SCIENTIFIC PAPER

MATHEMATICAL MODELING AS A TOOL IN KOMBUCHA BEVERAGES BIOACTIVE QUALITY CONTROL

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ABSTRACT

This study examined the application of mathematical models on total flavonoids content and sensory mark of kombucha beverages on winery effluent. Process parameters were as follows: 0, 3, 6 and 9 days of fermentation time; 20, 25 and 30 °C of fermentation temperature and 3, 5 and 7% of initial total reducing sugars. Total flavonoids were determined spectrophotometrically and sensory mark by a descriptive test and five points category scale. Total flavonoids content decreased during the applied kombucha fermentation process, which lasted for 9 days. On average, total sensory mark suggested that consume kombucha products are obtained after 3 days of the fermentation, regardless on the fermentation temperature or sugars content. In order to produce kombucha beverage with the highest bioactive quality, response surface methodology proposed the following process parameters: 3 days of fermentation, 7% of initial total sugars and 30 °C process temperature.

Highlights

- Total flavonoids content and sensory mark of kombucha beverages were determined.
- Influence of temperature, time and sugars content on flavonoids and sensoric was modeled.
- Response surface model to predict total flavonoids content in kombucha beverage was created.

Keywords: winery effluent, total flavonoids, kombucha, sensory characteristics, response surface methodology

INTRODUCTION

Black tea kombucha, a traditional beverage with sweet and acidic taste, is obtained after kombucha culture fermentation of black tea decoct, sweetened with sucrose. The applied culture is composed of bacteria (mainly acetic acid bacteria) and several yeasts species. Kombucha inoculum has the capability to metabolically transform a variety of substrates into alternative kombucha beverages. The applied fermentation media include various herbal teas, milk, beer, wine, fruit juices, different types of food industry by-products etc. [1]. In this investigation, black tea kombucha was used as the starter inoculum in the production of winery effluent based kombucha. Black tea was selected since it relates to the traditional kombucha production.

The main constituents of kombucha beverages chemical composition are different organic acids, mainly acetic, sugars, such as sucrose, glucose and fructose, water soluble vitamins, ethanol, catalase, invertase, microelements, but various types of polyphenolic compounds as well [1,2].

Flavonoids biosynthesis begins with L-phenylalanine. In the reaction of nonoxidative deamination catalysed by phenylalanine ammonia lyase, cinnamic acid is produced. This reaction leads to the carbon transfer from the shikimate route to the various parts of the general phenylpropanoid metabolism. Flavan nucleus, that is composed of 15 carbon atoms organized in C6-C3-C6, i.e. A, B, and C rings represents the main structure of flavonoids [3]. Flavonoids possess health promoting potential, but they have an impact to sensory characteristics, as well. These compounds are responsible for bitter taste and tactile sensation of astringency of different types of beverages. Besides flavonoids, the astringency is caused by the presence of organic acids, too. Bitter taste and astringency are usually regarded as unpleasant. Flavonoids have impact on the colour of the product, as well [3]. Flavonoids are known for their pronounced antioxidant activity and ability to prevent and suppress different chronic diseases [4]. It is very well known that sensory properties are the main product characteristic that influence its consumption.

Flavonoids are a result of secondary plant metabolism and therefore they are often studied as a part of chemical composition of kombucha beverages produced using herb-based fermentation media [5-7].

In order to improve kombucha fermentation process, perform a scale-up of this process, determine the influence of process parameters to the composition of the final product or to predict the textural characteristics of kombucha fermented milk products, as well as to optimize process conditions in order to obtain kombucha products with the best antioxidant features, different mathematical models were applied [8-12].

Different mathematical techniques may be used to examine the impact of numerous operational factors, as well as their interactions, on the efficiency of the fermentation process. For example, response surface methodology (RSM) and other experimental designs (e.g., Box-Behnken, Plackett-Burman, Taguchi design, etc.) can be remarkable instruments for maximizing the intended process and describing the individual and combined influence of the independent variables [9]. In kombucha fermentation numerous studies were conducted in order to optimize the production. Response surfaces were used to scale-up of black tea batch fermentation by kombucha [13]. RSM and neural networks were successfully used for modelling of antioxidant characteristics of kombucha fermented milk beverages with peppermint [12]. Using the Response Surface Methodology (RSM), the effect of fermentation time, sugar concentration, and herbal tea type on the antibacterial activity of Kombucha beverages were analyzed [14].

The aim of this paper was the application of mathematical models to total flavonoids content and sensory mark of winery effluent based kombucha products in favour to determine the production variables that will lead to the kombucha beverage with the highest bioactive quality and to propose a way of quality control of this type of products.

EXPERIMENTAL

Initial medium, fermentation media and process parameters

The initial medium was filtrated and then sterilized winery effluent obtained in the white wine production, after must flotation using gelatin. The plate filter press and the qualitative filter paper were applied for the filtration process. Sterilization was done in an autoclave (121 °C, 20 min). Fermentation media were prepared by the dilution of the initial medium (16.36% of total reducing sugars, 370.22 mg/L of total nitrogen and 250.00 mg/L of total phosphorus [15]) with boiled tap water to three different sugar levels (3, 5, and 7% of total reducing sugars). The fermentation process was done at three different temperatures (20, 25, and 30 °C) and the samples were collected at the start of the fermentation (day 0), after 3, 6 and 9 days.

Kombucha inoculum

Kombucha inoculum used in this investigation for the production of novel kombucha products, was obtained by three passages of the traditional kombucha inoculum [16,17] on the winery effluent with 7% of total reducing sugars, during 6 days, at 25 °C. Traditional kombucha inoculum was sourced from local household of kombucha consumer from the northern region of Serbia. The liquid part of the obtained inoculum was added in the amount of 10% (v/v) to the appropriate fermentation media. Winery effluent was fermented in sterilized glass beakers covered with sterile cheesecloth, in the incubator.

Total flavonoids analysis

Total flavonoids determination was performed using the spectrophotometric method by Markham, with some modifications [18]. Samples in the amount of 7 mL were mixed with 0.3 mL of 5% sodium nitrite solution. After 5 min, 0.3 mL of 10% aluminum chloride hexahydrate was added. After 6 min, 1 mol/L sodium hydroxide in the amount of 1 mL and 1.4 mL of distilled water were added to the mixture. The blank sample was prepared by replacing the sample with distilled water. Absorbance was measured at

510 nm, using the LLG-uni SPEC 2 LLG LABWARE spectrophotometer. Rutin was used as a calibration standard and results were given as rutin equivalents per mL of the sample (μg RE/mL).

Sensory characteristics analysis

Sensory characteristics determination was performed according to [19]. Five points category scale (5 - the highest and 1 – the lowest) and a descriptive test were applied. Appearance, color, odor and taste were examined. The qualified evaluators (4 persons) together with untrained consumers (3 persons) performed the sensory analysis.

Statistical analysis

Response surface methodology (RSM) on Design Expert v10.0.1 (Stat Ease Inc., Minneapolis, USA) was used in this study for regression and graphical analyses of the data. RSM has been discussed in detail elsewhere [12, 20]). Model diagnostics, as well as the optimization capabilities in Design-Expert software were used to optimize the bioactive quality control of kombucha production, through the mathematical modelling of total flavonoids content. Experimental results are selected for Box-Behnken to evaluate the applicability of RSM as statistical tools for total flavonoids content. ANOVA was used to asses model adequacy. The design has 15 experiments, with three replications in center point. In this design, treatment combinations are located in the middle of the process space's edges and in the center. In comparison to central composite designs, the designs offer restricted orthogonal blocking capabilities. These designs are rotatable (or almost rotatable), with three levels of each factor. The effects of temperature (20, 25 and 30 ˚C), initial total sugars (3, 5 and 7%) and time (3, 6 and 9 days) were investigated (Table 1). Selected responses were total flavonoids [μg RE/mL] and total sensory mark (1-5).

RESULTS AND DISCUSSION

Total flavonoids

Results of total flavonoids are presented in Table S1. of Supplementary material.

The initial medium contained 99.4±2.1 μg RE/mL of total flavonoids and this was the highest measured value in this investigation. The results of total flavonoids obtained for the fermentation media indicated that the higher content was related to the higher initial total sugars content and it was in the range from 24.8±0.3 to 51.8±0.5 μg RE/mL.

The total flavonoids content (for products with 7% of initial total sugars content), of samples obtained at 30 and 25°C, was the highest at the beginning of the fermentation (49.1±0.3 μg RE/mL) and the values decreased during the process. For products obtained at 30 °C, the lowest value was measured on third day of fermentation (31.1±0.7 μg RE/mL). Contents determined on sixth and ninth day were higher in comparison to the third day. For products obtained at 25 °C, total flavonoids decreased linearly until the sixth day when the lowest value was determined (33.2±0.7 μg RE/mL). After nine days of fermentation the value increased for around 18% in comparison to the sixth day. For products obtained at 20 °C, content decreased until the third day of fermentation. Mild increase (for around 9%) was established on the sixth day, in comparison to the third day of the process. The pronounced increase was established at the end of the fermentation (62.6±6.3 μg RE/mL) and the obtained value was for 75% higher in comparison to the sixth day.

The total flavonoids content (for products with 5% of initial total sugars content), of samples obtained at 30, 25 and 20°C, was the highest at the beginning of the fermentation (38.3±0.7 μg RE/mL). For products obtained at 30 °C, the content decreased after the ninth day and the lowest values were measured after the third and the sixth day of the process. The lowest content values were the same and for about 17% lower in comparison to the ninth day. For products obtained at 25 °C, the total flavonoids content decreased after the third and the sixth day. On the ninth day the content increased for 17% when compared to the sixth day. For products obtained at 20 °C, the value decreased after the third day for around 26%. On the sixth day, the value was the same as at the beginning, i.e. it increased for 36% in comparison to the third day. After nine days of fermentation the value decreased again for around 29% in comparison to the sixth day. The values determined on the ninth and third day were approximatelly the same.

The total flavonoids content (for products with 3% of initial total sugars content), of samples obtained at 30, and 25°C, was the highest at the start of fermentation (25.4±0.8 μg RE/mL) and the values decreased during the process. For products obtained at 30 °C, total flavonoids content was approximately the same on the third, sixth and ninth fermentation day. In comparison to the 0 fermentation day, the values determined on the third and sixth day were for around 36%, and after the ninth day for around 32% lower. For products obtained at 25 °C, the content linearly decreased until the sixth day. On the ninth day, the total flavonoids increased for around 11% in comparison to the sixth day. On the other hand, for products obtained at 20 °C, the total flavonoids content was the lowest at the beginning of the process and during the fermentation the increase in values was established. The value determined on the third day was for 100% higher in comparison to the start of the process. Contents measured on the sixth and ninth day were lower when compared to the third day. The determined values decreased after the third and the sixth day. The highest total flavonoids content showed the sample obtained after three days of the process and it amounted 50.4±6.8 μg RE/mL.

Of all of the examined variables, process temperature was the most influential one. The lowest examined (20 °C) was responsible for the highest values of total flavonoids in kombucha products.

Modern literature suggests that bacteria species (*E. coli*, *C. glutamicum*, *L. lactis*, and *Bacillus sp.*) can be applied in the flavonoids production [21]. The presence of flavonoids in kombucha products on winery effluent can be related to the applied fermentation medium (winery effluent obtained after must flotation in white wine production), i.e. to the used grapes. Since all of the kombucha products showed lower flavonoids content than the applied fermentation medium, it can be implied that kombucha metabolic activity led to the degradation of flavonoid compounds, probably by the enzymes secreted from kombucha microorganisms [22]. It is possible that the partial oxidation of flavonoids led to the formation of polymerized substances with higher molecular mass [19,5].

Existing literature gives the overview of total flavonoids content in different types of kombucha samples. The majority of papers on kombucha products with plant extracts showed the increase in total flavonoids values as the result of kombucha fermentation. Some investigations showed opposite trend, as well. These results suggested that the flavonoids content in kombuchas was influenced by the fermentation process and plant type [5].

Öztürk *et al.* [23] determined the total flavonoids content of traditional (with black tea) and alternative (with hawthorn, hop and madimak) kombucha products and established that the fermentation process enhanced the flavonoids content, which is in opposite to the results obtained in this study. The same trend was recognized by Kilic and Sengun [5], as well. This difference can be related to the fact that Öztürk *et al.* [23] and Kilic and Sengun [5] used higher contents of applied herbs, in comparison to the diluted winery effluent used in this examination. Higher values of total flavonoids in kombucha products were related to sucrose, as the carbon source, and nettle leaves in comparison to honey, as the carbon source, and Anatolian hawthorn [5]. On the other hand, Vitas *et al.* [19] obtained results on total flavonoids that correlated to the ones determined in this study, i.e. the kombucha fermentation also led to the decrease in values.

Modelling of total flavonoids content

The experimental design for process variables and responses are presented in Table 1. Values given in parenthesis are predicted by selected mathematical model. The design has 15 experiments, with three replications in center point.

Table 1.

Design Expert has a number of statistical tables that provide help to model selection for further study. Models that comply with the criteria are highlighted by the software and marked as "suggested". Model summary statistics gives several comparative measures for model selection. Ignoring the aliased model, for total flavonoids content and sensory mark the quadratic and linear models are suggested, respectively.

The experimental data for total flavonoids content were fitted with the second-degree polynomial equation to create models characterizing the influence of the aforementioned operational parameters on the kombucha fermentation. Table 2 shows the linear (b_1, b_2, b_3), quadratic (b_{11}, b_{22}, b_{33}), and interaction (b_{12}, b_{13}, b_{23}) model coefficients and corresponding p-values.

Table 2.

The obtained results have indicated statistical significance of linear and quadratic effects of initial total sugars. In the regression equation, interaction between initial total sugars and time is statistically significant. Temperature linear influence is more pronounced in comparison to the linear influence of time i.e. corresponding p-values is lower.

A summary of the analysis of variance of the second-degree polynomial model for total flavonoids content is given in Table S2. of Supplementary material. The model developed for total flavonoids content is significant with p-value 0.0103.

In addition to high coefficient of the determination value the proposed second-degree polynomial models had nonsignificant lack of fit (p –value 0.0531). Adequate precision is an indicator of signal to noise ratio and a ratio greater than 4 is desirable. The ratio of 10.94 has indicated an adequate signal for the response. As a result, these findings suggest that a regression model may be employed to analyze response trends. The impacts of chosen variables on total flavonoids content could be successfully described using a second-degree polynomial model.

Response surface plots were also created to better understand the interactions of independent variables - operating circumstances (temperature, initial total sugars, and time) to total flavonoids content. The response surface plots depict the effects of two independent variables on one response, while the third independent variable's value was fixed to the mean of the tested range of values.

The effects of initial total sugars and temperature are given in Figure 1. As it could be seen, total flavonoids content is at maximum value for higher total sugars content. Increase of initial total sugars resulted in increase of total flavonoids content for all selected fermentation temperatures.

Figure 1.

On the other hand, influence of temperature is different at low and high initial total sugars values. At low initial sugar values increase of temperature results in decrease of total flavonoids content, whilst at higher total sugars content increase of temperature results in increased total flavonoids content. Several studies have shown that the microbial spectrum of Kombucha consortium may vary and any change in the fermentation conditions might affect the final product [21]. These findings, i.e. decrease of total flavonoids content suggest that some microbial species in kombucha are involved in the conversion of flavonoids during fermentation; however, more research is needed to determine this [24,25]. The increase in total flavonoids may be caused by the activity of certain microorganisms that are able to break down the polyphenol compound, as flavonoids may also be produced from other polyphenols. Certain species of lactic acid bacteria has been known to have the capability to degrade polyphenols such as *L. hilgardii* which frequently found in wine [26,27].

Figure 2 shows the simultaneous influence of time and temperature on the total flavonoids content. Increase of temperature results in total flavonoids content insignificant decline for all selected fermentation duration periods. During fermentation slight decrease of total flavonoids was observed at all predetermined temperatures. The highest total flavonoids content showed the samples obtained after three days of the process.

Figure 2.

The results of total flavonoids obtained in this study were in accordance to the results obtained in the production of small white apricot wine in which case the highest content (around 40 μg RE/mL) of total flavonoids was determined on second day of the process [28]. On the other hand, Liang *et al.* [29] determined up to 100 times higher total flavonoids content in green tea-infused white wine. This could be attributed to the applied production process and the addition of green tea. Öztürk *et al.* [23] determined the total flavonoids content as quercetin equivalents in traditional black tea, hawthorn, hop and madimak-flavored kombucha. The obtained results suggested that the kombucha fermentation process led to the increase in flavonoids content. The madimak flavored kombucha proved to be the superior product because of the used fermentation medium, and not the performed fermentation process. Vitas *et al.* [18] concluded that alternative herb-based kombucha products had higher flavonoids content than the traditional tea-based ones. The results obtained by Vitas *et al.* [18] were in accordance to the results of the present study.

The effects of initial total sugars and time are given in Figure 3. Total flavonoids have the highest value for the higher total sugars content. Increase of initial total sugars resulted in increase of total flavonoids content for all predetermined fermentation periods.

Figure 3.

During the process of fermentation total flavonoids did not vary significantly. Similar results were reported for kombucha fermentation process of green and pu'er teas where no significant changes in the total flavonoids [25].

Sensory mark

Results of sensory mark are presented in Table S3. of Supplementary material. It is very well known that the colour of the food product suggests the possible taste and the freshness of the products. Odour is related to the behaviour in food choice and taste influences the perception of flavor. In food products, flavonoids are usually responsible for the taste and colour [30].

Colour

Kombucha products obtained from fermentation medium with 7% of initial total sugars had the highest colour mark (5) on the sixth day at 30 °C. This was the only product whose colour mark was higher than the sample mark from the fermentation start. The lowest colour mark (2) showed products obtained at 25 °C (third and sixth day) and at 20 °C (sixth day). The most suitable temperature was 30 °C, and the least favourable temperature was 25 °C.

Kombucha products obtained from fermentation medium with 5% of initial total sugars had the highest colour mark (5) on the sixth day at 30 °C, as well as products with 7% of sugars. The lowest colour mark (1) had sample produced at 20 °C. The most suitable temperature was 30 °C, and the least favourable temperature was 20 °C. Products obtained at 30 °C (sixth and ninth day) and sample produced at 25 °C (ninth day) had higher colour mark than sample from the fermentation start.

Kombucha products obtained from fermentation medium with 3% of initial total sugars had the highest colour mark (4) on the sixth day at 30 °C, as well as products with 7 and 5% of sugars. The lowest colour mark (1) showed products obtained at 25 °C (sixth day) and 20 °C (sixth and ninth day). The most suitable temperature was 30 °C, and the least favourable temperature was 20 °C, as for the products with 7 and 5% of initial total sugars content. Only product obtained at 30 °C after six days had higher colour mark than sample from the beginning of fermentation.

Odour

Kombucha products obtained from fermentation medium with 7% of initial total sugars had the highest odour mark (4) on the third day at 30 and 20 °C. The lowest odour mark (1) showed products obtained at 30, 25 and 20 °C on the ninth day. The most suitable temperature was 30 and 20 °C, and the least favourable temperature was 25 °C. Samples produced at 30 °C (third and sixth day), 25 °C (third day) and 20 °C (third day) had higher odour marks than the fermentation start product.

Kombucha products obtained from fermentation medium with 5% of initial total sugars had the highest odour mark (5) on the third day at 30 and 20 °C, as the products with 7% of sugars. The lowest odour mark (1) showed products obtained at 30, 25 and 20 °C on the ninth day, as the products with 7% of sugars. The most suitable temperature was 30 and 20 °C, and the least favourable temperature was 25 °C, as for the products with 7% of sugars. Samples produced at 30 °C (third and sixth day), 25 °C (third day) and 20 °C (third and sixth day) had higher odour marks than the product from the beginning of fermentation.

Kombucha products obtained from fermentation medium with 3% of initial total sugars had the highest odour mark (4) on the third day at 30, 25 and 20 °C. The lowest odour mark (1) showed products obtained at 30, 25 and 20 °C on ninth day, as the products with 7 and 5% of sugars. All of the fermentation temperatures showed the same suitability for the production. Samples produced at 30, 25 and 20 °C on third and sixth day had higher odour marks than the fermentation start product.

Taste

Kombucha products obtained from fermentation medium with 7% of initial total sugars had the highest taste mark (5) on the third day at 25 °C. The lowest taste mark (1) showed products obtained at 30, 25 and 20 °C on the ninth day, as well as the sample from the fermentation start. The most suitable temperature was 25 °C, and the least favourable temperature was 30 and 20 °C. Samples produced at 30 °C (third and sixth day), 25 °C (third and sixth day) and 20 °C (third and sixth day) had higher taste marks than the fermentation start product.

Kombucha products obtained from fermentation medium with 5% of initial total sugars had the highest taste mark (5) on the third day at 30, 25 and 20 °C. The lowest taste mark (1) showed products obtained at 30, 25 and 20 °C on the ninth day, as well as the sample from the beginning of the process. All of the fermentation temperatures showed the same suitability for the production. Samples produced at 30 °C (third and sixth day), 25 °C (third and sixth day) and 20 °C (third and sixth day) had higher taste marks than the product from the fermentation start, as the products with 7% of sugars.

Kombucha products obtained from fermentation medium with 3% of initial total sugars had the highest taste mark (4) on the third day at 30 and 20 °C. The lowest taste mark (1) showed products obtained at 30, 25 and 20 °C on ninth day, as well as the sample from the beginning of the process. All of the fermentation temperatures showed the same suitability for the production. Samples produced at 30, 25 and 20 °C on third and sixth day had higher taste marks than the fermentation start product.

Cohen *et al.* [31] established that the sensory characteristics of kombucha products were more influenced by the process temperature than the sucrose content. On the basis of the formed metabolites, lower temperatures were more suitable since the obtained products were sweet. Higher temperatures lead to the astringency and sourness that are not pleasant. Cohen *et al.* [31] also suggested that higher sugar content was related to the higher preference of the kombucha products.

Modelling of total sensory mark

Total sensory mark was chosen for modelling as the most significant parameter of the sensory mark.

Table 3. shows the coefficients of regression equation for total sensory mark in terms of coded and real variable values, as well as the related p-values. The obtained results have indicated that the factor of initial total sugar can be removed from linear model, while keeping model adequacy. Statistically significant coefficient is associated with time of fermentation.

Table 3.

A summary of the analysis of variance of the linear model for total sensory mark is given in Table S4. of Supplementary material. The model developed for total sensory mark proved significant with p-value 0.0016. The "Lack of Fit F-value" of 0.58 implies that Lack of Fit is not significant relative to the pure error. Adequate precision of linear model for sensory characteristics, 9.608, indicates an adequate signal so this model can be used to navigate the design space. Sensory mark increases with the increase of temperature while it decreases with duration of fermentation process. Initial total sugar content did not influence total sensory mark.

In previously published paper [17], it was reported that on the basis of sensory mark, the consume day samples were products obtained after three days of fermentation. In this investigation, the more detailed insight into the sensory mark was given. The total sensory mark was higher (3) and (4) for products obtained after three days of fermentation at 30, 25 and 20 °C. These results indicated that regardless on the initial total sugars content and fermentation process temperature, kombucha products ready for consumption were produced after three days of the process. Traditional kombucha beverage is usually obtained after 7-14 days [1] and production of kombucha beverages on winery effluent leads to the significant reduction in the process duration and therefore to the economic saves, as well.

Optimization

Optimization of the operational parameters during kombucha fermentation was performed using desirability function approach. The optimization was aimed at maximization of total flavonoids and sensory mark. The method combines a number of responses into a single response called the desirability function. The selected responses are transformed to an individual desirability values in range from 0 to 1. The overall desirability of the process is computed as geometric mean of the individual desirability functions [20]. From the optimization results by the desirability function approach, it can be concluded that the optimal results in terms of selected goal were obtained at 30˚C with initial total sugars around 7% and fermentation period three days. The optimized values of independent variables would result in predicted values 44.98 µg RE/mL for total flavonoids and 3.6 for sensory mark. After the validation experiment, results are in good agreement with the optimized values given by the model i.e. total flavonoids content and sensory mark are 45.2 µg RE/mL and 4, respectively.

CONCLUSION

Total flavonoids content and sensory mark of winery effluent based kombucha was established. The highest flavonoids content was determined in the initial medium (99.4±2.1 μg RE/mL) and the kombucha fermentation process led to the decrease in its value. The highest content in consume day (three days) kombucha beverages amounted to 50.4±6.8 μg RE/mL and it was measured in the product obtained at 20 °C with 3% of initial total reducing sugars. The highest total sensory mark had consumed day kombucha beverages produced at 20 and 30 °C with 7, 5 and 3% of initial sugars, as well as the product obtained at 25 °C with 5% of sugars.

Results of statistical analysis by response surface methodology led to the conclusion that RSM is applicable for modeling of total flavonoids content in kombucha beverage. Therefore, they can be applied for quality evaluation in the production of kombucha beverages on winery effluent. The optimum production conditions for the kombucha beverages with the highest values of total flavonoids (44.98 μg RE/mL) and sensory mark (3.6) were: fermentation time of 3 days, initial total sugars content around 7% and temperature of 30˚C. Future research can be oriented towards determination of single flavonoids compounds, as well as other phenolic compounds.

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FIGURE CAPTION

Figure 1. Response surface plot representing the influence of initial total sugars and temperature on the total flavonoids content

Figure 2. Response surface plot representing the influence of time and temperature on the total flavonoids content

Figure 3. Response surface plot representing the influence of initial total sugars and time on the total flavonoids content

TABLE CAPTION

Table 1. Box-Behnken experimental design with three independent factors and the obtained results for total flavonoids content and sensory mark

Table 2. Coefficients of regression equation for total flavonoids content

Table 3. Coefficients of regression equation for total sensory mark

Table 1. Box-Behnken experimental design with three independent factors and the obtained results for total flavonoids content and sensory mark

Exp.	Factors – Independent variables			Responses – Dependent variables	
	Temperature [˚C]	Initial total sugars [%]	Time [days]	Total flavonoids [μg RE/mL]	Total sensory mark
1	20	3	6	37.2 (34.1)	2 (2)
2	20	5	3	28.6 (31.0)	4 (3)
3	20	5	9	27.0 (27.2)	2 (2)
4	20	7	6	36.1 (36.6)	2 (2)
5	25	3	3	23.2 (23.9)	3 (3)
6	25	3	9	21.7 (24.6)	2 (2)
7	25	7	9	38.8 (38.1)	2 (2)
8	25	7	3	44.3 (41.4)	3 (3)
9	30	3	6	15.9 (15.4)	3 (3)
10	30	5	3	22.9 (22.7)	4 (4)
11	30	5	9	26.4 (24.0)	2 (2)
12	30	7	6	40.8 (43.9)	3 (3)
13	25	5	6	24.1 (24.0)	2 (3)
14	25	5	6	23.0 (24.0)	2 (3)
15	25	5	6	24.9 (24.0)	3 (3)

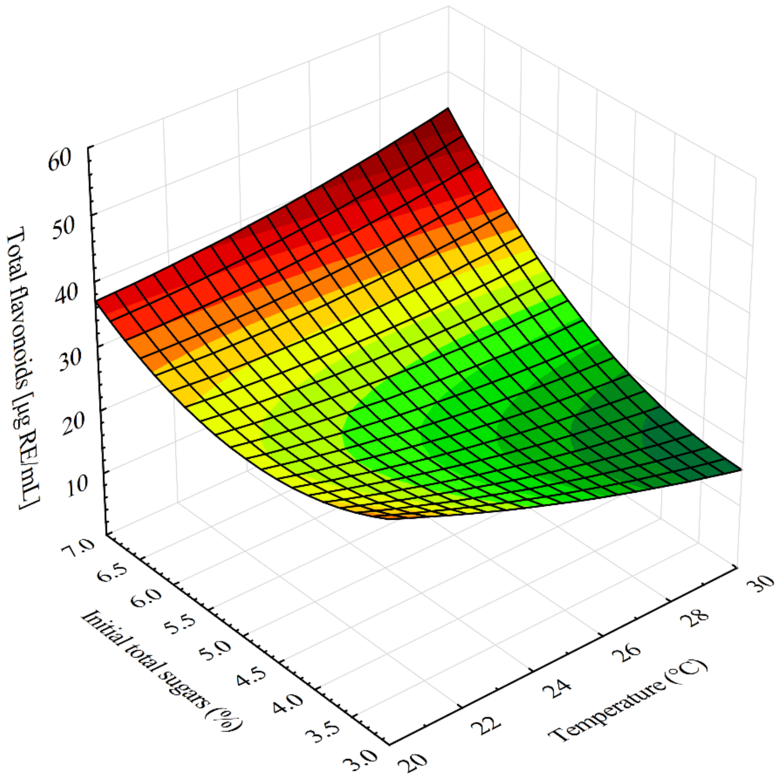
* values in parenthesis are predicted by selected mathematical model

Table 2. Coefficients of regression equation for total flavonoids content

Effects	Coefficient		p-value
	Actual	Coded	
Intercept			
b_0	191.3	24.00	<0.0001
Linear			
b_1	-7.057	-2.863	0.05205
b_2	-29.220	7.750	<0.0001
b_3	-2.654	-0.638	0.5964
Quadratic			
b_{11}	0.055	1.362	0.4492
b_{22}	1.784	7.137	0.0077
b_{33}	0.09583	0.863	0.6256
Interaction			
b_{12}	0.650	6.500	0.0096
b_{13}	0.085	1.275	0.4604
b_{23}	-0.167	-1.000	0.5583

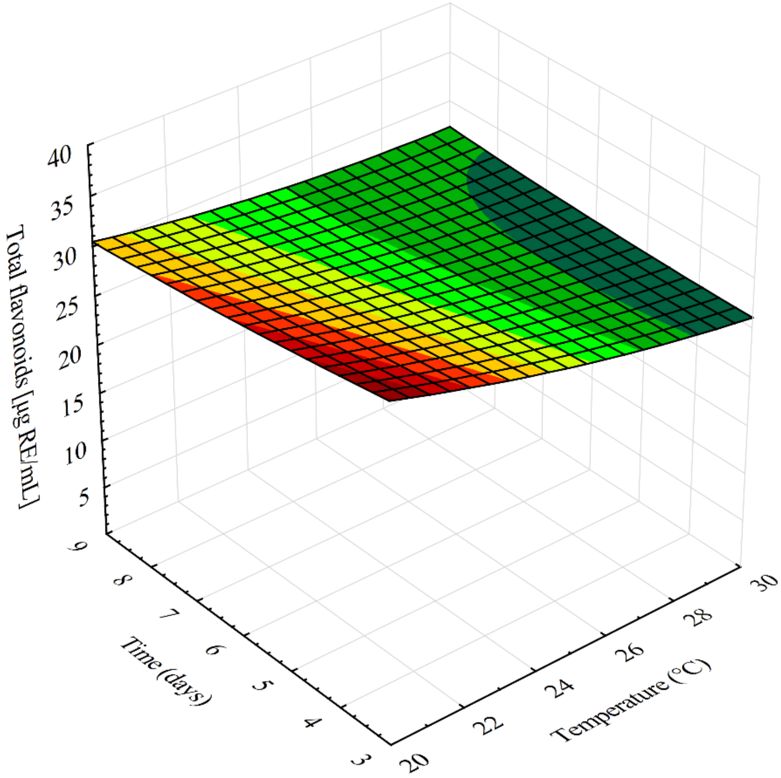
Table 3. Coefficients of regression equation for total sensory mark

Effects	Coefficient		p-value
	Actual	Coded	
Intercept			
b_0	2.8500	2.600	<0.0001
Linear			
b_1	0.0500	0.250	0.1546
b_3	-0.2500	-0.7500	<0.0001



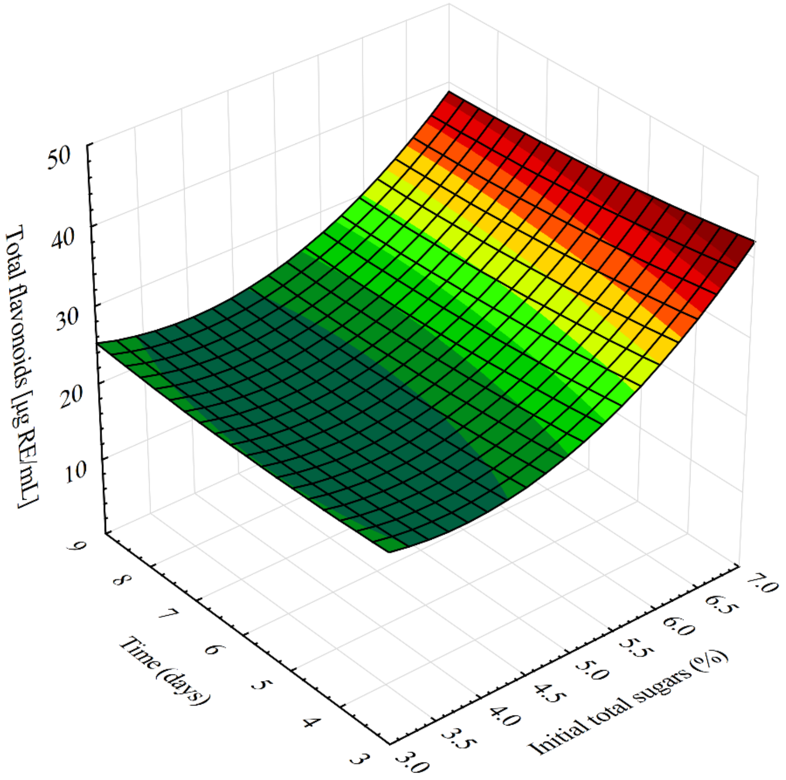
Total flavonoids [µg RE/mL]
60
50
40
30
20
10
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Initial total sugars (%)
20
22
24
26
28
30
Temperature (°C)

Figure 1



40
35
30
25
20
15
10
5
Total flavonoids [µg RE/mL]
9
8
7
6
5
4
3
Time (days)
20
22
24
26
28
30
Temperature (°C)

Figure 2



Total flavonoids [µg RE/mL]
50
40
30
20
10
9
8
7
6
5
4
3
Time (days)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
Initial total sugars (%)

Figure 3