SCIENTIFIC PAPER

INFLUENCE OF MAIN PRODUCTION VARIABLES TO NUTRITIONAL CHARACTERISTICS OF WINERY EFFLUENT KOMBUCHA

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Abstract

Determination of sugar, nitrogen and phosphorus consumption by kombucha culture is necessary in order to gain additional insight into the kombucha metabolic pathways, nutritional value of the produced beverages, and to set basis for optimising the conditions for large scale production.

Kombucha beverage was made using effluent obtained from grape must clarification phase of white wine production. The fermentation medium was prepared by diluting the sterilized initial medium with approximately 16% of total reducing sugars to 3, 5, and 7% of total reducing sugars. The duration of the fermentation was nine days at 20, 25, and 30 °C. Sugars, nitrogen and phosphorus content was measured using DNS, Kjeldahl and molybdenum blue method, respectively.

The highest sugars content was in the initial medium (16.34%) and the lowest was after nine days of fermentation at 30 °C with 3% initial sugars content (0.17%). Residual levels of sugars, nitrogen and phosphorus after three days were modelled using response surface methodology. The proposed mathematical models for sugars and nitrogen content showed an exceptional correlation with the experimentally obtained values.

With this study the insight into kombucha's consumption of basic nutrients, nutritional value of the obtained products and optimization of products composition was provided.

Keywords: kombucha; nutrient utilisation; sugars; nitrogen; phosphorus

Highlights

- Total sugars, nitrogen and phosphorus consumption rates were determined.
- Influence of temperature on sugar, nitrogen and phosphorus content was observed.
- Sugar was the most influential nutrient for kombucha winery effluent fermentation.

INTRODUCTION

Kombucha is a fermented beverage produced by a symbiotic culture of yeasts and bacteria (SCOBY) using medium rich in nutrients [1]. Traditional kombucha is made using black or green tea (1.5 g/L) decocts sweetened by sucrose (7% w/v) with a typical fermentation period of 7 days at 25 °C [2,3]. Tea is used as a nitrogen source [4], while sucrose is a source of carbon, being hydrolysed to glucose and fructose [5]. Symbiotic metabolic interactions between species in SCOBY influence kombucha's physical, chemical, biological and sensory properties [6]. Two phases can be observed during kombucha fermentation, sour liquid beverage and floating cellulose pellicle [7].

Health benefits associated with kombucha consumption have been attributed to the polyphenolic compounds, organic acids, vitamins, amino acids, antibiotics, and micronutrients synthetized during kombucha fermentation [8]. Kombucha beverage is nowadays available in many forms and flavours, which together with confirmation of its beneficial properties by many researchers contributed to its popularity and increased widespread availability [9]. Particularly in the United States, kombucha has been popularized by social media, highlighting its health benefits [10]. The recommended daily consumption of kombucha beverage is around 120 mL for healthy adults, according to the USA Centers for Disease Control and Prevention.

It is very well known that nutrients represent the substances present in food which determine biological activity, and are vital for the human body. They are categorized as carbohydrates, proteins, minerals, fats and vitamins. They are involved in building all parts (proteins), producing energy (carbohydrates) and keeping the good working order (minerals) of the human organism.

The carbon availability is an essential growth factor for any microbial culture. Previous study demonstrated that kombucha products can be successfully obtained by using winery effluent as an alternative substrate for the fermentation process [32]. For kombucha cultivated on winery effluent, the main carbon sources are simple sugars, i.e. glucose and fructose.

Although, at the very early stadium of the grape berry development there is a certain amount of sucrose, at later stadiums sucrose is almost completely hydrolysed to glucose and fructose, but that ratio is never 1:1. At the beginning, the glucose content is higher, and it drops during the harvest, whilst the fructose content is constant, so at the end, the grape contains negligible more fructose than glucose [11]. Scientific research established that the carbon source influences different metabolic pathways activation in kombucha culture microorganisms. For example, fructose and sucrose stimulate ethanol synthesis, while glucose has the opposite effect. Sucrose also enables the biosynthesis of lactic acid [12].

Nitrogen is an essential element for all forms of life and is the structural component of amino acids [13]. Only six members of *Acetobacteriacaeae* are able to fix nitrogen, among which *Gluconobacter kombuchae* sp. nov. was isolated from kombucha tea [14]. Several strains of *Komagataeibacter* spp.

express nitrogen-fixation with *K. hanseii* RG3 displaying ability to synthetize cellulose beside fixation of nitrogen [15]. The Recommended Dietary Allowance of nitrogen for adult both men and women is 800 mg/day of body weight [16].

Phosphorus is an essential mineral element found in rocks, soil, plants and animal tissues, while in aquatic systems phosphorus plays a critical role in the process of eutrophication, as the nutrient that limits the growth of phytoplankton. Phosphorus homeostasis in the body is controlled by hormonal and renal control systems [17,18]. It makes up from 0.65 to 1.1% of adult body, 85% of which is in bones and Recommended Dietary Allowance for both men and women older than 19 years is 700 mg/day [19]. Presence of carbon, phosphorus and nitrogen in kombucha beverage on winery effluent was also related to its very well-known presence in wastewater used for fermentation.

The aim of this article was to determine the basic nutrients consumption by kombucha winery effluent based culture in order to gain additional understanding of the kombucha metabolic pathways, nutritional composition of the beverage and to set basis for the optimal conditions for the kombucha on winery effluent production on larger scale.

MATERIALS AND METHODS

Kombucha starter culture

Traditional kombucha culture, which represented the basis for this investigation, consisted of two bacterial strains of the *Acetobacter* genera and five yeast strains (*Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp., and *Zygosaccharomyces* sp.) [20,21].

Kombucha starter culture used in this investigation for obtaining novel kombucha products, was produced by three passages of the traditional kombucha culture, on the winery effluent with 7% of total reducing sugars at 25 °C, during 6 days. Only the liquid part of the obtained kombucha starter culture was added in the amount of 10% (v/v) to the appropriate fermentation medium. Winery effluent was cultivated in the incubator in sterilized glass beakers covered with sterile cheesecloth.

Initial medium, fermentation medium and process parameters

The initial medium (IM) was filtrated and sterilized winery effluent generated after must flotation using gelatine, in the white wine production. The plate filter press and the filter paper were used for filtration. Sterilization was performed in an autoclave (121 °C, 20 min).

The initial medium contained 16.34% of total reducing sugars. The fermentation medium was prepared by the dilution of the initial medium with boiled tap water to three different sugars levels (3, 5, and 7% of total reducing sugars). These sugars levels referred to the initial total reducing sugars content.

The fermentation process was performed 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.

The initial medium and fermentation mediums were analysed as well.

Determination of total reducing sugars content

Total reducing sugars content was determined by the Miller method [22]. The reaction mixture consisted of 0.5 mL of the sample and 0.75 mL of the DNS reagent. After heating to the boiling point for 5 min, the mixture was cooled to room temperature and 3.75 mL of the distilled water was added. The sample reaction mixture was homogenized and the absorbance was measured at 540 nm (*LLG-uniSPEC2 Spectrophotometer,* Meckenheim, Germany). Blank sample used distilled water instead of the sample. The ripe grape contains almost equal amounts of glucose and fructose. Since both of these sugars react in the same way with the DNS reagent, glucose was selected for the preparation of the calibration curve [23,24]. Results were expressed as grams of glucose per litre of the sample.

Determination of total nitrogen content

Total nitrogen content was determined by the Kjeldahl method [25,26]. The appropriate volume of the sample (1 or 2 mL, depending on the sample) was transferred to the Kjeldahl flask, and 2 mL of concentrated sulphuric acid and 1 g of catalyst mixture were added. The obtained reaction mixture was heated until the solution changes colour to slightly green. The prepared sample was transferred to the distillation flask by using the small amount of distilled water. To the Erlenmeyer flask, 10 mL of 0.01 mol/L HCl and 3 drops of the mixed indicator were added. To the distillation flask, gradually, 33% sodium hydroxide was added until the alkaline reaction (the flask content is dark blue and all of the nitrogen is extruded in the form of ammonia). Distillation process was conducted for 6 minutes, in total. After that, the unreacted excess of HCl is retitrated with 0.01 mol/L of sodium hydroxide, in the presence of the mixed indicator, until the colour changes to green. Results were expressed as milligrams of nitrogen per litre of the sample.

Determination of total phosphorus content

Phosphorus was analysed by spectrophotometry using the molybdenum blue method [25]. Five mL of the samples were dried until dryness at 115 ^{o}C and allowed to cool to room temperature. Dry residue of each sample was digested using 5 mL of concentrated sulphuric acid and 15 drops of nitric acid. The obtained mixture was set on a hot plate until the clear or slightly green colour was obtained (after around 1 h). After cooling, digested samples were transferred to the volumetric flask and neutralized by adding several drops of 30% NaOH (1% solution of phenolphthalein was used for the indication), and finally diluted with doubly distilled water to 100 mL. Furthermore, certain volume of diluted sample (5, 10, 15, or 20 mL depending on the sample) was transferred to the 100 mL volumetric flask, and 5 mL 5% ammonium heptamolybdate, 1 mL 11% sodium sulfite and 1 mL 0.5% hydroquinone were added. After 45 min the absorbance was measured at 750 nm (UV-2100 Spectrophotometer,

Cole Parmer, Vernor Hills, IL, USA) and the results were expressed as mg/L. The calibration curve was defined with KH_2PO_4 from 0.05 to 0.4 mg P.

Statistical analysis

Statistical analysis and graphical representation of the data were performed using Statistica 13.5 software (StatSoft, USA). The coefficient of determination (R^2), model p-value (p = 0.05), and F-value were used to evaluate the adequacy of the model. Response Y was fitted by RSM using second-degree polyoma:

$$Y = b_0 + \sum b_i X_i + \sum {b_{ii}}^2 {X_{ii}}^2 + \sum b_{ij} X_i X_j \quad (1)$$

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 (20, 25, 30 °C) and X_2: initial total reducing sugars content (3, 5 and 7%). By sensory analysis in previous experiments it was determined that kombucha beverage after three days was suitable for consumption, therefore RSM modelling was performed only on those samples.

All analyses were performed in triplicate, and the data reported represent the average values of three determinations ± standard deviation (mean±StDev).

RESULTS AND DISCUSSION

Total reducing sugars content in kombucha products on winery effluent

The total reducing sugars content of kombucha products on winery effluent and the fermentation mediums were given in Table 1. It is very well known that the bacterial strains of *Acetobacter* genera, yeasts *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae* and *Zygosaccharomyces* use sugars as the main nutrient [37,38]. ~~Uninoculated~~ Fermentation medium, only for fermentations with 7% of initial total reducing sugars content, showed a slightly higher value than expected (7.57%), potentially because the homogenization was not good enough after the dilution of the sterilized effluent with the boiling tap water. Second homogenization was performed after the inoculation, when the value dropped by around 10%, in regards to the 7% of the initial content, which was in accordance with the expectations. During the entire course of the fermentation, sugars content decreased continually, but in different dynamics, depending on the initial content.

The most pronounced decrease was established after three days of fermentation, at 30 °C, when the initial sugars content was the lowest; from 2.9% on day 0 to just 0.4%. For the samples with 5% of initial total reducing sugars content, the decrease was over 40% during the first three days of the process. On the contrary, when the initial total reducing sugars content was the highest, the decrease was merely from 6.3 to 6.1%. This fact indicated the possibility of substrate inhibition. Therefore, it is more reasonable to use fermentation mediums with slightly lower reducing sugars content, because it

stimulates the bioprocess dynamics, lowers the initial costs and provides the beverage with lower sugars content, despite the fact that some authors successfully produced kombucha beverage with 10% of the initial sugar [27]. The differences in sugars metabolism can be also explained by the fact that winery effluent contains glucose and fructose as carbon source. Therefore, there is no need for the invertase activity by the kombucha culture in order to break sucrose to glucose and fructose, which are further metabolized by kombucha.

Table 1.

At the fermentation temperature of 25 °C, when the reducing sugars content was taken into account, the bioprocess dynamics were the most pronounced. After just three days of fermentation, the initial total sugars content of 6.3% was reduced to almost one third. The same pattern was established when the initial content was 2.9%. For the products with 4.7% of the initial content, the total residual reducing sugars content was decreased by over 50%. At the fermentation temperature of 20 °C, the kombucha culture showed similar activity: in the products with 3% of the initial sugars content, the residual sugars content decreased by almost 50%, for products with 5% of the initial sugars content, the residual sugars content was lowered for 40%. Products with 7% of the initial sugars content had the least pronounced decrease, which amounted to around one quarter.

After nine days of fermentation, at 25 and 30 °C, for products with 5 and 3% of the initial total reducing sugars content, the residual total reducing sugars content was below 1%.

The consume day kombucha products contained just 0.84 to 12.22 g/200 mL (i.e. a glass of the beverage), which was significantly lower than commercially available non-alcoholic beverages, that commonly contain over 20 g/200 mL of sugar [39].

Experiments by other authors, that obtained kombucha products with different herbal teas, indicated a significantly less pronounced lowering in total sugars content. From 10% at the start of the fermentation process, only the kombucha product with yerba mate, after 30 days, showed 50% lower content. The rest of the kombucha samples had sugars content that amounted to almost 75% of the initial value [28]. Kombucha beverage with seaweed (*Porphyra dentata*) produced at 25 °C, had mild increase in sugars content in comparison with the beginning of the process, until the 16th day, when the sugars started to decrease, but after 22 days it was still higher than at the fermentation start. At the fermentation temperature of 30 °C, with minor oscillations, the decrease was constant and after 20 days it amounted, to approximately, one fifth of the initial content [29]. Leonarski *et al.*, 2021 [30] demonstrated that kombucha microorganisms showed preference to glucose as carbon source, when acerola by-product, which contains same amounts of glucose and fructose, was used as the fermentation medium. The sugars consumption was directly proportional to acerola by-product content and different studies confirmed that yeasts species metabolize glucose over fructose. Products

with high sugar content can be applied in bacterial cellulose production [31], which is also generated as a result of kombucha metabolic activity [2].

Modelling of residual total reducing sugars content in kombucha beverages on winery effluent after three days of fermentation

Results measured for the residual total reducing sugars content after three days of kombucha fermentation were modelled by response surface methodology (Figure 1a). Results of the ANOVA analysis of variance, regression equation coefficients and their p-values were presented in Table 2.

Table 2.

The model was significant, at the confidence interval of 95%, with a F-value of 5890.123. The coefficient of determination was 0.999, which indicated the excellent reliability of the model (Table 2). Statistically significant influence on the model showed linear effects of the initial total reducing sugars content and fermentation temperature, as well as their interaction. Both were expected, since the residual total reducing sugars content had to be correlated with the initial, and the bioprocess temperature significantly influences the velocity of all metabolic functions. Higher initial total reducing sugars content, as expected, provided higher residual total reducing sugars content. The influence of temperature was not that clear (Table 2).

The lowest value of the residual total reducing sugars content had samples produced at 25 °C and the measured content was as follows: 1.1% for products with 3% of the initial sugars and almost equal values were established for samples with 7 and 5% of the initial total reducing sugars – 2.2 and 2.1%. The highest content showed products obtained at 30 °C (the highest value was 6.1% for samples with 7% of the initial sugars). This fact suggested that 7% of initial sugars and fermentation temperature of 30 °C were above the optimal conditions for the kombucha culture metabolic activity. Since the sugars amount in the beverage is highly important for the consumer, it can be concluded that a fermentation temperature of 25 °C is the most suitable. It is reasonable that the majority of the population wants to lower indiscriminate calories consumption, as well as the simple sugars calories that possess high glycaemic index.

Total nitrogen content in kombucha products on winery effluent

After the carbon, nitrogen is the most important macroelement, necessary for protein synthesis in all cells. Source of nitrogen greatly affects the production of bacterial cellulose by kombucha. Research has demonstrated that ~~with~~ yeast extract powder (freeze-dried) ~~being~~ was the most suitable, both in shaking and static conditions while yeast extract, beef extract, tryptone, or peptone caused low level of bacterial cellulose production [15]. It is very well known that yeasts, such as *Saccharomyces cerevisiae*, need nitrogen and phosphorus for their metabolic activity, beside carbon [38]. The total nitrogen content was given in Table 3. The initial medium contained 370.22 mg/L of total nitrogen.

Table 3.

Contrary to expectations, the total nitrogen content in fermentation mediums was inversely proportional to the total reducing sugars content, meaning that the dilution process caused the increase in the values. The addition of kombucha inoculum to fermentation mediums (day 0) showed no clear influence to nitrogen content, which remained the same for samples with 7% of initial sugars. For products with 5 and 3% of initial sugars the nitrogen values were lower than the ones measured in fermentation mediums and higher for samples with 5% of initial sugars. Values for day 0 products were in the range 106.68-144.33 mg/L. In consume day samples, the highest total nitrogen content showed samples with 7% of the initial total reducing sugars content, when the fermentation was performed at 30 and 20 °C, and it amounted 269.83 and 225.90 mg/L, respectively. At the 25 °C, the highest nitrogen content was measured for samples with 5% of initial total reducing sugars (288.65 mg/L).

During the fermentation, the total nitrogen content changed improperly, without the clear pattern in relation to the fermentation temperature or the initial total sugars content. Despite that, samples with 7% of initial total reducing sugars produced at 25 °C showed a linear increase in content during the fermentation. This trend and decrease in values, respectively, was established for products with 5 and 3% of initial total reducing sugars after six and three days of the process, with the decrease in fermentation temperature. The highest value was established for the kombucha product obtained at 25 °C that contained 5% of the initial total sugars content, after nine days of the process (480 mg/L), which was only sample with higher nitrogen content than initial medium. The lowest value was measured after six days of fermentation at 30 °C with 3% of initial total reducing sugars content (40.79 mg/L). Research conducted by [40] suggested that nitrogen content control was crucial for the metabolism of nitrogen by yeasts.

The increase in nitrogen content during fermentation compared to the initial fermentation medium is a consequence of the growth of microorganisms, bacteria and yeasts during cultivation, which is very intensive. This is confirmed by the previously published results, which indicate a much higher biomass growth of winery effluent kombucha compared to the traditional, ~~substrate with~~ black tea kombucha [32]. The above facts indicate that kombucha cultivated on winery effluent uses relatively small amounts of nitrogen for its growth and metabolism. Food waste can be a source of significant amount of nitrogen (2-15 g/L) and phosphorus (0.5-1 g/L) and therefore be used in value-added products production [33], such as kombucha. Also, microorganisms use nitrogen to produce different types of biofuels [33].

Modelling of residual total nitrogen content in kombucha beverages on winery effluent after three days of fermentation

Results of the ANOVA analysis of variance, regression equation coefficients and their p-values were presented in Table 2. Results measured for the residual total nitrogen content after three days of kombucha fermentation were modelled by response surface methodology (Figure 1b). The model showed excellent reliability and it can be regarded that it had completely described the system, at a confidence interval of 95% and a very high F-value (1483.773) (Table 2).

Statistically significant parameters of the model were just the quadratic effects of both process parameters. The fermentation temperature had more influence (Table 2). From the graphical representation of the model, it can be observed that the highest total nitrogen content was predicted at the fermentation temperature of 25 °C. This can be explained by the fact that the biomass production, determined in previous study, was lower at that temperature and the amount of nitrogen that is in cellulosic pellicle was lower [32]. Therefore, the higher nitrogen content is in the kombucha beverage.

Total phosphorus content in kombucha products on winery effluent

Phosphorus is naturally occurring in many different foods; mostly in the form of phosphates and phosphate esters [34]. The total phosphorus content (Table 4) of 250.00 mg/L was determined in the initial medium. The dilution of the initial medium correlated with the phosphorus values measured for the fermentation mediums. The addition of kombucha inoculum to fermentation mediums caused increase in phosphorus values, and with lowering of the initial sugars, the phosphorus values were higher. This fact is an indication of phosphorus presence in the added kombucha inoculum, which could be related to the degradation of ATP and/or the increase in number of microorganisms. The range of all other samples was between 80.33 mg/L after 9 days at 30 °C with 3% initial total reducing sugars content to 262.41 mg/L also after 9 days at 30 °C, but with 7% initial total reducing sugars content. Samples with 5 and 7% total initial sugars content had higher phosphorus content than in the fermentation medium, after nine days, when fermentation temperature was 25 and 30 °C, while for 3% of initial total reducing sugars phosphorus content was higher only at 25 °C. All kombucha products with 7% of initial sugars obtained at 30 °C had higher phosphorus content than samples from the fermentation start (day O). This fact was established for samples produced at 25 °C after 6 and 9 days, as well. The products obtained at 20 °C had lower content than fermentation start products. Samples with 5 and 3% of initial sugars after 6 days of the process at 20 °C, as well as the product with 3% of the initial sugars after 9 days at 25 °C showed also higher phosphorus content than the products from the beginning of the process.

Other authors measured 6.637 mg/L of phosphorus at the beginning of the fermentation (day 0) of kombucha using black tea and cane sugar, which reduced slightly, to 6.448 mg/L after 14 days. Green tea kombucha contained less phosphorus, 4.201 mg/L at the beginning and 5.332 mg/L after 14 days of fermentation. The highest content was determined in the white tea kombucha, 8.865 mg/L at the beginning (day 0) and 10.31 mg/L after 14 days of fermentation [35].

Table 4.

During the fermentation, the total phosphorus content, as nitrogen, changed improperly, without the clear pattern in relation to the fermentation temperature or the initial total sugars content. Samples produced at 25 °C with 5% of initial sugars showed linear decrease in values during the fermentation time, whilst products with 3% of initial sugars had increase in values during the process. After three days of fermentation the increase in fermentation temperature caused the increase in phosphorus content for beverages with 5% of initial sugars. This trend was also established for products with 7% of initial sugars after six and nine days of fermentation. For products with 3% of initial sugars, after six days of the process, the increase in temperature caused the lowering of phosphorus content.

Relatively high phosphorus contents during fermentation indicate a similarity with nitrogen as a nutrient, where it can also generally be considered that the phosphorus content required for kombucha activity is relatively low. Phosphorus was determined in green crop fractions and it was established that juice, deproteinised juice and leaf protein concentrate contain phosphorus in low amounts. Pressed crop showed the highest values. Distribution of phosphorus was not uniformed [36].

Modelling of residual total phosphorus content in kombucha beverages on winery effluent after three days of fermentation

Results of ANOVA analysis of variance, regression equation coefficients and their p-values were presented in Table 2. Results measured for the residual total phosphorus content after three days of kombucha fermentation were modelled by response surface methodology (Figure 1c). The model did not show reliability, explaining only around two third of modelled values with a F-value of 8.89 (Table 2).

Statistically significant parameters were quadratic effect of initial total reducing sugars content and the interaction between it and fermentation temperature (Table 2). From the graphical display of model (Figure 1c), it can be concluded that samples with 7 and 3% initial total sugars had lower phosphorus content which indicates that at those conditions phosphorus consumption rate was higher. Contrary to that, higher temperature meant lower phosphorus intake with higher values measured at higher temperatures, for all three sugars contents.

Fig. 1

CONCLUSION

Sugars content was changing through the fermentation according to the expectation, i.e. to the pattern established for the traditional kombucha fermentation. After three days of the process, temperature did not show significant effect, although samples fermented at 25 °C had the lowest residual total reducing sugars content regardless of initial total reducing sugars content, compared to those fermented at 20 and 30 °C.

Up to our knowledge, this is the first extensive measurement of nitrogen and phosphorus contents in kombucha products on an alternative medium, through fermentation. Changes were inconsistent.

Based on the obtained results, this study suggested that the most significant nutrient for kombucha culture activity on winery effluent was carbon, i. e. the sugars consumption. The comprehensive nitrogen and phosphorus analysis indicated that these elements play a minor role in kombucha metabolism of winery effluent.

Further studies are necessary to elucidate which microorganisms utilize examined nutrients and in what amount.

By the determined contents of carbon, nitrogen and phosphorus in winery effluent based kombucha beverages, it can be concluded that these products could represent a part of a daily well balanced nutrition of healthy adults.

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FIGURE CAPTION

Figure 1. Response surface that describes the influence of fermentation temperature and initial total reducing sugars content on the a) residual total reducing sugars content in kombucha beverages on winery effluent after three days of fermentation, b) residual total nitrogen content in kombucha beverages on winery effluent after three days of fermentation, c) residual total phosphorus content in kombucha beverages on winery effluent after three days of fermentation

Table 1. Total reducing sugars content of kombucha products on winery effluent and the fermentation mediums

Total reducing sugars content (g/L)[a]				
		initial total reducing sugars content (%)		
t (°C)	**τ (day)**	**7**	**5**	**3**
fermentation medium		75.73±0.31	50.23±0.56	29.89±0.41
day 0		63.08±0.62	46.93±0.56	29.17±0.06
30	3	61.09±0.37	28.43±0.50	4.19±0.08
	6	33.47±0.12	11.73±0.04	2.07±0.05
	9	12.39±0.05	9.37±0.02	1.68±0.01
25	3	22.29±0.34	21.17±0.41	10.82±0.34
	6	14.28±0.06	8.10±0.01	3.82±0.02
	9	10.35±0.10	4.05±0.07	3.24±0.03
20	3	54.07±0.65	30.11±0.62	16.80±0.16
	6	45.12±0.12	28.71±0.34	13.14±0.23
	9	36.31±0.78	15.29±0.55	5.99±0.14
[a]mean±StDev, n=3				

Table 2. Analysis of variance (ANOVA), regression equation coefficients and their p-values of the modelled response of the residual total reducing sugars, residual total nitrogen and residual total phosphorus content in kombucha beverages on winery effluent after three days of fermentation

Response	**Residual**			**Model**			**F- value**	***p*-value**	**R^2**
	DF	SS	MS	DF	SS	MS			
Residual total sugars content	18	3.230	0.181	8	8534.342	1066.793	5890.123	0.000	0.999
Residual total nitrogen content	8	162.424	9.024	8	107111	13388	1483.773	0.000	0.998
Residual total phosphorus content	24	26405.91	1100.246	5	48891	9778	8.89	0.00007	0.649

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

Effects		Intercept	linear		quadratic		interaction
		b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
Residual total sugars content	estimate	409.510	-31.405	0.574	-0.757	0.004	0.049
	p-value	0.000[a]	0.000[a]	0.000[a]	0.466838	0.626327	0.049[a]
Residual total nitrogen content	estimate	-381.14	5.987	0.063	17.243	-0.135	-0.086
	p-value	0.523	0.894	0.943	0.018[a]	0.022[a]	0.584
Residual total phosphorus content	estimate	56.372	-7.531	0.121	3.488	-0.083[a]	0.207[a]
	p-value	0.875	0.778	0.817	0.397	0.017[a]	0.035[a]

[a]Effects are statistically significant, $p < 0.05$; 1 – temperature; 2 – initial sugars content

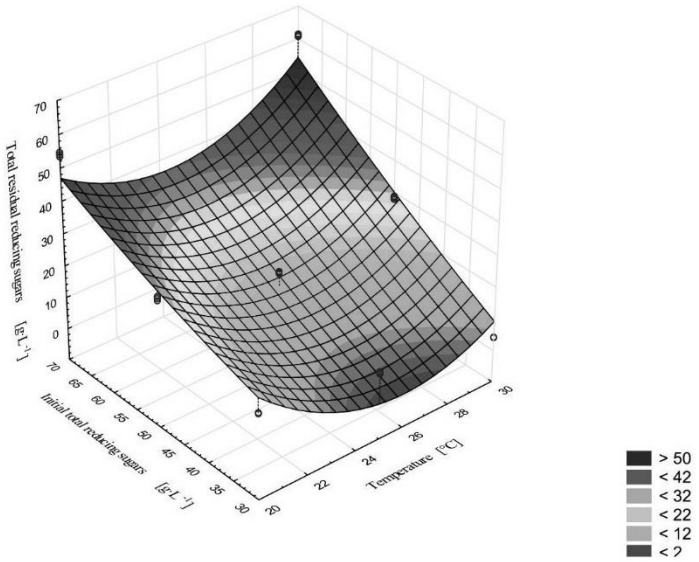
Table 3. Total nitrogen content of kombucha products on winery effluent and the fermentation mediums

Total nitrogen content (mg/L)[a]				
		initial total reducing sugars content (%)		
t (°C)	**τ (day)**	**7**	**5**	3
fermentation medium		106.68±3.14	166.29±1.57	261.98±1.57
day 0		106.68±0.00	144.33±3.14	109.81±3.14
30	3	269.83±3.14	203.94±3.14	166.29±0.00
	6	294.93±3.14	156.88±0.00	40.79±1.57
	9	288.65±0.00	200.80±0.00	47.06±1.57
25	3	109.81±3.14	288.65±0.00	164.72±4.71
	6	149.03±1.57	181.98±1.57	100.40±6.28
	9	194.53±1.57	480.04±3.14	323.16±1.57
20	3	225.90±3.14	181.98±3.14	87.85±3.14
	6	247.86±3.14	214.92±3.14	47.06±3.14
	9	228.25±0.78	181.98±1.57	144.33±3.14
[a]mean±StDev, n=3				

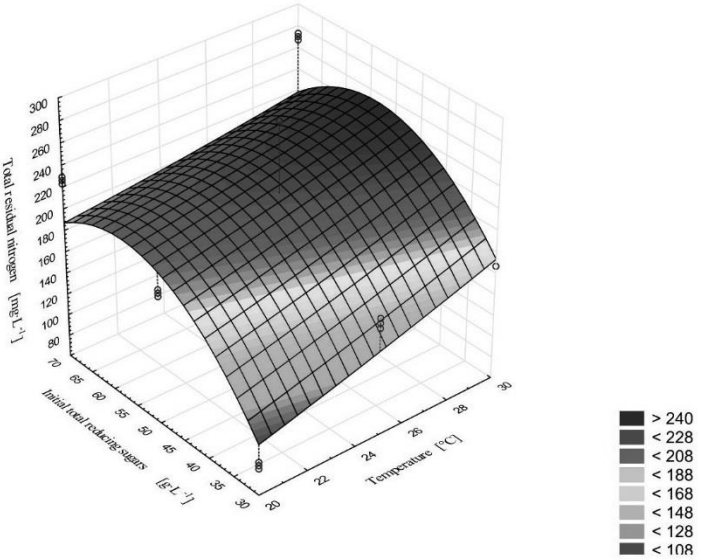
Table 4. Total phosphorus content of kombucha products on winery effluent and the fermentation mediums

Total phosphorus content (mg/L)[a]				
		initial total reducing sugars content (%)		
t (°C)	**τ (day)**	**7**	**5**	**3**
fermentation medium		159.28±13.61	148.50±12.32	145.39±0.35
day 0		176.56±14.17	202.48±18.07	218.84±16.88
30	3	242.29±25.56	199.70±21.54	144.80±17.12
	6	230.87±13.47	202.17±13.84	149.89±8.18
	9	262.41±7.12	158.23±9.13	80.33±1.44
25	3	85.58±1.99	195.24±11.12	152.67±9.85
	6	222.57±12.75	183.22±13.84	205.91±9.89
	9	213.86±9.93	172.11±4.98	232.76±23.43
20	3	108.52±2.81	121.19±16.92	90.31±3.45
	6	166.10±11.54	247.57±12.10	253.12±6.60
	9	156.84±17.92	118.88±12.45	128.14±12.52
[a]mean±StDev, n=3				

a)


Total residual reducing sugars [g·L⁻¹]
Initial total reducing sugars [g·L⁻¹]
Temperature [°C]
> 50
< 42
< 32
< 22
< 12
< 2

b)


Total residual nitrogen [mg·L⁻¹]
Initial total reducing sugars [g·L⁻¹]
Temperature [°C]
> 240
< 228
< 208
< 188
< 168
< 148
< 128
< 108

c)

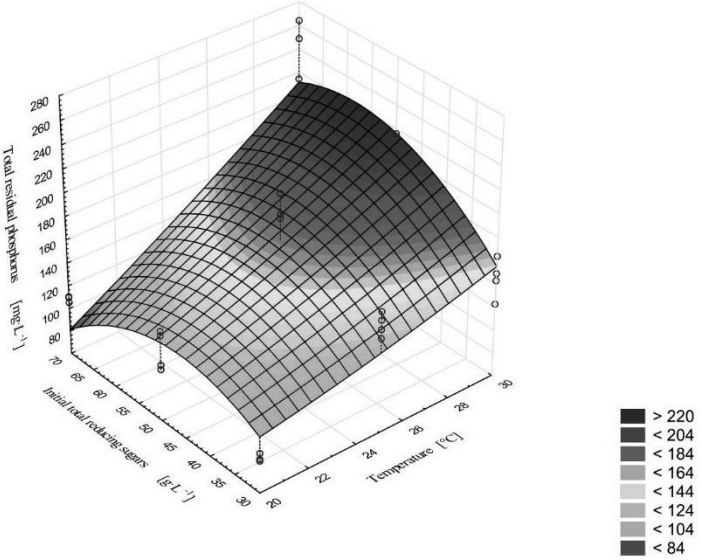

Total residual phosphorus [mg·L⁻¹]
Initial total reducing sugars [g·L⁻¹]
Temperature [°C]
> 220
< 204
< 184
< 164
< 144
< 124
< 104
< 84

Fig. 1