Effect of fermentation temperature in kombucha production on the concentration of organic acids, residual sugars and ethanol

GABRIELA KRÁTKÁ – NATÁLIE ALFERYOVÁ – IVETA HORSÁKOVÁ – EVA ŠVIRÁKOVÁ – RUDOLF ŠEVČÍK

Summary
Kombucha is a traditional beverage prepared by fermenting tea using a symbiotic culture of bacteria and yeasts. In this experimental work the concentration of lactic and acetic acid (analysed by isotachophoresis), saccharose, glucose and fructose (analysed by high-performance liquid chromatography-refractive index detector) and ethanol (analysed by solid-phase microextraction followed by gas chromatography-mass spectrometry) were determined in kombucha samples fermented at 20 °C, 25 °C and 30 °C. Active acidity (pH) was measured during fermentation, and after 7 days, the pH values of all samples ranged between 2.99 and 3.01. Lactic acid bacteria (LAB) and yeast counts were determined on day 0 and day 7. LAB counts remained the same (10^6 CFU·ml^{-1}) in all samples, whereas the yeast counts increased from 10^4 CFU·ml^{-1} (day 0) to 10^6 CFU·ml^{-1} (day 7) in all samples. The highest concentration of lactic acid (129.4 ± 6.5 mg·l^{-1}) and ethanol (21.6 ± 2.7 ml·l^{-1}), and the lowest concentration of sugars (11.9 ± 1.2 g·l^{-1}) were detected in the samples fermented at 25 °C. On the contrary, the sharpest drop in pH, the lowest concentration of lactic acid (36.9 ± 1.8 mg·l^{-1}) and ethanol (1.72 ± 0.01 mg·l^{-1}), and the highest concentration of acetic acid (17.7 ± 0.9 g·l^{-1}) were detected in the samples fermented at 30 °C.

Keywords
kombucha production; fermentation temperature; nutritional compounds; functional beverage; symbiotic culture

According to the Merriam-Webster dictionary [1] 'kombucha' is defined as 'fermented beverage prepared by fermenting black or green tea with sugar' and 'symbiotic culture of bacteria and yeast (SCOBY)' as 'a gelatinous mass of symbiotic bacteria (such as *Acetobacter xylinum*, according to the new taxonomy as *Komagataeibacter xylinus* [2]) and yeasts (such as the *Brettanomyces* and *Saccharomyces* genera) grown to produce a fermented beverage held to confer health benefits' [3]. To date, numerous review studies have been published that define kombucha in various ways. However, these definitions share several common characteristics, including the fact that it is a fermented drink made from tea and a sugar source using a SCOBY. Its typical features include acidity, fizziness and a slightly sweet taste [3].

The first step in making kombucha is to prepare the sweetened tea brew. Typically, black tea, green tea or a combination of the two is used. A cellulose biofilm containing the SCOBY culture, referred to as 'tea fungus' [4–6], is added to the sweetened tea after it cools to room temperature. This is followed by a fermentation process that usually takes 7–14 days. The final products are the fermented beverage (which is consumed) and the cellulosic biofilm (referred to as *Medusomyces gisevii*) that is not consumed but can be used to ferment a new batch of kombucha [5, 7, 8].

The SCOBY typically consists of acetic acid bacteria (such as *Komagataeibacter xylinus*, *K. rhaeticus, K. saccharivorans, K. intermedius*, and *K. kombuchae, Gluconobacter* spp., *Acetobacter* spp. and *Tanticharoenia* spp.), lactic acid bacteria (including *Lactobacillus* spp., *Lactococcus* spp. and *Oenococcus* spp.) and osmophilic yeasts (such as *Zygosaccharomyces bailii*, *Z. kombuchaensis, Torulaspora* spp., *Kluyveromyces* spp., *Dekkera* spp., *Pichia* spp., *Schizosaccharomyces pombe, Brettanomyces bruxellensis, B. anomalus,*

Gabriela Krátká, Natálie Alferyová, Iveta Horsáková, Eva Šviráková, Rudolf Ševčík, Department of Food Preservation, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 16000 Prague 6, Czech Republic.

Correspondence author:
Gabriela Krátká, e-mail: gabriela.kratka@vscht.cz

© 2025 National Agricultural and Food Centre (Slovakia)

Hanseniaspora valbyensis, H. vineae and *Saccharomyces cerevisiae*) [3, 9–11]. The variability of microorganisms present in the 'mother' culture leads to fluctuations in the nutritional and bioactive compound concentration of kombucha. The chemical composition of kombucha is highly diverse, containing an array of bioactive and nutritionally valuable compounds, primarily of microbial origin [9, 11, 12]. Key constituents include organic acids (such as acetic acid and lactic acid), vitamins (including the B vitamin group and vitamin C), amino acids, enzymes, ethanol and polyphenols, as well as carbon dioxide [5, 6, 9]. These compounds contribute to the characteristic taste and aroma of kombucha, and some are associated with potential positive health effects [5, 13].

The final composition and characteristics of kombucha are influenced not only by the microbial consortium but also by several factors, including the type of tea (which contributes polyphenols and antioxidant effects), the source of saccharose (such as refined sugar, cane sugar or honey), the temperature and duration of fermentation, water quality and other variables [12, 14]. These factors can also impact the activity of the microorganisms present and their metabolic pathways, which ultimately determine the final chemical composition of kombucha [13]. Both green and black tea are derived from the leaves of the *Camellia sinensis* plant; however, the distinct processing methods applied to the leaves result in varying flavours and different phenolic profiles [15–17]. Consequently, the choice of tea used to prepare the brew is crucial for the resulting taste and aroma of kombucha [7, 8].

Due to the complexity of interactions among the various factors influencing the fermentation process, this study aims to investigate how regulating fermentation temperature can affect the present microorganisms and, consequently, the chemical composition of kombucha. Microorganisms and the products of their metabolic activity are crucial to the desired organoleptic properties; therefore, it is essential to evaluate the nutritional benefits of fermentation and ensure the health safety of kombucha beverages.

MATERIAL AND METHODS

Sample preparation

The tea brew was prepared from 7 g of a mixture of loose-leaf black and green tea, i.e. 50 % black Georgian Likhauri, 25 % green Georgian Schvitobauri and 25 % oolong Ming Jian Dong Pian Si Ji Chun (GoodLok, Prague, Czech Republic) and 1 500 ml of hot water. The mixture was left to steep for 12 min and then poured through a sieve to remove the tea leaves. Subsequently, 112.5 g of saccharose was dissolved in the tea brew (concentration of 75 $g \cdot l^{-1}$). The tea brew was cooled to 35 °C before adding the kombucha 'mother' culture (GoodLok) at a concentration of 10 % v/v of the tea brew. After mixing, the inoculated tea brew was divided into six 250 ml sterile glass bottles, which were covered with sterile pulp (aerobic fermentation) and transferred to thermostats set at 20 °C, 25 °C and 30 °C. After 96 h, the glass bottles were sealed with tight lids (to reduce oxygen) and left for the fermentation process to continue until 168 h.

Active acidity (pH)

From each sample (both parallels), a volume of 10 ml was taken with a sterile pipette into a beaker, and the pH value was measured every 24 h (except at 120 h and 144 h). The pH metre SevenCompact S210 (Mettler Toledo, Columbus, Ohio, USA) with the combined electrode featuring an integrated temperature probe InoLab Expert PRO-ISM (Mettler Toledo, Pt wire with reference electrolyte KCl 3 $mol \cdot l^{-1}$ + free Ag^{+}) was used for the measurement. Each sample was measured by three consecutive pH meter readings and the result was expressed as the average value.

Microbial analyses

Microbial analyses were performed on days 0 and 7 of the fermentation process. After a series of decimal dilutions, the samples were transferred to a sterile petri dish and overlaid with a suitable culture medium. The determination of lactic acid bacteria counts was carried out on De Man–Rogosa–Sharpe (MRS) agar (Merck, Darmstadt, Germany) incubated for 5 days at 35 °C under anaerobic conditions. Yeasts and moulds (YM) were incubated on yeast–glucose–chloramphenicol (YGC) agar (Merck) at 25 °C for 7 days. For each sample, the microbial count was determined and expressed as a colony forming units per millilitre.

Organic acids

The concentrations of lactic acid and acetic acid were determined using isotachophoresis (Villa Labeco, Spišská Nová Ves, Slovakia). The leading electrolyte consisted of a mixture of hydrochloric acid (10 $mmol \cdot l^{-1}$), aminocaproic acid (22 $mmol \cdot l^{-1}$) and hydroxyethylcellulose (0.5 $ml \cdot l^{-1}$). The final electrolyte was caproic acid (1.26 $ml \cdot l^{-1}$). Calibration curves were constructed for the measurements using a mixed solution of lactic acid (lithium lactate salt ≥ 98%,

Sigma Aldrich, St. Louis, Missouri, USA) and acetic acid (p.a. crystalline sodium acetate, Lachema, Brno, Czech Republic) at concentrations of 5 mg·l^{-1}, 20 mg·l^{-1} and 50 mg·l^{-1}.

Residual sugars

Each sample was deaerated using the ultra-sonic Kraintek K12 device (Kraintek, Hradec Králové, Czech Republic). The samples were diluted tenfold with distilled water and subsequently transferred to vials through a nylon membrane filter with a pore size of 0.45 μm (Agilent Technologies, Santa Clara, California, USA). The analysis of fructose, glucose and saccharose concentration in the samples was conducted using the Agilent 1260 Infinity high-performance liquid chromatography (HPLC) system equipped with a refractive index detector (Agilent Technologies). The analysis parameters included an injection volume of 10 μl, a ZORBAX Original NH2 column (Agilent Technologies), a mobile phase consisting of a mixture of acetonitrile (70 % v/v) and de-ionised water (30 % v/v), a flow rate of 1 ml·min^{-1}, a column temperature of 30 °C and a cell temperature of 40 °C. The chromatograms were evaluated using the OpenLAB CDS programme (Agilent Technologies). The calibration series was prepared from a mixed standard of saccharose (≥ 99.5%), glucose (D(+)glucose, ≥ 99.5%) and fructose (D-fructose, ≥ 99%), all by Sigma Aldrich, at concentrations of 0.5 g·l^{-1}, 1.0 g·l^{-1}, 2.0 g·l^{-1} and 4.0 g·l^{-1}.

Ethanol

The concentration of ethanol was measured using solid-phase microextraction followed by gas chromatography coupled with mass spectrometry (SPME-GC-MS) analysis, as described by CHAN et al. [18] in their 2021 study, with modifications made to suit our laboratory conditions. Quantification of ethanol in the samples was conducted using both external and internal standards. The calibration series for the external standard was prepared from a stock solution of ethanol (96 % v/v) by diluting it to concentrations of 0.5 ml·l^{-1}, 1.0 ml·l^{-1}, 2.0 ml·l^{-1} and 3.0 ml·l^{-1}. For the external standard, these calibration solutions were brought to a specified volume using distilled water, while for the internal standard, they were supplemented with a diluted solution of the sample.

Into the 10 ml vial, 4 ml of sample or calibration series was pipetted, and the vial was sealed and tempered at 35 °C for 1 min. Subsequently, the SPME-GC-MS analysis was performed under the following conditions: the divinylbenzene/caraboxene/polydimethylsiloxane (DVB/CAR/PDMS) fibre (50/30 μm), the gas chromatograph GC-MS 7890/5975C (Agilent Technologies), desorption time of 120 s, the HP-5MS column (30 m × 250 μm × 0.25 μm), initial temperature of 35 °C (hold for 5 min), temperature ramp of 10 °C·min^{-1} to 90 °C (hold for 0 min), total analysis time of 10.5 min and mobile phase helium (grade 5.5) with a flow rate of 1.8 ml·min^{-1}.

RESULTS AND DISCUSSION

Change of active acidity

The results of the change in active acidity (pH) in kombucha samples during fermentation at 20 °C, 25 °C and 30 °C over 7 days are presented in Fig. 1. The results are expressed as the mean of three consecutive pH meter readings performed at room temperature (± 20 °C).

The sharpest decrease in pH values was observed in kombucha samples fermented at 30 °C. For samples fermented at 20 °C and 25 °C during the first 24 h, the pH values remained almost unchanged, likely due to the slower activation of microorganisms from the 'mother' culture. After 168 h, all samples reached pH values of 3.0–3.1. At the fermentation temperature of 30 °C, the steepest drop in pH values occurred during the first 48 h, suggesting that this temperature stimulates the microorganisms present to grow and produce metabolites more rapidly. In contrast, the most moderate decrease in pH occurred in the samples fermented at 20 °C. However, the pH drop was the steadiest in this case, and the trend of the pH curve indicates that if fermentation was not terminated by cooling, the pH value would continue to fall. At 25 °C and 30 °C, the rate of pH

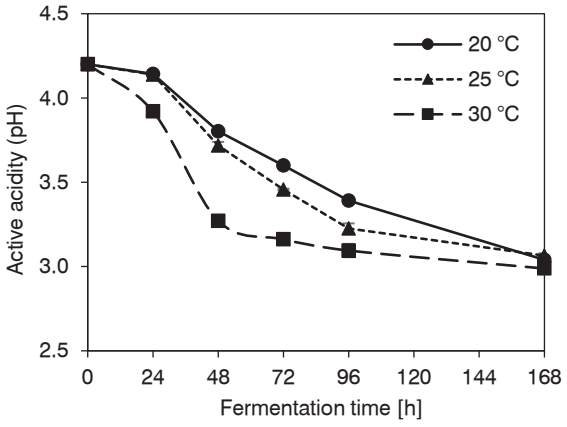

Fig. 1. Change in active acidity (pH) in kombucha samples.

decrease slowed down and the pH values gradually stabilised. At 96 h, when the samples were sealed and the environment shifted to anaerobic, a breaking point occurred in all three curves, resulting in a slowdown in the pH drop.

From the microbiological safety perspective, pH value is a very important parameter in the fermentation of kombucha [11]. Kombucha is not considered a health risk if the pH value is within the range between 2.5 and 4.2. Food and Drug Administration (FDA) Food Code Model recommendations set a pH ≤ 4.2 as the critical limit. In the case that pH does not decrease to this limit within 7 days of fermentation, the culture may be contaminated [19]. It is necessary to mention that the initial pH value is affected by the SCOBY inoculum. Cvetković et al. [20] observed during their study that the pH value of the sweetened black tea immediately after the inoculation (10 %) with the fermentation broth was 4.7. During the first 4 days of fermentation at 28 °C, the pH value dropped about 1.6 units, and no significant change in the pH value was observed after this phase [20]. This phenomenon was observed in other published studies [21, 22] and is in agreement with our measured data.

Microbial analysis

The counts of lactic acid bacteria and yeasts are presented in Tab. 1. No mould growth was detected in any of the samples. The lactic acid bacteria counts at the end of fermentation (10^6 CFU·ml^{-1}) were approximately the same as the initial count on day 0 (10^6 CFU·ml^{-1}) in all samples. On the other hand, the yeast counts increased from the initial count (10^4 CFU·ml^{-1}) and reached values two orders of magnitude higher at the end of the experiment (10^6 CFU·ml^{-1}), but there was no significant difference in yeast counts between the fermentation temperatures.

Fig. 2 shows the bacterial cellulose taken from the samples at the end of fermentation. Although we were unable to quantitatively determine the number of acetic acid bacteria using the plate method, it is possible to determine in which of the samples the greatest growth of acetic acid bacteria occurred based on bacterial cellulose, which is primarily produced by these bacteria. It is evident from Fig. 2 that the largest amount of cellulose is formed in the sample fermented at 30 °C, and conversely, only a very thin layer of cellulose is formed in the samples fermented at 20 °C and 25 °C.

Tab. 1. Counts of lactic acid bacteria and yeast in the kombucha samples fermented at various temperatures for 7 days.

Fermentation temperatue	Lactic acid bacteria [CFU·ml^{-1}]		Yeast [CFU·ml^{-1}]	
	Day 0	Day 7	Day 0	Day 7
20 °C	2.3×10^6	2.0×10^6	6.0×10^4	2.8×10^6
25 °C		2.0×10^6		1.9×10^6
30 °C		1.8×10^6		1.4×10^6

It is very difficult to compare the results of microbial analysis with other studies, as the SCOBY used during the experiments can differ significantly in the composition of microorganisms. But for example, studies published by Meng et al. [23] and Savary et al. [24] presented similar counts of lactic acid bacteria and yeast as we determined in our samples (10^6 CFU·ml^{-1}). De Filippis et al. [25] reported in their study that in the samples fermented at 30 °C, there is a higher viable count of acetic acid bacteria compared to samples fermented at 20 °C, which was also observed in our experimental work through the comparison of biofilm production.

Lactic acid and acetic acid

The results of lactic acid and acetic acid concentrations in the fermented kombucha samples are shown in Tab. 2. The measurements were performed at the end of the fermentation process, and each sample was measured in two parallels.

Based on the results presented in Tab. 2, it can be concluded that acetic acid bacteria were the predominant microorganisms in the microbial consortium of kombucha samples fermented at 30 °C. The samples fermented at this temperature exhibited the highest concentration of acetic acid (17 701.1 ± 885.1 mg·l^{-1}) and the lowest concentration of lactic acid (36.9 ± 1.8 mg·l^{-1}). A similar concentration of acetic acid was reported by Chakravorty et al. [26], who detected an acetic acid concentration of 16.57 ± 0.9 g·l^{-1} in kombucha fermented at 28 ± 2 °C for 21 days. In our experiments, the highest concentration of lactic acid (129.4 ± 6.5 mg·l^{-1}) and the lowest concentration of acetic acid (2 692.0 ± 134.1 mg·l^{-1}) were observed in kombucha samples fermented at 25 °C.

The study by Jayabalan et al. [27] investigated the effect of different types of tea on the nutrient concentration in kombucha. Specifically, during fermentation conducted at 24 ± 3 °C for 18 days, kombucha prepared from green tea achieved a lactic acid concentration of 0.12 ± 0.02 g·l^{-1} and an acetic acid concentration of 8.36 ± 0.87 g·l^{-1}, while samples prepared from black tea exhibited a lactic acid concentration of 0.18 ± 0.08 g·l^{-1} and an acetic acid concentration of 4.69 ± 0.54 g·l^{-1} [27]. Contradictory results were observed by Kaewkod et al. [28] in their study,

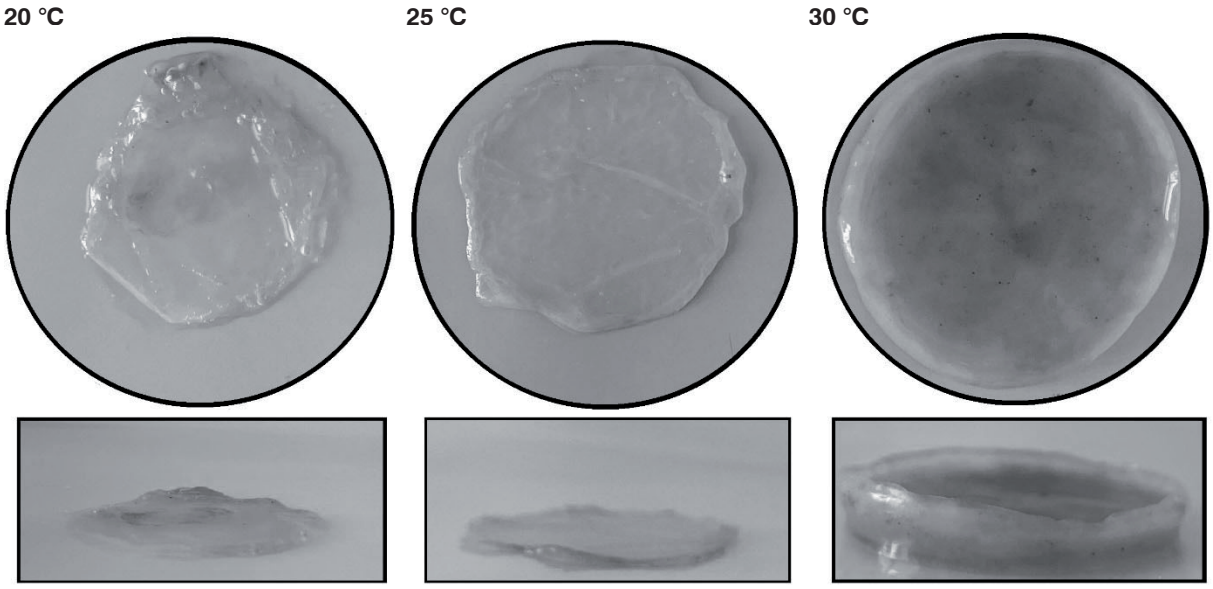

Fig. 2. Photo documentation of bacterial cellulose formed in kombucha samples after 7 days of fermentation.

Tab. 2. Concentrations of lactic acid and acetic acid, residual sugars and ethanol in the kombucha samples fermented at various temperatures for 7 days.

	Fermentation temperature		
	20 °C	25 °C	30 °C
Organic acids			
Lactic acid [mg·l^{-1}]	104.9 ± 5.2	129.4 ± 6.5	36.9 ± 1.8
Acetic acid [mg·l^{-1}]	6 361.4 ± 318.1	2 692.0 ± 134.1	17 701.1 ± 885.1
Residual sugars			
Fructose [g·l^{-1}]	8.20 ± 0.35	5.15 ± 0.14	11.50 ± 0.30
Glucose [g·l^{-1}]	5.60 ± 0.43	2.22 ± 0.12	6.64 ± 0.26
Saccharose [g·l^{-1}]	4.55 ± 0.25	4.51 ± 0.17	3.99 ± 0.20
Total [g·l^{-1}]	18.35	11.88	22.13
Ethanol [ml·l^{-1}]	19.0 ± 1.0	21.6 ± 2.7	17.2 ± 0.1

in which they fermented kombucha for 15 days at room temperature and used a different type of tea (green, oolong, black) for the brew. They determined the highest concentration of acetic acid (11.15 ± 0.00 g·l^{-1}) in kombucha prepared from the black tea and identified the acetic acid and gluconic acid as the major organic acids in the kombucha samples, regardless of the type of tea they were made from [28]. In our study, we used a mixture of all three types of tea mentioned above, but after fermentation at 20 °C and 25 °C, which can be considered as room temperature, we determined a lower concentration of acetic acid. This phenomenon is caused by the shorter fermentation time (7 days). On the contrary, after fermentation at 30 °C, the concentration of acetic acid in our samples was higher, and therefore it is obvious that with the higher temperature, the fermentation process is accelerated, and the metabolic activity of the acetic acid-producing bacteria is supported.

Residual sugar concentration

The results of the residual sugar concentrations in the fermented kombucha samples are presented in Tab. 2. The analysis was conducted after the fermentation process, with each sample measured in duplicate.

Results presented in Tab. 2 show that the highest concentration of residual sugars was detected in the kombucha samples fermented at 30 °C (22.13 g·l^{-1}). In the kombucha samples fermented at 25 °C, the concentration of residual sugars was nearly half as much (11.88 g·l^{-1}). This

indicates that at 25 °C, the kombucha microbial consortium was predominantly composed of microorganisms that utilised carbohydrates as a source of energy during their fermentative metabolism. Because this temperature is optimal for yeast growth, yeasts were likely the dominant microorganisms in the samples fermented at 25 °C. This hypothesis is further supported by the ethanol concentration results presented in Tab. 2, which show that the kombucha samples fermented at 25 °C contained the highest levels of ethanol, the characteristic product of yeast fermentative metabolism. Several studies published to date support this statement. For example, a similar phenomenon was observed and reported by Villarreal-Soto et al. [14]. On the other hand, Aung and Eun [28] observed during their experiment that the reducing sugar concentration after 7 days of fermentation at 25 °C was almost the same as at 30 °C, but after 22 days, the concentration of reducing sugars was significantly lower in samples fermented at 30 °C. The difference between the mentioned study and our results can be caused by the fact that they used brew from a lave (*Porphyra dentata*) [29].

Ethanol concentration

The results of ethanol concentrations in the fermented kombucha are presented in Tab. 2. The measurement was performed at the end of the fermentation process, and each sample was measured in duplicate.

Based on the analysis of ethanol concentration detected using the SPME-GC-MS method, the highest concentration of ethanol was found in samples fermented at 25 °C (21.6 ± 2.7 ml·l^{-1}), which corresponds to increased yeast metabolic activity at this temperature, as discussed above. The lowest ethanol concentration was observed in samples fermented at 30 °C (17.2 ± 2.7 ml·l^{-1}). This confirms the hypothesis that in our samples fermented at 30 °C, the metabolic activity of acetic acid bacteria was higher than in other samples, because acetic acid bacteria utilise ethanol in their metabolism. Jakubczyk et al. [30] also measured higher ethanol concentrations in kombucha samples prepared from different types of tea fermented at 28 ± 1 °C for 7 days, specifically 30.0 ± 0.0 ml·l^{-1} for black tea and 32.5 ± 5.0 ml·l^{-1} for green tea. However, after 14 days of fermentation, a decrease in ethanol concentration was recorded: 2.5 ml·l^{-1} for black tea and 12.5 ml·l^{-1} for green tea [30]. This suggests that the alcohol concentration in kombucha beverages can be reduced by choosing an appropriate fermentation temperature and by prolonging the fermentation time.

CONCLUSION

The results of this study indicate that the temperature of kombucha fermentation significantly affected its chemical composition. Although the kombucha samples were prepared using the same ingredients and the same 'mother' culture, varying temperatures during fermentation allowed different microorganisms to thrive, leading to the production of characteristic chemical compounds as a result of their metabolic activities.

As we assumed, the acetic acid bacteria grew best in kombucha samples fermented at 30 °C, which contained the highest concentrations of acetic acid and residual sugars and the lowest concentration of ethanol. Conversely, lactic acid bacteria and yeasts, which were predominant in the kombucha samples fermented at 25 °C, demonstrated considerable tolerance to an environment with a high concentration of lactic acid and an elevated level of ethanol.

Acknowledgements

This work was supported by the grant of Specific university research – grant No A1_FPBT_2024_008 (University of Chemistry and Technology Prague, Prague, Czech Republic).

REFERENCES

1. Kombucha. In: Merriam-Webster. Dictionary [online]. Springfield : Merriam-Webster, 2024 [cited 4 December 2024]. <https://www.merriamwebster.com/dictionary/kombucha>
2. Yamada, Y. – Yukphan, P. – Lan Vu, H. T. – Muramatsu, Y. – Ochaikul, D. – Tanasupawat, S. – Nakagawa, Y.: Description of *Komagataeibacter* gen. nov., with proposals of new combinations (*Acetobacteraceae*). The Journal of General and Applied Microbiology, *58*, 2012, pp. 397–404. DOI: 10.2323/jgam.58.397.
3. Kim, J. – Adhikari, K.: Current trends in kombucha: Marketing perspectives and the need for improved sensory research. Beverages, *6*, 2020, article 15. DOI: 10.3390/beverages6010015.
4. de Miranda, J. F. – Ruiz, L. F. – Silva, C. B. – Uekane, T. M. – Silva, K. A. – Gonzalez, A. G. M. – Fernandes, F. F. – Lima, A. R.: Kombucha: A review of substrates, regulations, composition, and biological properties. Journal of Food Science, *87*, 2022, pp. 503–527. DOI: 10.1111/1750-3841.16029.
5. Dutta, H. – Paul, S. K.: Kombucha drink: Production, quality, and safety aspects. In: Grumezescu, A. M. – Holban, A. M. (Eds.): Production and management of beverages. Sawston : Woodhead Publishing, 2019, pp. 259–288. ISBN: 9780128157008. DOI: 10.1016/b978-0-12-815260-7.00008-0.
6. Kapp, J. M. – Sumner, W.: Kombucha: a systematic

review of the empirical evidence of human health benefit. Annals of Epidemiology, *30*, 2019, pp. 66–70. DOI: 10.1016/j.annepidem.2018.11.001.
7. Jayabalan, R. – Waisundara, V. Y.: Kombucha as a functional beverage. In: Grumezescu, A. M. – Holban, A. M. (Eds.): Functional and medicinal beverages. Vol. 11. The science of beverages. Cambridge : Academic Press, 2019, pp. 413–446. ISBN: 9780128163979. DOI: 10.1016/B978-0-12-816397-9.00012-1.
8. Nyiew, K. Y. – Kwong, P. J. – Yow, Y. Y.: An overview of antimicrobial properties of kombucha. Comprehensive Reviews in Food Science and Food Safety, *21*, 2022, pp. 1024–1053. DOI: 10.1111/1541-4337.12892.
9. Cardoso, R. R. – Neto, R. O. – dos Santos D'Almeida, C. T. – do Nascimento, T. P. – Pressete, C. G. – Azevedo, L. – Martino, H. S. D. L. – Cameron, L. C. – Ferreira, M. S. L. – de Barros, F. A. R.: Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. Food Research International, *128*, 2020, article 108782. DOI: 10.1016/j.foodres.2019.108782.
10. Harrison, K. – Curtin, C.: Microbial composition of SCOBY starter cultures used by commercial kombucha brewers in North America. Microorganisms, *9*, 2021, article 1060. DOI: 10.3390/microorganisms9051060.
11. Villarreal-Soto, S. A. – Beaufort, S. – Bouajila, J. – Souchard, J. P. – Taillandier, P.: Understanding kombucha tea fermentation: a review. Journal of Food Science, *83*, 2018, pp. 580–588. DOI: 10.1111/1750-3841.14068.
12. Leal, J. M. – Valenzuela Suárez, L. – Jayabalan, R. – Huerta Oros, J. – Escalante-Aburto, A.: A review on health benefits of kombucha nutritional compounds and metabolites. CyTA – Journal of Food, *16*, 2018, pp. 390–399. DOI: 10.1080/19476337.2017.1410499.
13. Jayabalan, R. – Malbaša, R. – Lončar, E. S. – Vitas, J. – Sathishkumar, M.: A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Comprehensive Reviews in Food Science and Food Safety, *13*, 2014, pp. 538–550. DOI: 10.1111/1541-4337.12073.
14. Villarreal-Soto, S. A. – Beaufort, S. – Bouajila, J. – Souchard, J. P. – Renard, T. – Rollan, S. – Taillandier, P.: Impact of fermentation conditions on the production of bioactive compounds with anticancer, anti-inflammatory and antioxidant properties in kombucha tea extracts. Process Biochemistry, *83*, 2019, pp. 44–54. DOI: 10.1016/j.procbio.2019.05.004.
15. Sang, S. – Lambert, J. D. – Ho, C. T. – Yang, C. S.: The chemistry and biotranformation of tea constituents. Pharmacological Research, *64*, 2011, pp. 87–99. DOI: 10.1016/j.phrs.2011.02.007.
16. Zhang, L. – Ho, C.-T. – Zhou, J. – Santos, J. S. – Armstrong, L. – Granato, D.: Chemistry and biological activities of processed *Camellia sinensis* teas: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety, *18*, 2019, pp. 1474–1495. DOI: 10.1111/1541-4337.12479.
17. Tanaka, T. – Kouno, I.: Oxidation of tea catechins: Chemical structures and reaction mechanism. Food Science and Technology Research, *9*, 2003, pp. 128–133. DOI: 10.3136/fstr.9.128.
18. Chan, M. – Sy, H. – Finley, J. – Robertson, J. – Brown, P. N.: Determination of ethanol content in kombucha using headspace gas chromatography with mass spectrometry detection: Single-laboratory validation. Journal of AOAC International, *104*, 2021, pp. 122–128. DOI: 10.1093/jaoacint/qsaa094.
19. Food code 2009. In: FDA [online]. Silver Spring : Food and Drug Administration, 2009 [cited 18 April 2025]. <http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM2019396.htm>
20. Cvetković, D. – Markov, S. – Djurić, M. – Savić, D. – Velićanski, A.: Specific interfacial area as a key variable in scaling-up Kombucha fermentation. Journal of Food Engineering, *85*, 2008, pp. 387–392. DOI: 10.1016/j.jfoodeng.2007.07.021.
21. Chen, C. – Liu, B. Y.: Changes in major components of tea fungus metabolites during prolonged fermentation. Journal of Applied Microbiology, *89*, 2000, pp. 834–839. DOI: 10.1046/j.1365-2672.2000.01188.x.
22. Sreeramulu, G. – Zhu, Y. – Knol, W.: Kombucha fermentation and its antimicrobial activity. Journal of Agricultural and Food Chemistry, *48*, 2000, pp. 2589–2594. DOI: 10.1021/jf991333m.
23. Meng, Y. – Wang, X. – Li, Y. – Chen, J. – Chen, X.: Microbial interactions and dynamic changes of volatile flavor compounds during the fermentation of traditional kombucha. Food Chemistry, *430*, 2024, article 137060. DOI: 10.1016/j.foodchem.2023.137060.
24. Savary, O. – Mounier, J. – Thierry, A. – Poirier, E. – Jourdren, J. – Maillard, M. B. – Penland, M. – Decamps, C. – Coton, E. – Coton, M.: Tailor-made microbial consortium for Kombucha fermentation: Microbiota-induced biochemical changes and biofilm formation. Food Research International, *147*, 2021, article 110549. DOI: 10.1016/j.foodres.2021.110549.
25. De Filippis, F. – Troise, A. D. – Vitaglione, P. – Ercolini, D.: Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. Food Microbiology, *73*, 2018, pp. 11–16. DOI: 10.1016/j.fm.2018.01.008.
26. Chakravorty, S. – Bhattacharya, S. – Chatzinotas, A. – Chakraborty, W. – Bhattacharya, D. – Gachhui, R.: Kombucha tea fermentation: Microbial and biochemical dynamics. International Journal of Food Microbiology, *220*, 2016, pp. 63–72. DOI: 10.1016/j.ijfoodmicro.2015.12.015.
27. Jayabalan, R. – Marimuthu, S. – Swaminathan, K.: Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. Food Chemistry, *102*, 2007, pp. 392–398. DOI: 10.1016/j.foodchem.2006.05.032.
28. Kaewkod, T. – Bovonsombut, S. – Tragoolpua, Y.: Efficacy of kombucha obtained from green, oolong, and black teas on inhibition of pathogenic bacteria, antioxidation, and toxicity on colorectal cancer cell

line. Microorganisms, *7*, 2019, article 700. DOI: 10.3390/microorganisms7120700.
29. Aung, T. – Eun, J. B.: Impact of time and temperature on the physicochemical, microbiological, and nutraceutical properties of laver kombucha (*Porphyra dentata*) during fermentation. LWT – Food Science and Technology, *154*, 2022, article 112643. DOI: 10.1016/j.lwt.2021.112643.
30. Jakubczyk, K. – Kałduńska, J. – Kochman, J. – Janda, K.: Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black and red tea. Antioxidants, *9*, 2020, article 447. DOI: 10.3390/antiox9050447.

Received 27 January 2025; 1st revised 23 April 2025; accepted 13 May 2025; published online 6 June 2025.