Chemometric approach to texture profile analysis of kombucha fermented milk products

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Revised: 4 November 2014 / Accepted: 10 November 2014 / Published online: 18 November 2014
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Abstract In the present work, relationships between the textural characteristics of fermented milk products obtained by kombucha inoculums with various teas were investigated by using chemometric analysis. The presented data which describe numerically the textural characteristics (firmness, consistency, cohesiveness and index of viscosity) were analysed. The quadratic correlation was determined between the textural characteristics of fermented milk products obtained at fermentation temperatures of 40 and 43 °C, using milk with 0.8, 1.6 and 2.8% milk fat and kombucha inoculums cultivated on the extracts of peppermint, stinging nettle, wild thyme and winter savory. Hierarchical cluster analysis (HCA) was performed to identify the similarities among the fermented products. The best mathematical models predicting the textural characteristics of investigated samples were developed. The results of this study indicate that textural characteristics of sample based on winter savory have a significant effect on textural characteristics of samples based on peppermint, stinging nettle and wild thyme, which can be very useful in the determination of products texture profile.

Keywords Peppermint · Stinging nettle · Wild thyme · Winter savory · Hierarchical cluster analysis · Chemometrics

Introduction

Kombucha represents a symbiotic association of acetic acid bacteria and yeasts. It is capable to transform sweetened black tea or another similar substrate into a refreshing, acidic and slightly carbonated beverage. Traditionally, a variety of health effects are associated with this beverage (Malbaša et al. 2011). Investigations showed that it is possible to obtain a fermented milk product when kombucha beverage is used as a new type of starter culture in milk fermentation process (Belloso-Morales and Hernández-Sánchez 2003; Malbaša et al. 2009; Vitas et al. 2013a). Herbal extracts of winter savory, wild thyme, peppermint and stinging nettle are applicable in kombucha fermentation process and obtained beverage can be used for fermented milks production (Vitas et al. 2011). New studies stress out the importance of fermented milk product's texture to its acceptance by consumer (Bruzone et al. 2013) and suggest texture profile analysis as fast and appropriate for determination of milk gel strength (Kumar and Mishra 2003; do Espírito Santo et al. 2012).

Chemometric methods allow the simultaneous evaluation of an almost unlimited amount of data, highly facilitating the clarification of theoretical and practical problems. The data flood generated by modern analytical instrumentation is one reason, that analytical chemists in particular develop chemometric applications. A chemometric method in analytics is the discipline that uses mathematical and statistical methods to obtain relevant information on material systems.

Chemometric analysis is undisputable of great importance in modern sciences. It means performing calculations on measurements of chemical data. Chemometric techniques are applied to explain both descriptive and predictive problems in experimental life sciences, especially in chemistry and biochemistry. In descriptive applications, properties of chemical systems are modeled with the intent of learning the underlying relationships and

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structure of the system. In predictive applications, properties of chemical systems are modeled with the intent of predicting new properties or behaviour of interest (Katritzky and Lobanov 1995; Eriksson et al. 2008; Miller and Miller 2010). Chemometric analyses are particularly appropriate to areas such as food investigations, plant extracts and biological investigations (Farkas et al. 2004; Liu et al. 2008; Podunavac Kuzmanović and Cvetković 2011).

The objective of this study was to investigate the usefulness of chemometric analysis in the prediction of textural characteristics of fermented milk products obtained by kombucha inoculums with peppermint, stinging nettle and wild thyme, testing only the textural characteristic of product with winter savory.

Materials and methods

Materials

Kombucha inoculums

Native local kombucha culture contains at least five yeast strains (*Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bispurus*, *Torulopsis* sp. and *Zygosaccharomyces* sp.) and primary bacterium belongs to the strains of the genus *Acetobacter* (Malbaša et al. 2011). Total count of bacteria was approximately 6.2×10^5 cells/mL of substrate and total count of yeasts was approximately 3.2×10^4 cells/mL of substrate.

Fermentation liquid of kombucha obtained after 7 days long fermentation at 25 °C on extracts (2.25 g/L) of peppermint (*Mentha piperita*, Lamiaceae), wild thyme (*Thymus serpyllum*, Lamiaceae), winter savory (*Satureja montana*, Lamiaceae) and stinging nettle (*Urtica dioica*, Lamiaceae), sweetened with sucrose (7%), represented the four different kombucha inoculums that were used as starter cultures for milk fermentation.

Herbal teas were purchased in the local health food store and they are used because of the many beneficial effects to humans, which are very well known. They include their antioxidant, antimicrobial, antidiarrheal, antihypertensive, anti-inflammatory and other biological activities.

Milk

For the production of fermented milk products in the laboratory, pasteurized, homogenized cow's milk with 0.8, 1.6 and 2.8% milk fat, from the manufacturer AD IMLEK Beograd, branch Novosadska mlekar, Novi Sad (Serbia), was used.

Production of kombucha fermented milk products

Fermented milk products were produced from milk described in section 2.1.2., as follows: to the 500 mL of milk, 10% (v/v) of the appropriate kombucha inoculum was added. The milk fermentation process was performed at 40 and 43 °C and it lasted until the pH value of 4.5 was reached. Milk gel was then cooled to the temperature of 8 °C, homogenized by mixer and the samples were stored in refrigerator. The obtained products were marked as KWS (kombucha fermented milk products with winter savory), KP (kombucha fermented milk products with peppermint), KWT (kombucha fermented milk products with wild thyme) and KSN (kombucha fermented milk products with stinging nettle). These fermented milk products resemble the most to kefir, because kefir is also produced with mixed culture of bacteria and yeasts.

Methods

Texture profile analysis of kombucha fermented milk products

Texture profile analysis was performed by determination of textural characteristics (firmness, consistency, cohesiveness and index of viscosity) using Texture analyzer TA.HD.plus (Stable Micro System, Godalming, UK). A single compression test, with a back extrusion cell (A/BE) disc (diameter 35 mm; distance 30 mm; speed 10 mm s^{-1}) and an extension bar, using 5 kg load cell. Tests are carried out in a standard size back extrusion container (50 mm diameter). Option *Return to Start* was used.

Analyses were performed at 5 °C, in triplicate, after production of samples and results are given in Table 1.

Statistical analysis

In this study HCA was performed using NCSS 2007 and GESS 2006 Statistical Software in order to confirm the grouping of compounds already based on textural characteristics of fermented milk products (firmness, consistency, cohesiveness and index of viscosity). Clustering is based on the Euclidean distance and single linkage algorithm (Kovačević et al. 2013a; Miller and Miller 2010). Also, the complete regression analysis was carried out by PASS 2005, GESS 2006 and NCSS Statistical Softwares.

For the testing the validity of the predictive power of selected models the leave-one-out technique (LOO) was used (Golbraikh and Tropsha 2002). The developed models were validated by the calculation of the following statistical parameters: $PRESS$, SSY , S_{PRESS} , r^2_{CV} and r^2_{adj} (Kovačević et al. 2013b).

Table 1 Textural characteristics of fermented milk products obtained by kombucha inoculums with herbal extracts

Conditions		Firmness				Consistency			
		KWS	KP	KSN	KWT	KWS	KP	KSN	KWT
40°C	0.8 % MF	10.541	17.244	17.789	16.376	496.679	507.762	500.883	473.264
	1.6 % MF	15.688	14.866	13.987	13.874	437.25	432.197	433.863	368.041
	2.8 % MF	10.438	16.574	18.216	15.812	277.005	375.515	421.968	319.203
43°C	0.8 % MF	18.295	21.305	18.306	20.426	495.154	527.214	484.707	509.702
	1.6 % MF	12.294	13.199	15.538	12.872	320.095	375.795	412.056	333.043
	2.8 % MF	17.096	16.969	14.629	15.542	465.322	454.954	458.386	406.448
		Cohesiveness				Index of viscosity			
40°C	0.8 % MF	-6.149	-6.593	-9.490	-6.234	-8.121	-8.813	-8.293	-7.602
	1.6 % MF	-5.496	-5.635	-8.047	-5.01	-1.213	-1.238	-2.88	-2.594
	2.8 % MF	-6.519	-6.922	-10.38	-4.259	-1.749	-1.999	-6.985	-1.049
43°C	0.8 % MF	-10.382	-9.604	-9.514	-10.258	-7.138	-8.389	-5.522	-7.574
	1.6 % MF	-7.379	-7.191	-7.045	-7.715	-2.159	-2.361	-1.95	-2.572
	2.8 % MF	-12.251	-11.237	-9.084	-10.617	-9.95	-9.403	-4.596	-7.175

These parameters were calculated from following Eqs. (1, 2, 3, 4 and 5):

$$PRESS = \sum (Y_{\text{obs}} - Y_{\text{calc}})^2 \quad (1)$$

$$SSY = \sum (Y_{\text{obs}} - Y_{\text{mean}})^2 \quad (2)$$

$$S_{\text{PRESS}} = \sqrt{\frac{PRESS}{n}} \quad (3)$$

$$r^2_{\text{CV}} = 1 - \frac{PRESS}{SSY} \quad (4)$$

$$r^2_{\text{adj}} = 1 - (r^2) \left(\frac{n-1}{n-p-1} \right) \quad (5)$$

Where Y_{obs} , Y_{calc} and Y_{mean} are observed, calculated and mean values; n , number of compounds; p , number of independent parameters.

PRESS is an acronym for prediction sum of squares. It is used to validate a regression model with regards to predictability. To calculate *PRESS*, each observation is individually omitted. The remaining $n-1$ observations are used to calculate a regression and estimate the value of the omitted observation. This is done n times, once for each observation. The difference between the actual Y value, Y_{obs} , and the predicted Y , Y_{calc} is called the prediction error. The sum of the squared prediction errors is the *PRESS* value. The smaller *PRESS* is, the better the predictability of the model. Its value being less than *SSY* points out that the model predicts better than chance and can be considered statistically significant. *SSY* are the sums of squares associated with the corresponding sources of variation. These values are in terms of the dependent variable, y .

The *PRESS* value above can be used to compute an r^2_{CV} statistic, called r^2 cross validated, which reflected the production ability of the model (Golbraikh and Tropsha 2002). This is a good way to validate the prediction of a regression model without selecting another sample or splitting data. It is very possible to have a high r^2 and a very low r^2_{CV} . When this occurs, it implies that the fitted model is data dependent. This r^2_{CV} ranges from below zero to above one. When outside the range of zero to one, it is truncated to stay within this range. Adjusted r -squared is an adjusted version of r^2 . The adjustment seeks to remove the distortion due to a small sample size.

In many cases r^2_{CV} and r^2_{adj} is taken as a proof of the high predictive ability of regression models (Golbraikh and Tropsha 2002). A high value of these statistical characteristic (>0.5) is considered as a proof of the high predictive ability of the model.

Table 2 Statistical parameters of the relationships between the textural characteristics

Independent variable	Dependent variable	$Y(\text{firmness})=a(\text{firmness})^2+b(\text{firmness})+c$						Model
		a	b	c	r^2	F	s	
KWS	KP	0.396	−10.844	86.973	0.973	54.204	0.577	1
	KSN	0.289	−8.390	74.328	0.917	16.588	0.716	2
	KWT	0.388	−10.663	85.294	0.961	36.944	0.665	3
KWS	Y	$Y(\text{consistency})=a(\text{consistency})^2+b(\text{consistency})+c$						
	KP	a	b	c	r^2	F	s	4
	KSN	0.005	−3.212	890.094	0.977	63.595	12.572	5
KWS	KWT	0.004	−2.765	885.573	0.978	66.785	6.806	6
	Y	$Y(\text{cohesiveness})=a(\text{cohesiveness})^2+b(\text{cohesiveness})+c$						
	KP	a	b	c	r^2	F	s	7
KWS	KWT	−0.002	0.740	−1.749	0.991	166.067	0.258	8
	Y	$Y(\text{index of viscosity})=a(\text{index of viscosity})^2+b(\text{index of viscosity})+c$						
	KP	a	b	c	r^2	F	s	9
KWS	KWT	0.091	1.973	1.202	0.998	875.881	0.206	10
	Y	$Y(\text{index of viscosity})=a(\text{index of viscosity})^2+b(\text{index of viscosity})+c$						
	KWT	0.106	1.877	0.728	0.932	20.479	1.013	10

Results and discussion

Regression analysis

In this investigation, 24 samples were produced. All obtained kombucha fermented milk products had physico-chemical and sensory characteristics that are typical for this group of products (Vitas et al. 2013b). They had uniform colour and no separated whey on the surface. Their aroma was similar to yoghurt and kefir, but also they had the aroma of the herbal extract used for the production of kombucha inoculums.

We focused our research on developing the chemometric models which relate the textural characteristics of fermented milk products obtained by kombucha inoculums with peppermint, stinging nettle and wild thyme and textural characteristics of fermented milk products obtained by kombucha

inoculum with winter savory. To obtain the quantitative effects above listed textural characteristics, chemometric regression analysis was operated. Therefore, a complete regression analysis was made resorting to quadratic relationships. The statistical quality of the generated models, as depicted in Table 2, were determined by statistical measures: squared correlation coefficient (r^2), standard error of estimate (s), and F-test (Fisher's value) (F) for statistical significance. The correlation coefficient is a relative measure of fit by the regression equation. Correspondingly, it represents the part of the variation in the observed data that is explained by the regression. The correlation coefficient values closer to 1.0 represent a better fit of the regression, and high values of the F -test indicate that the model is statistically significant. Standard deviation expresses the variation of the residuals or the variation about the regression line, and should have a low value for the regression

Table 3 Cross-validation parameters

Independent variable	Dependent variable	$PRESS$	SSY	$PRESS/SSY$	S_{PRESS}	r^2_{CV}	r^2_{adj}	Model
KWS	KP	3.5852	37.2105	0.0963	0.7730	0.9037	0.9551	1
	KSN	9.9058	18.5614	0.5337	1.2849	0.4663	0.8618	2
	KWT	6.6302	34.0793	0.1946	1.0512	0.8054	0.9350	3
	KP	1880.4	20576.74	0.0914	17.7031	0.9086	0.9616	4
	KSN	655.1	6326.47	0.1035	10.4491	0.8965	0.9634	5
	KWT	11740.4	29460.8	0.3985	44.2350	0.6015	0.8872	6
	KP	1.0432	22.3289	0.0467	0.4169	0.9533	0.9851	7
	KWT	12.8630	35.5383	0.3619	1.4642	0.6381	0.7954	8
	KP	0.6770	74.7248	0.0091	0.3359	0.9909	0.9972	9
	KWT	17.0977	45.0782	0.3793	1.6881	0.6207	0.8863	10

Table 4 Textural characteristics screening summary

Conditions		Model 7		Model 9	
		Cohesiveness _{pred.}	Residuals	Index of viscosity _{pred.}	Residuals
40 °C	0.8 % MF	−6.7147	0.5657	−9.1182	0.9972
	1.6 % MF	−5.9824	0.4864	−1.1011	−0.1119
	2.8 % MF	−6.9671	0.4481	−2.3784	0.6294
43 °C	0.8 % MF	−9.0404	−1.3415	−8.9453	1.8073
	1.6 % MF	−7.1737	−0.2052	−2.9489	0.7899
	2.8 % MF	−10.3169	−1.9341	−8.9018	−1.0481

to be significant. Thus, the standard deviation is an absolute measure of quality of fit and should have a low value for the regression to be significant.

The data from Table 2 indicate the small effect of variation of temperature and milk fat on relationships between textural characteristics of fermented milk products with kombucha inoculums cultivated on herbal teas.

Models 7 and 9 indicate that the *cohesiveness* (for model 7) and *index of viscosity* (for model 9), as textural characteristics of fermented milk products obtained at the defined fermentation temperatures, using milk with various milk fat content and kombucha fermented milk products inoculum cultivated on winter savory is contributing positively to the textural characteristics of other samples. All the models have given significant information to build strategy for simple determination of textural characteristics of products with inoculums cultivated on herbal extracts (peppermint, stinging nettle and wild thyme).

According to calculated statistical parameters, it can be concluded that obtained polynomial models have very good basic statistical properties (low *s*, high r^2 and *F* values). Also, all the models show high squared correlation coefficient, higher than 0.877. But, only high correlation coefficient is not enough to select the equation as a model and hence various statistical approaches were used to confirm the robustness and practical applicability of the equations. For the testing the quality of the predictive power of the models the LOO procedure was used (Table 3).

LOO cross-validation measures indicate a very good prediction ability of the obtained predicted models (high r^2_{CV} and r^2_{adj} values, and low *PRESS*).

To confirm the predictive power of a model, the textural characteristics of fermented milk products obtained by kombucha inoculums with stinging nettle, wild thyme and peppermint included in the study was calculated by models 7 and 9. These results were compared with the experimental values (Table 4).

It indicates the good predictability of the established models. In order to investigate the existence of a systemic error in developing mathematical models, the residuals of predicted textural characteristics based on samples with peppermint, wild thyme and stinging nettle were plotted against the observed values (Fig. 1).

The propagation of the residuals on both sides of the zero axis indicates that no systemic error in the development of regression models exists, as suggested by Jalali-Heravi and Kyani (Jalali-Heravi and Kyani 2004). It proves the usefulness of the derived mathematical models.

The presented results indicate that these models can be successfully applied to predict the textural characteristics of fermented milk products obtained by kombucha inoculums with stinging nettle, wild thyme and peppermint from the textural characteristics of one fermented milk product obtained by kombucha inoculum with winter savory.

Fig. 1 Plot of the residual values against the experimentally observed textural characteristics (cohesiveness and index of viscosity) for models 7 and 9

Fig. 2 Clustering of samples based on firmness

Fig. 4 Clustering of samples based on cohesiveness

Hierarchical cluster analysis

HCA is a method for dividing a group of objects into classes so that similar objects are in the same class (cluster). The groups are not known prior to the mathematical analysis and no assumptions are made about the distribution of the variables. Cluster analysis searches for objects which are close together in the variable space (Miller and Miller 2010). The data in each cluster share some common trait, often proximity according to some defined distance measure. However, HCA does not explain which exact variables are responsible for grouping of the data in each cluster. In HCA grouping of the data is presented graphically as a dendrogram (Miller and Miller 2010).

HCA performed on textural characteristics of investigated samples resulted in four dendrograms (Figs. 2, 3, 4 and 5).

From the presented dendrogram, it can be seen that products containing the peppermint and wild thyme are similar regarding firmness parameter (Fig. 2). According to this

parameter, product with stinging nettle is similar to products with peppermint and wild thyme. Product with winter savory does not belong to any cluster.

Previous investigation demonstrated that products with winter savory have more pronounced antioxidant characteristics in comparison to products with wild thyme, peppermint and stinging nettle (Vitas et al. 2013b).

Presented dendrogram shows two clusters according to consistency (Fig. 3). First cluster is made of products with peppermint and stinging nettle, while products with winter savory and wild thyme belong to the second cluster. There is significant dissimilarity between these two clusters.

The dendrogram presented in Fig. 4 shows that there is one cluster that contains products with winter savory, peppermint and wild thyme, while product with stinging nettle does not belong to any cluster. According to cohesiveness, products with winter savory and peppermint are very similar.

Clustering of the analysed samples based on index of viscosity is completely the same as clustering based on

Fig. 3 Clustering of samples based on consistency

Fig. 5 Clustering of samples based on index of viscosity

cohesiveness (Fig. 5). Hence, there is just one cluster with products with winter savory, peppermint and wild thyme, while product with stinging nettle stands alone.

Conclusions

In this study the focus of chemometric analysis was performed to estimate the relationships between the textural characteristics of fermented milk products obtained at fermentation temperatures of 40 and 43 °C, using milk with 0.8, 1.6 and 2.8% milk fat and kombucha inoculums cultivated on peppermint, wild thyme, stinging nettle and winter savory. For this purpose the quadratic correlation and HCA were performed. Accurate mathematical models were developed for predicting textural characteristics of fermented milk products. The validity of the models has been established by the determination of suitable statistical parameters. High agreement between experimental and predicted values, obtained in the validation procedure, indicated the good quality of the models.

Acknowledgments This paper was performed within the framework of the research projects No.172012, TR 31055 and III 46009 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia and the project No. 114-451-1156/2014-02, financially supported by the Provincial Secretariat for Science and Technological Development of Vojvodina.

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