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ANN and RSM modelling of antioxidant characteristics of kombucha fermented milk beverages with peppermint

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Jasmina Vitas, Radomir Malbaša, Aleksandar Jokić, Eva Lončar, Spasenija Milanović*

University of Novi Sad, Faculty of Technology Novi Sad,
Bulevar cara Lazara 1, 21000 Novi Sad, Republic of Serbia

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Abstract

Antioxidant activity to stable DPPH radical (AA_{DPPH}) and unstable hydroxyl radicals ($AA._{OH}$) and nutraceuticals (monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and ascorbic acid) content of kombucha fermented milks with peppermint (KFM-P) were modelled and optimised. Beverages were produced by the addition of 10 % of kombucha peppermint inoculum to the milk containing 0.8, 1.6 and 2.8 % milk fat at 37, 40 and 43 °C. Response surface methodology (RSM) indicated opposite response surfaces for AA_{DPPH} and $AA._{OH}$ PUFAs and ascorbic acid, as most significant and influential factors, were included in graphical optimization and gave the working region for obtaining products of highest antioxidant quality: lower temperatures and milk fat up to 1.8 %; higher temperatures and milk fat of maximum 1.6 %. ANN modelling of antioxidant characteristics of kombucha fermented milk beverages with peppermint was, as expected, more accurate than RSM.

Key words: kombucha, antioxidants, peppermint, fermented milk, RSM and ANNs

Introduction

Kombucha is a popular traditional fermented beverage which is usually prepared at home and is associated to various health promoting characteristics, which are mainly reported by the users. Metabolic activity of symbiotic association of bacteria and yeasts is responsible for the production of kombucha beverage, usually after 7 days long fermentation process of sweetend black or green tea. Beneficiary effects of kombucha are attributed to the chemical composition that includes: organic acids (acetic, gluconic, glucuronic, tartaric, malonic, oxalic, lactic, malic, citric, succinic), tea polyphenols (theaflavins, thearubigins, EGCG, ECG, EC, EGC), vitamins (C, B_1, B_2, B_6) and a range of other micronutrients (such as iron, zinc, copper) produced during fermentation. Alternative substrates (herbal extracts, fruit juices, coffee, milk etc.) can also be applied for kombucha fermentation (Malbaša et al., 2009; Jayabalan et al., 2014; Jayabalan et al., 2016). Milk fermentation process leads to a variety of useful compounds released from metabolic activity, such as organic acids, bioactive peptides or folic acid, that cause the increase of nutritional and health value of fermented milk products (Sánchez et al., 2009). Antioxidant potential is an indicator that a food product has prospective functional effect. Antioxidant activity of traditional yoghurt (Parrella et al., 2012; Ye et al., 2013) and some novel types such as hickory-black soybean yoghurt (Ye et al., 2013) were established. Scientific results indicated the possibility for production of kombucha fermented milk beverages by applying various types of kombucha starters. These products should be considered as the novel group of fermented milks, because of the complexity of used starter and quality characteristics of final product (Malbaša et al., 2009; Vitas et al., 2013; Malbaša et al., 2014). The products obtained by kombucha cultivated on herbal teas possess antioxidant characteristics (Vitas et al., 2013;

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*Corresponding author/Dopisni autor: E-mail: vitasj@uns.ac.rs

Malbaša et al., 2014). New studies reported that peppermint has potential to be a source of dietary bioactive compounds that can improve health. Peppermint possesses anti-proliferative and strong anti-inflammatory ability (Lv et al., 2012).

In order to transfer the production from laboratory scale to industry level statistical packages were applied. Response surface methodology (RSM) and artificial neural networks (ANNs) represent statistical software used in modelling the area of food science and technology (Nunes et al., 2015). RSM is a statistical technique useful for the analysis and modelling in which a response of interest is influenced by several variables. The final goal of RSM is to optimize the response. Optimization can be carried out in numerical or graphical way.

ANNs are promising tools for different processes modelling. In many studies, they have demonstrated superior results compared to conventional modelling techniques. Even though, ANNs are known as black-box models (they do not provide insight amongst parameters relationships), but they are able to map relationship between input and output data without prior understanding of the process. ANN models are built upon the input and output observations without the detailed understanding of the complex physical laws of investigated process under and are able to provide reasonably accurate model for the process. Predictions in food (processing, quality, shelf-life) and fermentation (biotechnology) technology are often performed by neural nets (Bhotmange and Shastri, 2011). ANNs were successfully implemented in the modelling of the antioxidant activity and phenolic compounds in bananas that undergo drying process (Guiné et al., 2015).

The aim of this paper was to set grounds for scale-up production of kombucha fermented milk beverages with peppermint (KFM-P). These products possess antioxidant activity towards DPPH and ·OH radicals as well as MUFAs, PUFAs and ascorbic acid content were determined, which are well-known antioxidant nutraceuticals. To our knowledge, RSM and ANNs were employed for the first time for kombucha fermented milk products fortified with peppermint. Process optimization was also performed with the goal to determine the conditions for production of beverages with the best antioxidant quality. The contribution of fermentation process parameters (milk fat content and fermentation temperature) in the production was established.

Materials and methods

Kombucha starter

The local kombucha culture contains bacteria of the genus *Acetobacter* and following yeast strains: *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp. and *Zygosaccharomyces* sp. (Malbaša et al., 2009).

Kombucha starter was fermentation broth of kombucha obtained after 7 days long fermentation process on peppermint (*Mentha piperita*) extract (2.25 g/L) sweetened with sucrose (7 %), at 25 °C. The total yeasts count was 1.45×10^5 cells mL^{-1} while total acetic acid bacteria count was 1.87×10^5 cells mL^{-1}.

Milk

Kombucha fermented milk beverages were produced on laboratory scale using pasteurized and homogenized cow's milk with 0.8, 1.6 and 2.8 % milk fat.

Fermentation process

Kombucha starter with peppermint was cultivated on milk (0.8, 1.6 and 2.8 % milk fat) at fermentation temperatures of 37, 40 and 43 °C. The kombucha inoculum was added in the amount of 10 % (v/v) and fermentation was stopped when the pH value of 4.5 was reached. The obtained milk gel was then cooled to the temperature of 8 °C, homogenized by mixer, and the samples were stored in refrigerator, at 4 °C until the analyses, which were performed after production (Malbaša et al., 2009).

Determination of pH, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and ascorbic acid content

pH values were measured by a pH-meter (PT-70, Boeco, Germany). MUFAs and PUFAs content were determined using the gas chromatography-mass spectrometry (GC-MS) method (Malbaša et al., 2011). Used technique includes extraction of fat from samples, preparation of fatty acids methyl esters and the GC-MS analysis of the obtained esters. Hewlett-Packard (HP) 5890 Series II gas chromatograph coupled with HP 5971A mass spectrometer detector was used. Capillary column Supelco fused silica SP-2560 (100 m x 0.25 mm, internal diameter; thickness of stationary liquid phase film 0.20 µm) was applied. Injection volume was 1 µL, injector temperature was 230 °C and split ratio was 1:40. Carrier gas was helium with 0.58 mL/min constant flow. Temperature program was set as follows: initial temperature 100 °C, held for 5 min and increased for 6 °C/min until the final 240 °C (20 min). Mass spectrometer possess ionic source and works on the principle of electron ionization. Temperature of quadrupole was 180 °C. Data acquisition was performed in scan mode (in range 50-400 m/z). Multi-Standard solution of 37 fatty acids methyl esters (37 component FAME Mix, 47885-U) from Supelco, Bellefonte, PA, USA was used, as well as 'Wiley' commercial data base of mass spectra. With modified method of 100 % MUFAs and PUFAs were quantified. Ascorbic acid content was determined using the HPLC method (Vitas et al., 2013). Sample preparation includes ascorbic acid extraction with 3 % *m*-phosphoric acid (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) in 8 % acetic acid (J.T. Baker, Deventer, Holland) solution, filtration and filtration of the extract through 0.45 µm membrane filter prior to HPLC analysis. Agilent 1100 Series HPLC with UV-DAD detector was used. Isocratic elution with 0.1 mol/L ammonium-acetate (pH=5.1) as mobile phase at flow rate of 0.4 mL/min and C-8 column (150 x 4.6 mm; pore diameter 5 µm) was used. Injection volume was 20 µL and detection wavelength was set at 254 nm. Ascorbic acid standard (J.T. Baker, The Netherlands) was used for external standard method quantification and content was expressed in mg/L.

Antioxidant activity to DPPH (AA_{DPPH}) and hydroxyl radicals ($AA_{\cdot OH}$)

AA_{DPPH} was determined according to Živković et al. (2009), with some modifications, that included the use of 120 µM DPPH standard solution and 0,5 mL of sample addition and 1.5 mL of methanol addition to reaction mixture. Sample preparation included absolute ethanol (Zorka Pharma Hemija d.o.o., Šabac) addition (1:1, v/v), after which the sample was kept for 20 min in the freezer and centrifuged (4000 g) for 30 min at 4 °C, and repetition of the process. Obtained supernatant was used for further analysis. 1 mL of DPPH· radical standard (Sigma-Aldrich® CHEMIE GmbH, Steinheim, Germany) solution in methanol (120 µM) was added to 1.5 mL methanol and 0.5 mL of sample supernatant. The reaction tubes were kept in the dark for 45 min at room temperature. Absorbance was measured at 515 nm. Antioxidant activity was expressed as inhibition percent (%).

$AA_{\cdot OH}$ was determined according to Deeseenthum and Pejovic (2010). The 75 µL of sample was mixed with 450 µL of sodium phosphate (Merck, Alkaloid, Skoplje, Macedonia) buffer (0.2 mol/L, pH=7,00), 150 µL of 2-deoxyribose (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) (10 mmol/L), 150 µL of EDTA disodium salt dihydrate (Lach-Ner, Neratovice, Czech Republic) (10 mmol/L), 150 µL of $FeSO_4$ x $7H_2O$ (Zdravlje, Leskovac, Serbia) (10 mmol L^{-1}), 150 µL of H_2O_2 (Zorka Pharma, Šabac, Serbia) (10 mmol L^{-1}), and 525 µL of double distilled water. Samples were incubated at 37 °C for

Table 1. Regression equation coefficients of modelled responses for KFM-P

Effects	AA_{DPPH} (%)		$AA_{\cdot OH}$ (%)		MUFAs (%)		PUFAs (%)		Ascorbic acid (mg/L)	
	estimate	p-value	estimate	p-value	estimate	p-value	estimate	p-value	estimate	p-value
intercept										
b_0	442.043	0.442	103.457	0.430	353.912	0.047*	150.843	0.008*	-3692.540	0.039*
linear										
b_1	-22.078	0.442	-4.315	0.503	-17.159	0.051	-7.352	0.009*	185.86	0.038*
b_2	67.898	0.097**	-19.573	0.057**	-12.484	0.137	-2.299	0.189	42.660	0.525
quadratic										
b_{11}	0.270	0.450	0.040	0.609	0.213	0.052	0.091	0.009*	-2.320	0.038*
b_{22}	-11.017	0.033*	-0.151	0.836	2.403	0.033*	0.397	0.066**	-4.100	0.553
interaction										
b_{12}	-0.786	0.318	0.559	0.033*	0.050	0.749	0.012	0.719	-0.740	0.628

*Effects are statistically significant, p = 0.05; ** Effects are statistically significant, p = 0.10

4 h after which 750 μL of trichloroacetic (J. T. Baker, Deventer, The Netherlands) (2.8%) acid and 750 μL of thiobarbituric (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) (0.1 %) acid was added. Afterwards, the samples were kept in boiled water for 10 min. Absorbance was measured at 520 nm. 96 % ethanol (Reahem, Novi Sad, Serbia) was used as control. Antioxidant activity was expressed as inhibition percent (%). Chemicals were of analytical, HPLC and GC purity grade.

Sensory analysis

Sensory marks were obtained according to the method described by Malbaša et al. (2014). Briefly, qualified and untrained consumers determined the sensory characteristics of beverages. Flavour, consistency, odour, colour and appearance were sensory parameters that were examined. Descriptive test and five-point category scale (1 - lowest mark; 5 - highest mark) was used.

Antioxidant activities, MUFAs, PUFAs and ascorbic acid content were presented as differences between the values obtained for kombucha fermented milk beverages with peppermint and milk.

Statistical analysis

All experiments and analysis in this study were carried out in triplicate, after production of the samples. Results were averaged and the reproducibility of the results was good. Deviations between parallel experiments were in the range of ±5.2 %.

In this paper, for the description of the responses Y by RSM (AA_{DPPH}, $AA_{\cdot OH}$, MUFAs, PUFAs, and ascorbic acid content), a second-degree polynomial model was fitted to data:

$$Y = b_o + \sum b_i X_i + \sum b_{ii}^2 X_{ii}^2 + \sum b_{ij} X_i X_j \quad (1)$$

where b_0 represents intercept, b_i represents the linear, b_{ii} the quadratic and b_{ij} the interaction effect of the factors. The factor variables and their ranges were: X_1: fermentation temperature (37, 40, 43 °C) and X_2: milk fat (0.80, 1.60 and 2.80 %).

Statistical analysis and graphical representation of the data were performed using Statistica 9.1 software (StatSoft, USA). Coefficient of determination (R^2) and model p-value ($p=0.05$) were used for evaluation of the adequacy of the model.

Optimal values of processing variables were determined by Design-Expert v.7.1.5; Stat-Ease, Minneapolis, MN, USA. In order to implement ANN modeling, MATLAB R2012b neural network toolbox was used.

Results and discussion

Production of KFM-P

The production process was initiated by the addition of kombucha starter with peppermint to the milk heated at appropriate process temperature. This provided the total count of bacteria around 1.87×10^4 cells $mL^{-1}$and total number of yeasts was approximately 1.45×10^4 cells $mL^{-1}$of substrate. Microorganisms number was lower in comparison to our other investigations (Vitas et al., 2013; Malbaša et al., 2014). This fact suggests weaker metabolic activity of kombucha on peppermint extract in comparison to winter savoury, stinging nettle (Vitas et al., 2013) and wild thyme (Malbaša et al., 2014) extract.

All fermentation processes were monitored by measuring pH values during time period. The fermentation was stopped when the pH of 4.5, characteristic for kefir and yoghurt production, was reached. Both applied parameters (milk fat content and fermentation temperature) had influence on the process duration. Production lasted the shortest (9 h 30 min) for sample produced at 43 °C from milk with 1.6 % milk fat. The longest production (17 h) was for samples obtained at 37 °C from milk with 0.8 and 2.8 % milk fat. Fermentation progress corresponds to sigmoidal curvature which is in accordance with the production of these types of beverages (Malbaša et al., 2009; Vitas et al., 2013; Malbaša et al., 2014). When taking into account milk fat content, higher temperature caused the shortening of production process, which is also in accordance to our previous investigations (Vitas et al., 2013; Malbaša et al., 2014).

Modelling by RSM approach

Results of the RSM for AA_{DPPH}, $AA_{\cdot OH}$, MUFAs, PUFAs and ascorbic acid content of KFM-P are given in Table 1. The coefficients in Table 1 are related to actual variables. The ANOVA results for selected responses are reported in Table 2. Relatively high values of R^2, obtained for all responses, indicate good fit of experimental data to Eq. 1. All polynomial models tested for the selected responses were significant at 95 % confidence level, with the model F-value 27.502, 8.739, 7.973, 41.408 and 13.914 for AA_{DPPH}, $AA_{\cdot OH}$, MUFAs, PUFAs and ascorbic acid content, respectively.

RSM of MUFAs, PUFAs and ascorbic acid content

MUFAs and PUFAs are found in milk and different milk products, including fermented milks. MUFAs and PUFAs possess unsaturated bonds and therefore have antioxidant potential. For the response of MUFAs in KFM-P coefficient of determination was 0.940 which indicated that only 6.0 % of the variations could not be explained by the model. Significant effect at the level 0.05 was quadratic effect of milk fat. The effects of independent variables on MUFAs in KFM-P are shown in Fig. 1a. MUFAs content increased in the temperature range of 42-43 °C and at lowest values of milk fat. From the presented results it was evident that the obtained model predicted highest values of MUFAs at milk fat content of 0.8-1.0 % and fermentation temperature of 37-38 °C. This prediction was in accordance with results obtained when wild thyme kombucha starter was used for production (Malbaša et al., 2014) indicating that starter variety had no influence on MUFAs content.

PUFAs are well known as good radical scavengers (Maadane et al., 2015). For the response of PUFAs in KFM-P coefficient of determination was 0.980 which indicated that only 2.0 % of the variations could not be explained by the model. Significant effects at the level 0.05 were linear and quadratic effects of fermentation temperature. Quadratic effect of milk fat was significant at level 0.10. The effects of independent variables on PUFAs in KFM-P are shown in Fig. 1b. It was evident that obtained model predicted highest values of PUFAs at milk fat content of 0.80 % and fermentation temperature of 37-38 °C. Predictions for MUFAs and PUFAs content were very similar. PUFAs estimation was completely opposite with its predictions in products with winter savory, stinging nettle (Vitas et al., 2013) and wild thyme (Malbaša et al., 2014). of the application of kombucha peppermint starter lead to the highest content of PUFAs at lowest milk fat content and fermentation temperature.

Ascorbic acid is hydrosoluble vitamin and nonenzymatic antioxidant. Its antioxidant activity is based on a strong reducing characteristics. For the response of ascorbic acid in KFM-P, R^2 was 0.912, which indicated that less than 8.8 % of the variations could not be explained by the model. Significant effects at the level 0.05 were linear and quadratic effects of fermentation temperature. The effects of independent variables on the content of ascorbic acid in KFM-P are presented in Fig. 1c. Highest values of selected response were predicted at fermentation temperature in the range of 39-41 **°C**. Beyond this range, model predicted lower content of ascorbic acid at all applied values of milk fat. Kombucha starter with peppermint used in this study, as well as inoculums with winter savoury, stinging nettle (Vitas et al., 2013) and wild thyme (Malbaša et al., 2014) caused different prediction of highest values of ascorbic acid content for every group of the obtained fermented milk products. These results suggested that ascorbic acid content was strongly dependent upon the used starter culture.

Table 2. Analysis of variance (ANOVA) of modelled responses for KFM-P

Response	Source								
	Residual			Model			F-value	p-value	R^2
	DF	SS	MS	DF	SS	MS			
AA_{DPPH} (%)	3.000	47.281	15.760	6.000	2600.606	433.434	27.502	0.010	0.958
$AA_{\cdot OH}$ (%)	3.000	2.443	0.814	6.000	42.698	7.116	8.739	0.052	0.972
MUFAs (%)	3.000	2.239	0.746	6.000	35.705	5.951	7.973	0.058	0.940
PUFAs (%)	3.000	0.108	0.036	6.000	8.904	1.484	41.408	0.006	0.980
Ascorbic acid (mg/L)	3.000	206.914	68.971	6.000	5757.926	959.654	13.914	0.027	0.912

DF - degree of freedom, SS - sum of squares, MS - mean squares

RSM of AA_{DPPH} and $AA_{\cdot OH}$

DPPH method is very well established for the estimation of the antioxidant activity of different food products. The R^2 for the response of AA_{DPPH} in KFM-P was 0.958. This value indicated high correlation between observed and predicted values and means that less than 4.2 % of the variations could not be explained by the model. p-values suggested that at level 0.05 the most important factor was quadratic effect of milk fat content. Also linear effect of the same independent variable was significant at level 0.10. The effects of fermentation temperature and milk fat on AA_{DPPH} are shown in Fig. 2a. It was observed that obtained model predicted increase of AA_{DPPH} values during decrease of fermentation temperature at all applied milk fat contents. Nevertheless, highest values of AA_{DPPH} were predicted at fermentation temperature of 37 °C and milk fat content in the range 1.4-2.2 %. The values of milk fat content and process temperature predicted in order to obtain the highest value of AA_{DPPH} in KFM-P differ from the values characteristic for the kombucha fermented milk beverages with winter savoury, stinging nettle (Vitas et al., 2013) and wild thyme (Malbaša et al., 2014). This indicated that used starter had influence on AA_{DPPH}.

Hydroxyl radical is the most potent and highly reactive radical that belongs to the group of reactive oxygen species, which are responsible for initiation of oxidative stress. It reacts with every biomolecule from its surrounding. $^{\cdot}OH$ radicals were generated in Fenton's reaction (Fe^{2+} reacts with hydrogen-peroxide, in the presence of EDTA), which is characteristic for human organism. Formed radicals react with deoxyribose and produced malondialdexyde gives with thiobarbituric acid a pink compound. The goodness of fit of the model for the response of $AA_{\cdot OH}$ in KFM-P was checked by the determination coefficient which was 0.972, indicating that less than 2.8 % of the variations could not be explained by the model. The most important factor influencing $AA_{\cdot OH}$ was interaction between milk fat and fermentation temperature. Positive interaction between the two independent variables (data reported in Table 1) permit to assess that there was a synergetic effect of milk fat and fermentation temperature on $AA_{\cdot OH}$. Beside this, linear effect of milk fat was significant at level 0.10. The effects of independent variables on $AA_{\cdot OH}$ in KFM-P are shown in Fig. 2b. Shown

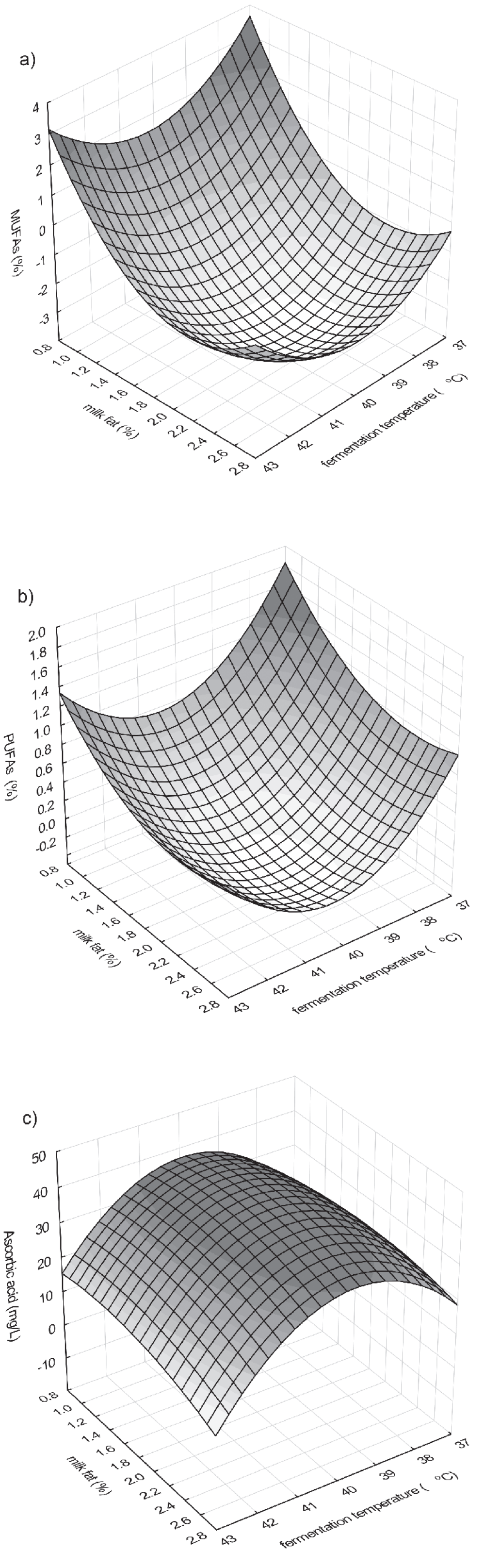

Figure 1. The effects of milk fat and fermentation temperature on MUFAs (a), PUFAs (b) and ascorbic acid content (c) for KFM-P

$AA_{\cdot OH}$ in KFM-P also increased. Presented model predicted highest values of $AA_{\cdot OH}$ for milk content in range of 2.4-2.8 % and fermentation temperature between 41.5 and 43 °C. These results were in correlation with results obtained when winter savoury, stinging nettle (Vitas et al., 2013) and wild thyme (Malbaša et al., 2014) were used for inoculums preparation, suggesting that type of starter did not influenced $AA_{\cdot OH}$ of these types of products.

Simultaneous optimization of multiple responses

Optimization of multiple response variables by using RSM offers two approaches: numerical and graphical optimization. During numerical optimization individual responses are combined into overall desirability function, which has range from 0 (the least desirable result of optimization) up to 1 (all individual criteria for selected responses are completely fulfilled). In this study, numerical optimization was done by maximizing all process responses. In this case, maximum value of desirability function was 0.569, for temperature 37.4 °C and milk fat content 0.94 %. When compared to kombucha fermented milk products with stinging nettle (Vitas et al., 2013) it could be observed that MUFAs content had no influence on antioxidant activity. The optimization did not include MUFAs content and the obtained values for process parameters were 37 °C and 0.94 % milk fat, while desirability function was 0.52. The desirability function is shown in Fig. 3. The highest desirability function values were obtained for lower temperature values up to around 38.5 °C and milk fat content up to around 1.6 %.

Overlay plots are graphical representation of selected model responses with defined limit conditions of experimental factors to show the windows of operability where requirements simultaneously meet limit conditions of selected multiple responses. In this study, two responses were selected for graphical optimization, i.e. PUFAs and vitamin C. Lower limits for responses were set at the values of 0.5 % and 12 mg L^{-1}, for PUFAs and vitamin C; respectively. These limits were selected based on previously published results (Vitas et al., 2013; Malbaša et al., 2014), since these values were around minimum positive values measured in kombucha fermented milk products with winter savoury, stinging nettle and wild thyme.

Shaded (grey) areas on the overlay plot do not meet the selection criteria. The results presented in Fig. 3. it is obvious that there was a limiting response inPUFAs content. The clear (white) "window" shows where the set of experimental factors is positioned that satisfies requirements for both responses. As it can be seen from the Fig. 3 for all selected milk fat values were confined to lower temperature values. As for lower milk fat content up to 1.8 % the region of satisfactory response values was obtained even at higher temperatures. At higher temperatures milk fat content shouldn't be higher than 1.6 %

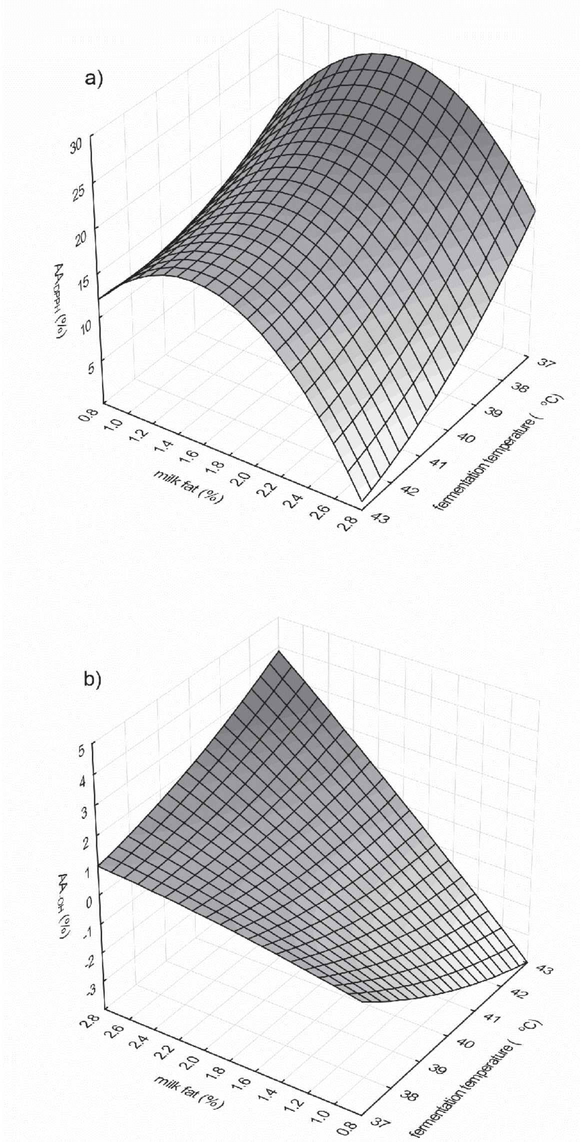

Figure 2. The effects of milk fat and fermentation temperature on AADPPH (a) and AA·OH (b) for KFM-P

Modelling by ANNs

In this study, feed-forward ANN was used with backpropagation training algorithm. This type of ANN consists of many computing elements, neurons, which are connected by weights, improved through a learning process. The hyperbolic tangent sigmoid function is the transfer function employed in the hidden and output layers. The Levenberg-Marquardt training algorithm was selected. The mean square error (MSE) and the R^2 between the experimental and calculated values were selected as the standard statistical indicators in order to determine the best ANN architecture. Since the neural network is highly dependent upon the initial weight values and in order to achieve the best results, the neural networks were run ten times and the average values of statistical indicators are used for comparing of network performances. In this paper, as input neurons, temperature (°C) and milk fat content (%) were selected. The network had five output neurons AA_{DPPH}, (%); $AA_{\cdot OH}$, (%); MUFAs, (%); PUFAs, (%) and ascorbic acid content (mg L^{-1}).

In order to find optimal the ANN architecture number of hidden neurons are varied. The optimal number of hidden layer neurons is case dependent and there is no straightforward method to adjust it (Ghafari-Nazari and Mozafari, 2012). Increase in the number of the hidden layer neurons leads to enhancement of the approximation ability of the neural networks. For the network with one hidden neuron average values of MSE and R^2 were 59.181 and 0.762, respectively. With increasing the number of hidden neurons predictive capabilities of ANN was improved up to five hidden neurons. Further increase in neuron number did not resulted in predictions improvement. So in this study ANN with architecture 2-5-5 was selected as optimal. In the case of five neurons in hidden layer averaged R^2 was 0.993, while average value of MSE was 0.713.

Comparison of RSM and ANNs

Prediction performance of RSM and ANN model are presented in Fig. 4. The predicted values were very close to the desired values in most cases. Compared to RSM model the ANN model predictions are distributed nearby to the line of perfect prediction. Thus it is obvious conclusion that the ANN model shows a higher generalization capacity than the RSM models. This higher predictive precision of the ANN can be attributed to its ability to approximate the nonlinearity of the system, whereas the RSM is restricted to a second-order polynomial. RSM model quality is possibility to estimate the significance of each coefficient, i.e. linear, quadratic and interactions effects of selected variables by Student's t-test and p-values.

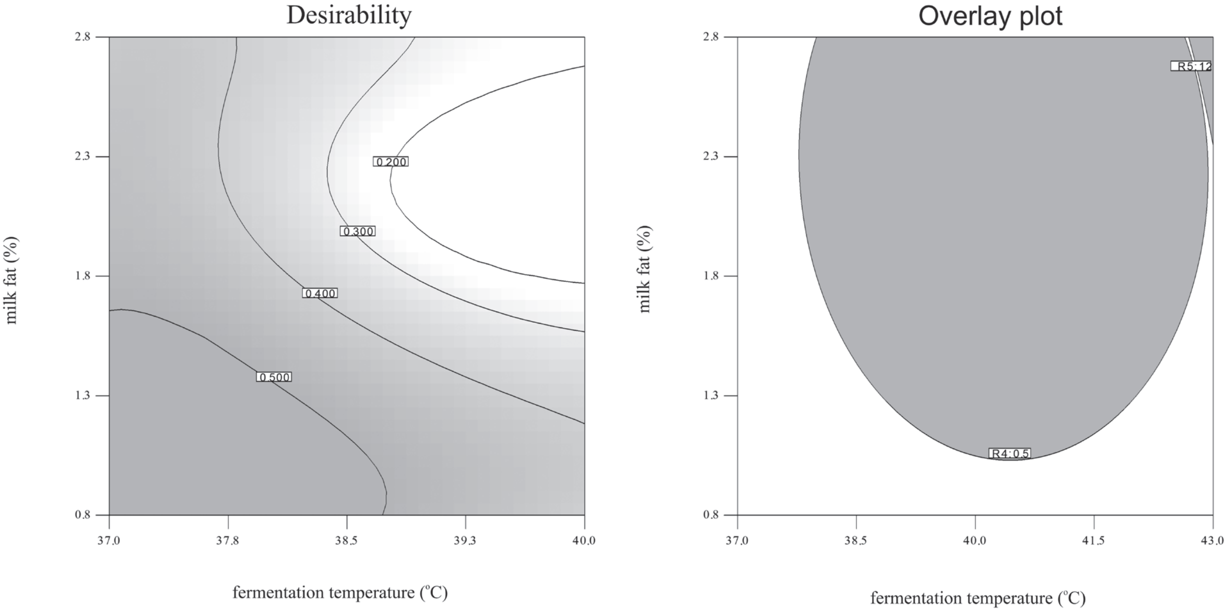

Figure 3. Desirability function and Overlay plot-for PUFAs and vitamin C responses

Sensory evaluation

The produced KFM-P samples were by their sensory attributes similar to yoghurt and kefir. All beverages were without separated whey and with colour and consistency characteristic for fermented milks. Odour and taste were pleasant and resembled to peppermint extract. Increase in fermentation temperature lead to higher sensory marks for all products, which is in accordance to Vitas et al. (2013) and Malbaša et al. (2014). Higher milk fat content also caused better sensory quality.

Conclusions

Kombucha fermented milks were produced using kombucha broth cultivated on sweetened peppermint extract, as starter culture. RSM and ANNs were successfully used for process modelling. In our case it was confirmed also that ANNs are superior modelling technique over RSM. Numerical and graphical optimization for maximization of antioxidant characteristics were also performed, indicating lower process temperature (up to 40 °C) and milk fat content (up to 1.3 %) as optimal. Response and prediction of MUFAs and PUFAs are very similar and for AA_{DPPH} and $AA_{\cdot OH}$ are completely different. Higher values of process temperature, regardless on milk fat content, lead to maximization of ascorbic acid content.

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Modeliranje antioksidativnih svojstava kombuha fermentiranih mliječnih napitaka s mentom primjenom ANNs i RSM metode

Sažetak

U ovom radu modelirana je i optimizirana antioksidativna aktivnost prema stabilnom DPPH radikalu (AA_{DPPH}) i nestabilnim hidroksi radikalima ($AA_{\cdot OH}$), kao i sadržaj nutraceutika (mononezasićene masne kiseline, polinezasićene masne kiseline i askorbinska kiselina) kombuha fermentiranih mliječnih proizvoda s mentom. Napici su proizvedeni dodavanjem 10 % kombuha startera s mentom u mlijeko koje je sadržavalo 0,8, 1,6 i 2,8 % mliječne masti pri 37, 40 i 43 °C. Metodologija površine odgovora (RSM) naznačila je suprotne površine odgovora za AA_{DPPH} i $AA_{\cdot OH}$. Sadržaj polinezasićenih masnih kiselina i askorbinske kiseline, kao najznačajnijih utjecajnih

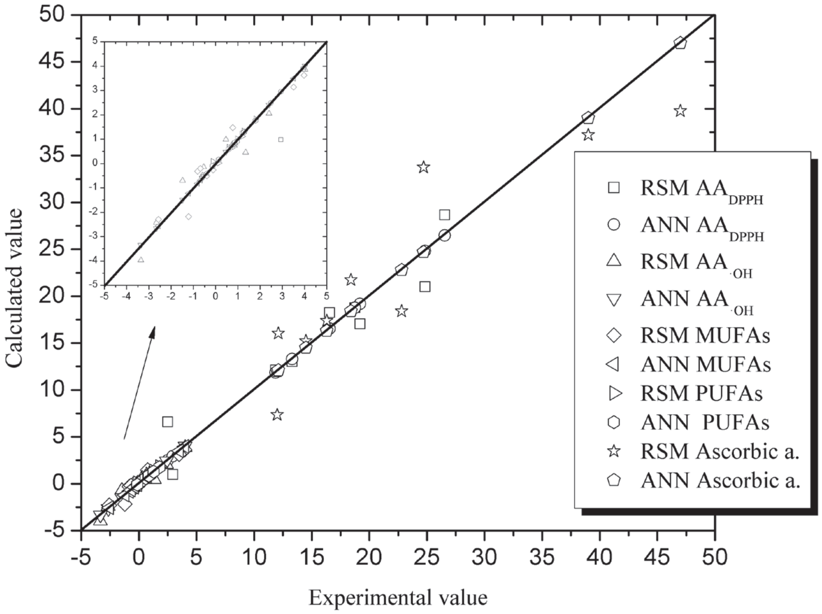

Figure 4. RSM and ANNs model prediction performance

čimbenika, uključeni su u grafičku optimizaciju i utvrđeno je radno područje za proizvodnju napitaka najveće antioksidativne kvalitete: niže temperature i mliječna mast do 1,8 %; više temperature i mliječna mast do maksimalno 1,6 %. Modeliranje antioksidativnih svojstava kombuha fermentiranih mliječnih napitaka s mentom primjenom umjetnih neuronskih mreža, kako je i očekivano, bilo je superiornije u poredbi s RSM.

Ključne riječi: kombuha, antioksidanti, menta, fermentirano mlijeko, RSM i ANNs

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