 1.

During the fermentation, pH value of juices decreased from 5.87 to 3.81. A decrease was found also in the concentration of reducing sugars determined according to Schoorl. The content of reducing sugars at the beginning of fermentation was 61.59 g/kg, after 150 h its decreased to 41.67 g/kg. The amount of sugars was calculated as glucose. The decrease of L-ascorbic acid concentration was from 430.76 mg/kg to 362.93 mg/kg. At the beginning of fermentation, the cabbage-carrot juice contained only

Table 1. Changes of pH, total acidity, reducing sugars, L-ascorbic acid and organic acids during the fermentation of the cabbage-carrot juices

Fermentation time (h)	pH	Total acidity (g/kg)	Reducing sugars (g/kg)	Acid			
				L-ascorbic (mg/kg)	lactic (g/dm^3)	acetic (g/dm^3)	citric (g/dm^3)
0	5.87	1.35	61.59	430.76	0.124	0.100	2.768
24	4.98	3.03	56.55	405.84	0.912	0.951	1.657
48	4.16	6.87	52.25	390.01	3.281	2.602	0.950
72	3.99	8.55	47.82	386.12	4.152	3.253	0.748
96	3.91	9.37	43.82	374.83	5.146	3.953	0.748
102	3.9	9.52	43.82	373.12	5.270	3.908	0.748
120	3.87	9.61	43.58	370.11	4.835	3.803	0.748
126	3.86	9.80	43.23	368.33	4.773	3.803	0.647
144	3.83	10.11	42.87	364.25	4.723	3.953	0.748
150	3.81	10.60	41.67	362.93	4.959	4.053	0.647

Table 2. Taste evaluation of the cabbage-carrot juices

Samples	Taste descriptors and mixture tastes (% of scale)							
	sweet	acid	cabbage	carrot	salty	cabbage-carrot	sweet acid	harmonic
KM0	97	19	65	87	21	69	37	62
KM24	85	39	81	80	32	89	61	70
KM48	67	50	85	63	35	86	50	76
KM72	43	61	90	60	34	79	55	86
KM96	40	69	91	55	30	67	61	94
KM102	40	71	92	50	29	64	66	87
KM120	34	80	87	48	25	60	52	85
KM126	30	81	79	42	24	50	50	82
KM144	28	90	79	33	24	46	52	81
KM150	26	90	78	32	20	42	53	70

small amounts of lactic and acetic acids (0.124, 0.1 g/l), citric acid was present in the concentration of 2.768 g/l. The highest increase of the lactic acid content was observed between 24th and 48th hour of fermentation (from 0.912 g/l to 3.281 g/l), that is an increase to 3.6-times the initial values. The highest increase of the acetic acid concentration was also observed at the same time of fermentation, from 0.951 g/l to 2.602 g/l. The highest decrease in the content of citric acid concentration was found at the start of fermentation, from 2.768 g/l to 1.657 g/l (a decrease of about 67.05%). The highest contents of lactic and acetic acids were present at the 102nd h of fermentation, the content of lactic acid having reached the value of 5.270 g/l (in comparison with the 0th h of fermentation it is an increase to 42.5 times the initial value). During the fermentation, the content of biogenic amines was below the detection limit of capillary isotachophoresis, the detection limit for this method being 2.32 mg/kg for cadaverine, 1.02 mg/kg for putrescine, and 1.59 mg/kg for histamine. HPLC method was also used for the determination of biogenic amines. The detection limit of this method was 1µg per cm^3 for putrescine and histamine, and 2 µg/cm^3 for tyramine, respectively.

Samples of cabbage-carrot juice were slightly turbid at the start of fermentation (0 and 24th h), between 48th and 144th h of fermentation they showed medial turbidity and were strongly turbid on 150th h. During the fermentation, the colour of samples changed from dark-pink (0 at 24th h) to light-orange with a yellow shade (102nd–150th h). The appearance of the samples at the initial hours of fermentation was adequate and between 96th and 150th h, it was good. A sediment was observed in all samples. In this work only the results of the taste evaluation of cabbage-carrot juices are given. From the results it can be seen that at the start of fermentation, the sweet taste prevailed in the samples (97% from the total intensity), its intensity decreasing in course of fermentation to the value of 26% (150th h). The acid taste was observed from the start of fermentation (19% from the total intensity) and its intensity increased in the course of fermentation reaching the highest value of 90% from the total intensity at 144th and 150th h of fermentation. The salty taste did not change markedly, the intensity of this taste had values from 20 to 35% from the total intensity. The cabbage taste reached the highest intensity on 102nd h of fermentation: 92%, the carrot taste decreased markedly during the fermentation from 87% (0th h) to 32% (150th h). As to the mixture tastes,

Table 3. Correlation coefficients for analytical variables

pH	Total acidity	Reducing sugars	L-ascorbic acid	Lactic acid	Acetic acid	Citric acid
1.0000	–0.9681	0.9428	0.9619	–0.9588	–0.9710	0.9943
	1.0000	–0.9864	–0.9767	0.9861	0.9959	–0.9419
		1.0000	0.9836	-0.9739	–0.9862	0.9166
			1.0000	–0.9491	–0.9725	0.9425
				1.0000	0.9952	–0.9319
					1.0000	–0.9455
						1.0000

Table 4. Correlation coefficients for sensory (taste) variables

Sweet	Acid	Sweet acid	Cabbage	Carrot	Cabbage-carrot	Salty	Harmonic
1.0000	-0.9779	–0.3699	–0.4664	0.9536	0.7150	0.2201	–0.6570
	1.0000	0.3704	0.3942	–0.9823	–0.4785	–0.2768	0.5572
		1.0000	0.8212	–0.2607	0.1327	0.4734	0.6136
			1.0000	–0.2795	0.2297	0.6695	0.8454
				1.0000	0.8088	0.3582	–0.4536
					1.0000	0.8210	–0.1081
						1.0000	0.3806
							1.0000

cabbage-carrot reached maximum intensity at 24th h of fermentation (89%), sweet acid at 102nd h (66%), and harmonic at 96th h (94%). The standard deviation of the evaluation of the individual taste descriptors varied from 2.30 to 5.06%.

Statistical evaluation of the results of the chemical and sensory analyses

Evaluation of analytical measurements of cabbage-carrot juice. The correlation analysis was used for the measurement of the linear association between variables. The correlation coefficients between the analytical variables are presented in Table 3. From the statistical point of view, the measurements of all the followed analytical variables were important (with a significance level $P < 0.01$ with a confidence level of 99%).

Using PCA (standardized input data), the original analytical variables were reduced to one principal component that accounted for 96.92% from the total variance. The most notable variables were: acetic acid (loading 0.373), lactic acid (loading 0.382), and total acidity (loading 0.382). By FA (factoring type: principal component), 1 factor was extracte that accounted for the same percentage of variance as PCA. Using the classical type of factoring, it arrived at a slight redistribution of the determined variance to the value of 97.01%.

CA divided samples into 2 groups (cluster 1: samples KM0 and KM24, cluster 2: samples KM48–KM150). In Fig. 1, the clusters of samples in axes of 2 selected variables (lactic and acetic acids) are shown. From Fig. 1 (plotting clusters of samples in axes of 2 selected variables) it can be seen that the samples contained in the first cluster were marked with a low content of acetic and lactic acids (initial fermentation hours). Samples KM120 and KM126 belonging to the second cluster overlapped each other and were very similar by lactic and acetic acid contents.

Taste evaluation. The correlation coefficients between the taste variables are presented in Table 4. The most important correlations were found between carrot and acid tastes (–0.9823), sweet and acid tastes (–0.9774), sweet and carrot tastes (–0.9536). The coefficients indicate that a high indirect dependence exists between these variables. Apart from these correlations, correlations between sweet acid and cabbage tastes, carrot and cabbage-carrot tastes, cabbage and harmonic tastes, and between cabbage-carrot and salty tastes also showed a statistical significance (with a significance level $P < 0.01$ with a confidence level of 99%).

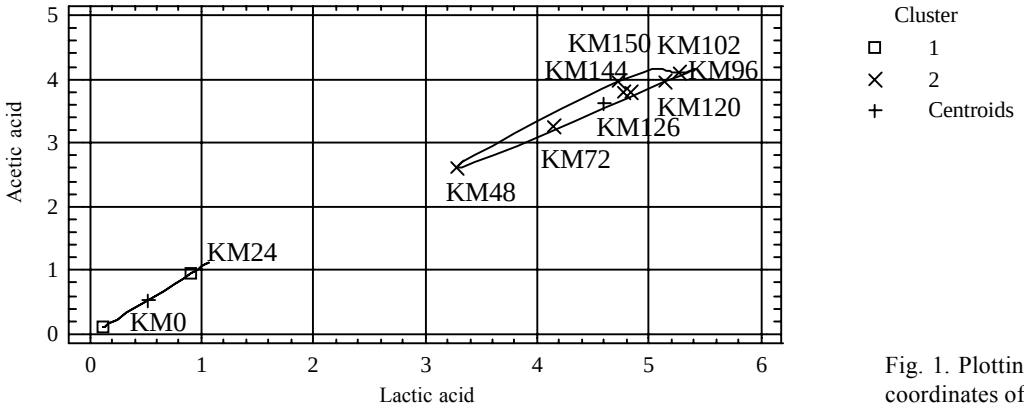

Fig. 1. Plotting of sample clusters in coordinates of two selected variables

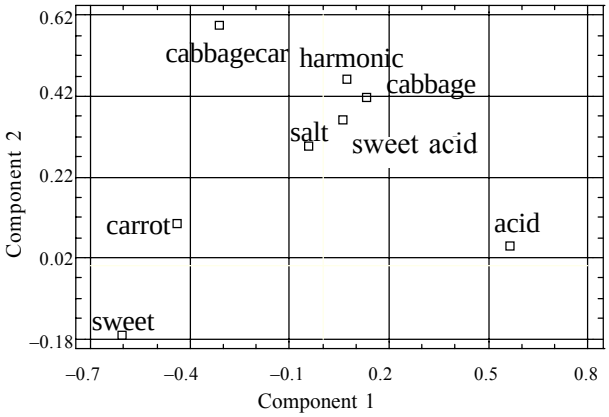

Fig. 2. Loadings of variables in coordinates PC1 and PC2 (nonstandardized entrance data)

PCA reduced the original 8 variables to 2 principal components that accounted for 97.28% of the total variance (PC1 86.625% and PC2 next 12.659%) – non-standardized input data. In Fig. 2 it can be seen that the positive part of the first principal component reflected mostly the acid variable (component weight 0.564). The negative part of the first principal component reflected mostly the sweet taste (component weight 0.604%) and it did not reflect the salty and cabbage taste. The second principal component described mostly cabbage-carrot (component weight 0.595) and cabbage (component weight 0.461) tastes and did not express the acid taste. The cabbage, sweet acid, harmonic, and acid tastes correlated positively together and correlated negatively with the sweet, carrot and cabbage-carrot tastes.

For a comparison, the results are showed of PCA analysis with the standardization of the input data. Standardization was carried out to convert all the data to the same unit to avoid a scale effect (in a common technique, from every number is subtracted the mean of all individuals for a variable and it is divided by the standard deviation). In this case, there were also 2 principal components extracted that accounted for 91.262% of the total variance of results (PC1 54.087% and PC2 37.176%) which means that the redistribution of the explained data was arrived at. In Fig. 3 it can be seen that PC1 mostly described carrot (component weight 0.446), sweet (component weight 0.471) and acid tastes (component weight –0.465) in the negative part and did not describe the salty taste (component weight 0.547).

FA similarly to PCA accounted for 91.262% of the total variance of results. F1 presented mostly acid (factor loading 0.968) and sweet (factor loading 0.928) taste, and in the negative part the sweet taste (factor loading –0.980). In Fig. 4 the scores of the samples are shown in coordinates of factors. In Fig. 4 it can be seen that F1 defined mostly the samples KM0 and KM24, and in the negative part the samples KM144 and KM150. F2 described mostly the samples KM0 and KM72–KM102. For the evaluation of the results by the cluster analysis, the cluster method of the nearest neighbour was used. For the measurement of the distance between the objects, the squared Euclidean method was used. The cluster procedure continued until the first cluster was formed. Fig. 5 shows a dendro-

Table 5. Clusters of samples and grouping of samples into individual clusters

Number of clusters	Cluster	Grouping of samples into the individual clusters
2	1	KM0
	2	KM24–KM150
3	1	KM0
	2	KM24–KM102
	3	KM120–KM150
4	1	KM0
	2	KM24–KM48
	3	KM72–KM102
	4	KM120–KM150

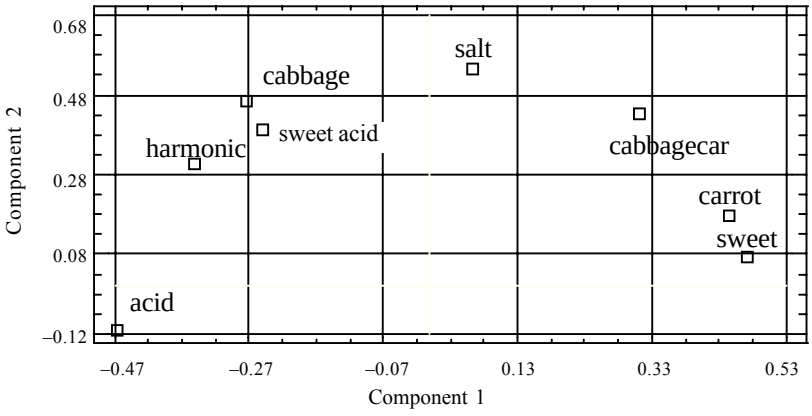

Fig. 3. Loadings of variables in coordinates PC1 and PC2 (standardized entrance data)

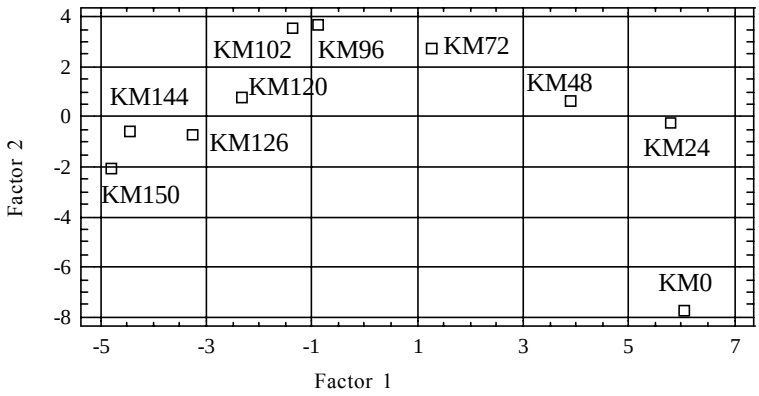

Fig. 4. Factor loadings of variables in coordinates F1 and F2

gram (tree chart). From this chart it is possible to see the gradual formation of the individual clusters. Sample KM126 with KM144 and sample KM96 with KM102 were the most similar. In this case, if the final number of clusters was to be 2, the sample KM0 would form an independent cluster and the samples KM24–KM150 would form the next cluster. If there were to be 3 clusters, both samples KM0 and KM24 would form independent clusters and samples KM48–KM150 would form one cluster.

For a comparison, the results are shown of PCA and FA application to 5 taste descriptors (mixture tastes not included). PCA reduced the original 5 variables to 2 independent components that accounted for 97.244% from the total variance (PC1 63.638% and PC2 33.56%). PC1 described mostly the acid taste (component weight 0.557) and in the negative part carrot (component weight –0.548) and sweet (component weight 0–0.556) tastes. PC2 expressed mostly the cabbage (component weight 0.729) and salty (component weight 0.674) tastes. In Fig. 6 are shown the scores of samples and loading of variables (5-taste descriptors) in axes PC1 and PC2. From Fig. 6 it seems that samples KM120–KM150 were the most acidic,

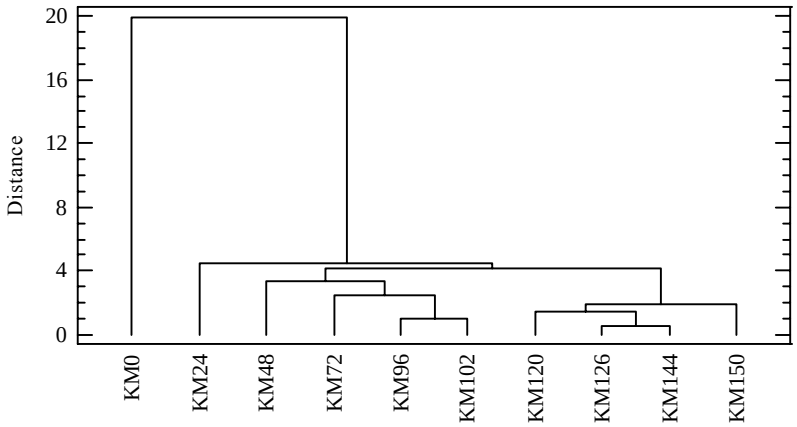

Fig. 5. Dendrogram (three graphs)

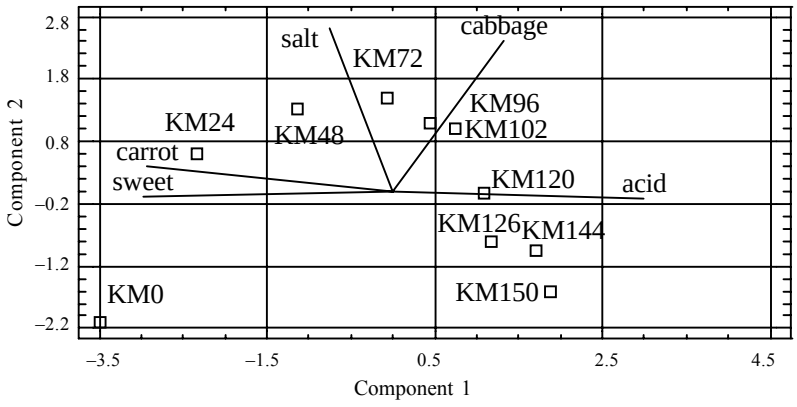

Fig. 6. Score of samples and loadings of variables (5-taste descriptors) in coordinates PC1 and PC2

samples KM0 and KM24 the most sweet (the start of fermentation) and samples KM72–KM102 had the cabbage taste most frequently. Using the 5 taste variables in comparison with the previous 8 variables, a great part of the results variance was defined and the component weight for the most variables described had higher values. We can note that using 5-taste descriptors is sufficient to define the total variance of the input data.

FA (factoring type: principal component), the same percentage of variance as in the case of PCA was found and also two factors were extracted. Using classical factoring type, the % of the defined variance changed only slightly (PC1 67.078 and PC 32.604%).

In the CA application we used the same cluster method and cluster procedure as in the previous case. In Table 5 are shown the clusters of samples and grouping of samples into individual clusters, in the cases when 2, 3, or 4 clusters were pre-elected. Samples KM96 with KM144 (likewise as at the 8 taste descriptors) were the most similar.

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Súhrn

KAROVIČOVÁ J., KOHAJDOVÁ Z. (2002): **Použitie PCA, FA, CA pre hodnotenie zeleninových štiav inokulovaných mliečnymi baktériami**. Czech J. Food Sci., **20**: 135–143.

Kapustovo-mrkvové šťavy boli inokulované *Lactobacillus plantarum* 92H o koncentrácii 8 × 10^6 CFU/ml a fermentované v termostate pri 24 °C 150 hodín. Počas fermentácie boli sledované analytické (pH, titračná kyslosť, redukujúce cukry, kyselina L-askorbová, mliečna, octová a citrónová, biogénne amíny) a senzorické (vzhľad, farba, zákal, sediment a chuť) parametre. Na vyhodnotenie výsledkov chemických a senzorických (chuť) analýz boli aplikované multivariačné štatistické metódy. PCA a FA

zredukovali pôvodných sedem analytických premenných na jeden nezávislý komponent (faktor), ktorý vysvetlil 96,92 % z celkovej variability výsledkov, a CA rozdelila vzorky do 2 skupín na základe obsahu kyseliny mliečnej a octovej. PCA a FA zredukovali 8 deskriptorov chutí a zložených chutí na 2 komponenty (faktory), ktoré vysvetlili 97,28 % z celkovej variability výsledkov.

Kľúčové slová: fermentácia; zeleninové šťavy; chemická analýza; senzorické hodnotenie; multivariačné štatistické metódy

Corresponding author:

Ing. JOLANA KAROVIČOVÁ, CSc., Chemicko-technologická fakulta STU, Radlinského 9, 812 37 Bratislava, Slovenská republika
tel.: + 421 2 59 32 56 83, fax: + 421 2 52 49 31 98, e-mail: karovicovaj@chtf.stuba.sk