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SCIENTIFIC PAPER

UDC 582.282.23:663.2

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

Article Highlights

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

Abstract

Determination of sugar, nitrogen, and phosphorus consumption by kombucha culture is necessary to gain additional insight into the kombucha metabolic pathways and nutritional value of the produced beverages and to set a basis for optimizing 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 were measured using DNS, Kjeldahl, and molybdenum blue methods, respectively. The highest sugar content was in the initial medium (16.34%) and the lowest was after nine days of fermentation at 30 °C with 3% initial sugar content (0.17%). Residual levels of sugars, nitrogen, and phosphorus after three days were modeled 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 insight into kombucha's consumption of basic nutrients, nutritional value of the obtained products, and optimization of product composition was provided.

Keywords: kombucha, nutrient utilisation, sugars, nitrogen, phosphorus.

Kombucha is a fermented beverage produced by a symbiotic culture of yeasts and bacteria (SCOBY) using a 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 hydrolyzed 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 synthesized during kombucha fermentation [8]. Kombucha beverage is nowadays available in many forms and flavors, which

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Paper received: 2 October, 2023

Paper revised: 25 December, 2023

Paper accepted: 16 January, 2024

<https://doi.org/10.2298/CICEQ231002001V>

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 beverages 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 that 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 studies 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 hydrolyzed 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 *Acetobacteriaceae* can 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 the ability to synthesize cellulose besides the fixation of nitrogen [15]. The Recommended Dietary Allowance of nitrogen for adults 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 an adult body, 85% of which is in bones and the Recommended Dietary Allowance for both men and women older than 19 years is 700 mg/day [19]. The presence of carbon, phosphorus, and nitrogen in kombucha beverages on winery effluent was also related to its very well-known presence in wastewater used for fermentation.

This article aimed to determine the basic nutrients consumed by kombucha winery effluent-based culture to gain an additional understanding of the kombucha metabolic pathways and nutritional composition of the beverage and to set the basis for the optimal conditions for the kombucha on winery effluent production on a 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 (*Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp., and *Zygosaccharomyces* sp.) [20,21].

The 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 sugar levels (3, 5, and 7% of total reducing sugars). These sugar levels referred to the initial total reducing sugar 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 analyzed as well.

Determination of total reducing sugar content

Total reducing sugar 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). The 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 liter 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 changed color to slightly green. The prepared sample was transferred to the distillation flask by using a 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). The 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 color changes to green. Results were expressed as milligrams of nitrogen per liter of the sample.

Determination of total phosphorus content

Phosphorus was analyzed by spectrophotometry using the molybdenum blue method [25]. Five mL of the samples were dried until dryness at 115 °C and allowed to cool to room temperature. The 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 a clear or slightly green color 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, a 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, Vernon Hills, IL, USA) and the results were expressed as mg/L. The calibration curve was defined with KH₂PO₄ 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:

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

where b_0 is the intercept, b_i , b_{ii} , and b_{ij} are the linear, quadratic, and interaction effects of the factors, respectively. The factor variables were the fermentation temperature, X_1 : 20, 25, and 30 °C, and the initial total reducing sugar content, X_2 : 3, 5, and 7%). As determined previously by sensory analysis, the kombucha beverage after three days was suitable for consumption, therefore RSM modeling was performed only on those samples.

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

RESULTS AND DISCUSSION

Total reducing sugar content in kombucha products on winery effluent

The total reducing sugar content of kombucha products on winery effluent and the fermentation mediums are given in Table 1. It is very well known that the bacterial strains of *Acetobacter* genera, yeasts *Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, and *Zygosaccharomyces* use sugars as the main nutrients [37,38]. Fermentation medium, only for fermentations with 7% of initial total reducing sugar

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 agreed with the expectation. During the entire course of the fermentation, sugar 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 sugar content was the lowest; from 2.9% on day 0 to just 0.4%. For the samples with 5% of initial total reducing sugar content, the decrease was over 40% during the first three days of the process. On the contrary, when the initial total reducing sugar 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 a slightly lower reducing sugar content, because it stimulates the bioprocess dynamics, lowers the initial costs, and provides the beverage with lower sugar content, although some authors successfully produced kombucha beverage with 10% of the initial sugar [27]. The differences in sugar metabolism can be also explained by the fact that winery effluent contains glucose and fructose as carbon sources. Therefore, there is no need for the invertase activity by the kombucha culture to break sucrose into glucose and fructose, which are further metabolized by kombucha.

At the fermentation temperature of 25 °C, when the reducing sugar content was taken into account, the bioprocess dynamics were the most pronounced. After just three days of fermentation, the initial total sugar

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

| t (°C) | τ (day) | Total reducing sugar content (g/L) ^a | | |
|---------------------|---------|-------------------------------------------------|------------|------------|
| | | Initial total reducing sugar content (%) | | |
| | | 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 |

^amean±StDev, n=3.

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 sugar 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 sugar content, the residual sugar content decreased by almost 50%, for products with 5% of the initial sugar content, the residual sugar content was lowered for 40%. Products with 7% of the initial sugar 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 sugar content, the residual total reducing sugar content was below 1%.

The consume day kombucha products contained 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, which 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 sugar 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 sugar content that amounted to almost 75% of the initial value [28]. Kombucha beverage with seaweed (*Porphyra dentata*) produced at 25 °C, had a mild increase in sugar 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, a fifth of the initial content [29]. Leonarski *et al.* [30] demonstrated that kombucha microorganisms showed a preference for glucose as a carbon source, when acerola by-product, which contains the same amounts of glucose and fructose, was used as the fermentation medium. The sugar 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 sugar content in kombucha beverages on winery effluent after three days of fermentation

Results measured for the residual total reducing sugar content after three days of kombucha fermentation were modeled 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.

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

Table 2. Analysis of variance (ANOVA), regression equation coefficients, and their *p*-values of the modeled 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 | <i>p</i> -value | R ² |
|-----------------------------------|-----------------|--------------------|--------------------|--------------------|--------------------|---------------------|--------------------|-----------------|----------------|
| | DF | SS | MS | DF | SS | MS | | | |
| Residual total sugar content | 18 | 3.230 | 0.181 | 8 | 8534 | 1067 | 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 | 26405.910 | 1100.246 | 5 | 48891 | 9778 | 8.890 | 0.000 | 0.649 |
| Effects | Intercept | | Linear | | Quadratic | | Interaction | | |
| | estimate | b ₀ | b ₁ | b ₂ | b ₁₁ | b ₂₂ | b ₁₂ | | |
| Residual total sugar content | estimate | 409.510 | -31.405 | 0.574 | -0.757 | 0.004 | 0.049 | | |
| | <i>p</i> -value | 0.000 ^a | 0.000 ^a | 0.000 ^a | 0.467 | 0.626 | 0.049 ^a | | |
| Residual total nitrogen content | estimate | -381.140 | 5.987 | 0.063 | 17.243 | -0.135 | -0.086 | | |
| | <i>p</i> -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 | | |
| | <i>p</i> -value | 0.875 | 0.778 | 0.817 | 0.397 | 0.017 ^a | 0.035 ^a | | |

DF - degree of freedom, SS - sum of squares, MS - mean squares. ^aEffects are statistically significant, *p* < 0.05; 1 - temperature; 2 - initial sugar content.

linear effects of the initial total reducing sugar content and fermentation temperature, as well as their interaction. Both were expected, since the residual total reducing sugar content had to be correlated with the initial, and the bioprocess temperature significantly influences the velocity of all metabolic functions. Higher initial total reducing sugar content, as expected, provided higher residual total reducing sugar content. The influence of temperature was not that clear (Table 2).

The lowest value of the residual total reducing sugar 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 sugar 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 calorie consumption, as well as the simple sugar calories that possess a 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. The source of nitrogen greatly affects the production of bacterial cellulose by kombucha. Research has demonstrated that yeast extract powder (freeze-dried) was the most suitable, both in shaking and static conditions while yeast extract, beef extract, tryptone, or peptone caused low levels of bacterial

cellulose production [15]. It is very well known that yeasts, such as *Saccharomyces cerevisiae*, need nitrogen and phosphorus for their metabolic activity, besides carbon [38]. The total nitrogen content is given in Table 3. The initial medium contained 370.2 mg/L of total nitrogen.

Contrary to expectations, the total nitrogen content in fermentation mediums was inversely proportional to the total reducing sugar 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 on nitrogen content, which remained the same for samples with 7% of initial sugars. For products with 5 and 3% initial sugar, the nitrogen values were lower than the ones measured in fermentation mediums and higher for samples with 5% initial sugar. 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%

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

| t (°C) | τ (day) | Total nitrogen content (mg/L) ^a | | |
|---------------------|---------|--------------------------------------------|-------------|-------------|
| | | initial total reducing sugar content (%) | | |
| | | 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 |

^amean±StDev, n=3.

of the initial total reducing sugar content, when the fermentation was performed at 30 and 20 °C, and it amounted to 269.8 mg/L and 225.9 mg/L, respectively. At 25 °C, the highest nitrogen content was measured for samples with 5% of initial total reducing sugars (288.7 mg/L).

During the fermentation, the total nitrogen content changed improperly, without a clear pattern concerning the fermentation temperature or the initial total sugar 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, were 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 sugar content, after nine days of the process (480 mg/L), which was the only sample with higher nitrogen content than the initial medium. The lowest value was measured after six days of fermentation at 30 °C with 3% of the initial total reducing sugar content (40.8 mg/L). Mendes-Ferreira *et al.* [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, 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 amounts 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 modeled by response

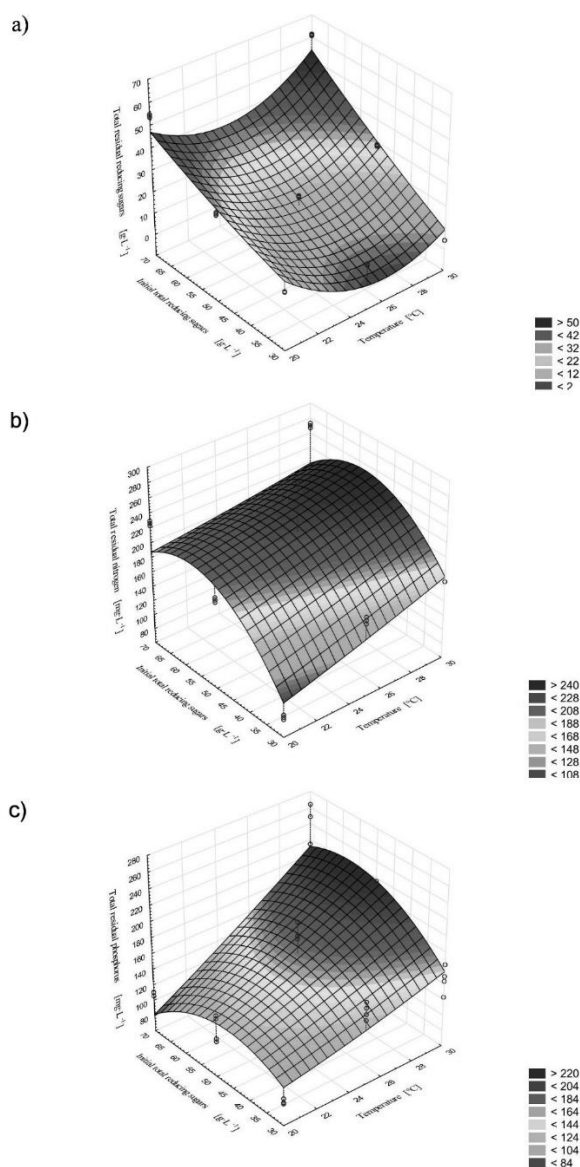


Figure 1. Response surface that describes the influence of fermentation temperature and initial total reducing sugar content on the (a) residual total reducing sugar content in kombucha beverages on winery effluent after three days of fermentation, (b) residual total nitrogen content in kombucha beverages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

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 a 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 250.0 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 an increase in phosphorus values, and with the 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 the 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 sugar content to 262.41 mg/L also after 9 days at 30 °C, but with 7% initial total reducing sugar content. Samples with 5 and 7% total initial sugar 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 0). 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].

During the fermentation, the total phosphorus content, as nitrogen, changed improperly, without a clear pattern in relation to the fermentation temperature or the initial total sugar content. Samples produced at 25 °C with 5% of initial sugars showed linear decrease

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

| t (°C) | τ (day) | Total phosphorus content (mg/L) ^a | | |
|---------------------|---------|----------------------------------------------|--------------|--------------|
| | | Initial total reducing sugar content (%) | | |
| | | 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 |

^amean±StDev, n=3.

in values during the fermentation time, whilst products with 3% of initial sugars had an increase in values during the process. After three days of fermentation, the increase in fermentation temperature caused an 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 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, deproteinized juice, and leaf protein concentrate contain phosphorus in low amounts. The pressed crop showed the highest values. The distribution of phosphorus was not uniform [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 modeled by response surface methodology (Figure 1c). The model did not show reliability, explaining only around two-thirds of modeled values with an *F*-value of 8.89 (Table 2).

Statistically significant parameters were the quadratic effect of the initial total reducing sugar content and the interaction between it and fermentation temperature (Table 2). From the graphical display of the 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 sugar contents.

CONCLUSION

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

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. sugar consumption. The comprehensive nitrogen and phosphorus analysis indicated that these elements play a minor role in the 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 the daily well-balanced nutrition of healthy adults.

ACKNOWLEDGMENT

This work was supported by the Ministry of

Science, Technological Development and Innovations, Republic of Serbia (Grant number 451-03-65/2024-03/200134.).

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UTICAJ GLAVNIH PROIZVODNIH PROMENLJIVIH NA NUTRITIVNE KARAKTERISTIKE KOMBUHE NA OTPADNOM TOKU IZ PROIZVODNJE VINA

Određivanje potrošnje šećera, azota i fosfora od strane kulture kombuhe je potrebno da bi se dobio dodatni uvid u metaboličke puteve kombuhe i nutritivnu vrednost proizvedenih napitaka kao i da bi se postavila osnova za optimizaciju uslova proizvodnje na industrijskom nivou. Kombuha napitak je dobijen korišćenjem otpadnog toka koji nastaje u fazi bistenja šire grožđa u proizvodnji belog vina. Fermentacioni medijum je pripremljen razblaživanjem sterilizovanog početnog medijuma, koji je imao oko 16% ukupnih redukujućih šećera, na sadržaj ukupnih redukujućih šećera koji je iznosio 3, 5 i 7%. Vreme trajanja fermentacije je bilo devet dana na temperaturi od 20, 25 i 30 °C. Sadržaj šećera, azota i fosfora je određen primenom DNS, Kjeldal i molibden plavo metode, redom. Najveći sadržaj šećera je izmeren u početnom medijumu (16.34%), a najmanji je određen u uzorku nakon devet dana fermentacije na 30 °C sa 3% početnog sadržaja šećera (0.17%). Preostali nivoi šećera, azota i fosfora u uzorcima nakon tri dana fermentacije su modelovani primenom metode odzivne površine. Predloženi modeli za sadržaj šećera i azota su pokazali izuzetnu korelaciju sa eksperimentalno dobijenim vrednostima. Ovim radom je obezbeđeno dobijanje uvida u potrošnju osnovnih nutrijenata od strane kulture kombuhe, nutritivnu vrednost i optimizaciju sastava kombuha proizvoda.

Ključne reči: kombuha, iskorišćenje hranljivih materija, šećeri, azot, fosfor.

NAUČNI RAD