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# THE INFLUENCE OF THE METHOD OF PREPARATION OF A FERMENTED TEA BEVERAGE ON THE SELECTED PHYSICOCHEMICAL AND SENSORY PROPERTIES

S u m m a r y

**Background.** In the article, changes in the chemical properties (pH, sugar content) of a fermented tea beverage during a fermentation process and the influence of the above properties on the sensory characteristics of the final product were analyzed.

**Results and conclusions.** On average, the sugar content decreased by 10.6 % on the sixth fermentation day, the decrease in sugar content at its highest on the sixth fermentation day was observed in the peppermint tea sample (18.7 %), while the reduction at its lowest was observed in green tea with additives (ginger and lemon – 8.0 %; ginseng and lemon – 6.5 %) and black tea (8.1 %). The average pH value was $3.13 \pm 0.15$ – i.e., lower than 4.2, and if that level is achieved, scientific literature recommends stopping the fermentation process due to the possible negative impact of the beverage on health; the pH of green tea samples without additives was higher than the average ($3.13 \pm 0.15$) – $3.20 \pm 0.12$ (peppermint), $3.23 \pm 0.15$ – green tea, while the pH of the black tea sample was $3.0 \pm 0.20$ – lower than all the samples tested. A positive correlation was specified between the sugar content, the pH change and temperature, however, it is of different strength: the relationship between the pH and temperature was medium ($r = 0.682$); while the relationship between the sugar content and temperature was low ($r = 0.305$).

**Key words:** a fermented tea beverage, pH, sugar content, flavor, odor

## Introduction

In recent years, growing public interest in healthy nutrition and natural products has led to an increase in the popularity of fermented beverages such as kombucha. Consumer interest in kombucha is most often attributed to its taste properties, antioxidant content and the perception of the functional benefits associated with the presence of live microbial cultures. Kombucha tea is a functional food that can lower blood pressure, has hypoglycemic effects and other health benefits [1, 20, 23]. This ferment-

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ed tea drink can be characterized by antioxidant and antimicrobial properties, which are determined by the bioactive compounds of the drink [5, 12, 24]. Differences in composition can cause different taste. A fermented tea drink is usually made from black sweetened tea, but nowadays various herbs and fruit are used. For example, using white hibiscus calyces results in milder, less sour taste, fresher aroma kombucha [3, 30]. The addition of additives such as ginkgo biloba leaves to the tea fermentation process can create a drink with stronger antioxidant properties and distinctive flavor [6, 14]. Depending on the type of tea, fermentation conditions and microbial community of kombucha SCOBY, the amount of the main active ingredients of kombucha products may vary [24]. For example, green and white tea kombucha can have higher antibacterial activities and black tea kombucha can have a greater variety of phenolic compounds [20]. This drink is valued for its potential probiotic properties, natural acidity and unique taste. Kombucha is manufactured using *Symbiotic Culture of Bacteria and Yeast* (abbreviated as SCOBY) by mixing various sweetened teas (e.g., green, black, white, red) with a starter culture from previous fermentation (about 10 %), which lowers the pH of sweetened tea – back slopping kombucha fermentation process [4, 5, 21]. During fermentation, which lasts up to 14 days, ongoing biochemical processes determine the chemical properties of the beverage – changes in the pH (safe pH range is 2.5 to 4.2), reduction in sugar content – and shape its sensory properties [14, 16]. During fermentation, biochemical processes occur in which microorganisms break down organic substances, mainly sugars, and convert them into other compounds such as alcohol, acids or gas [27, 29]. In the manufacture of fermented teas, especially kombucha, this process is carried out by SCOBY. The interaction between these microorganisms determines both chemical and sensory (taste, smell, color) changes during fermentation. Kombucha fermentation consists of a combination of three biochemical processes: alcoholic fermentation due to yeast activity, the formation of lactic and acetic acids (due to bacterial activity) [15]. At the beginning of fermentation, yeast (*Saccharomyces, Zygosaccharomyces, Brettanomyces*) uses sugars dissolved in tea – glucose and fructose – as a source of energy. Under anaerobic conditions, they carry out alcoholic fermentation, during which sugar is converted into ethanol and carbon dioxide [15, 18, 29].

During this process, a small amount of alcohol (usually up to 1 %) begins to form in the drink, and the $CO_2$ released gives kombucha a mild natural effervescence. In addition, yeast produces various by-products – esters and alcohols – which determine the complexity of the beverage aroma [13, 15].

In the second stage of fermentation, acetic acid bacteria (*Acetobacter, Gluconobacter, Komagataeibacter*) play a key role. They oxidize ethanol produced by yeast into acetic acid ($CH_3COOH$) and gluconic acid using oxygen [15].

This oxidation causes the pH of the beverage to decrease (usually from 5 ÷ 6 to 2.5 ÷ 3.5) and the sugar concentration to decrease, as it is consumed by the metabolism of microorganisms. Acetic acid gives it its characteristic sourness, while gluconic acid helps to stabilize the taste of the drink. The decreasing pH also acts as a natural preservative, preventing the growth of microorganisms and pathogenic bacteria. Lactic acid fermentation occurs when lactic acid bacteria break down glucose [15].

Other important processes also take place during fermentation:

- the polyphenolic compounds in tea partially break down or oxidize, therefore, changing the color of the drink and giving it its characteristic aroma.
- some bacteria produce group B vitamins, lactic acid, malic acid and gluconic acid which improve the nutritional value of the beverage.
- the carbon dioxide released partially dissolves in the beverage and gives it a refreshing taste [10, 19].

The fermentation process of tea is influenced by the following factors such as temperature, pH, oxygen content and dissolved $CO_2$ content. Changes in the above factors can affect the speed of fermentation, olfactory-gustatory sensation (taste and smell), chemical properties. The intensity of the processes is strongly influenced by temperature: at higher temperatures (25 ÷ 30 °C), fermentation occurs faster, the pH drops more rapidly and the drink becomes more acidic. At lower temperatures (18 ÷ 22 °C), the processes are slower – more sugar remains, the taste is milder and the smell is more subtle [15]. The sugar content in the final fermentation product can vary, depending on the metabolic processes that occur during the fermentation and the initial sugar concentration. The longer the tea is fermented, the greater quantity of organic acids is formed. During the fermentation process, the pH of the beverage can drop to as low as 2.5, given the formation of large amounts of acetic acid, which can cause a risk to consumers of the fermented tea beverage. In the literature, it is stated that when the pH reaches 4.2, the fermentation process must be stopped [7].

The changing chemical properties of fermented tea – sugar content, pH – affect the sensory properties of the final product. The changing pH of fermented tea affects the change of the beverage color. As the fermentation time of the beverage increases, its smell changes, the intensity of which is also influenced by organic compounds formed during the fermentation, such as acetic acid, gluconic acid, etc. After the fermentation process is complete, the primary aroma of the tea is masked and a sour smell appears, which is characteristic of the fermentation process. Specific aromas may also appear, such as fruit and citrus aromas [2, 11, 17].

Temperature is one of the main factors influencing these processes. Higher temperatures can inhibit the formation of some aromatic compounds, and the synthesis of alcohol can accelerate and, as a result, the smell of flowers and citrus fruits is felt more in kombucha [28]. Therefore, it is important to research how different fermentation temperatures affect both the chemical and sensory properties of kombucha. The above-mentioned studies help to better understand the mechanisms of fermentation and optimize beverage manufacturing conditions in order to achieve higher quality and appeal to consumers.

The aim of the research is to evaluate changes in the chemical properties (pH, sugar content) of the fermented tea beverage during the manufacturing process, taking into account the fermentation time, sample temperature, the presence of tea additives (ginger, lemon, ginseng) and the sensory properties of the final product (the fermented tea beverage – kombucha).

## Materials and methods

In the literature, it is stated that such factors as the type of tea used, the pH value, the fermentation time and temperature affect the tea fermentation process. Changes in these parameters affect the quality and sensory properties of the final product [2, 9, 24, 26].

The kombucha manufacturing process for the research was studied using the [8] described method. The study of changes in the acidity and sugar content of the fermented tea beverage depending on the temperature of the drink and the fermentation time was performed using different types of tea leaves: peppermint leaves (*Mentha piperita* L., 100 %, marked with the index "1"), green Ceylon tea (marked with the index "2"), green tea (95 %) with additives (ginger and lemon (ginger pieces 4.5 %, lemon peels 0.3 %, lemongrass 0.2 %)), marked with the index "3"), green tea (95 %) with additives (ginseng and lemon (lemon granules 4.9 %, ginseng root powder 0.1 %), marked with the index "4"), black tea (*Camellia sinensis*, marked with the index "5"). The initial tea samples were prepared using the same procedure. A volume of 500 $cm^3$ of hot (85°C) water was poured over 15 g of each type of tea leaves , and 35 g of white sugar (producer JSC Nordic Sugar Kedainiai) made from sugar beets was added. The solution was stirred until the sugar dissolved and cooled under natural conditions, reaching the temperature of 25 ÷ 30 °C. After removing the tea leaves, 56 $cm^3$ of fermented tea drink ("starter") was added to each initial sample – using back slopping kombucha fermentation process – it lowers the pH of the mixture to inhibit the growth of unwanted microorganisms [18, 26]. The solution was stirred, and 80 g of tea fungus was added.

Fermenting the tea beverage for six ÷ ten days results in a refreshing taste, while fermenting it for longer than ten days gives the beverage an unpleasant vinegar-like smell [8]. Taking this into account, the fermentation time for the tea drink was set at six days, and the hydrogen ion concentration, sugar content and solution temperature of the sample were measured every two days.

The concentration of hydrogen ions is measured using a pH meter HI 2210 HI 2211 (manufacturer Hanna Instruments Inc, origin country – the USA) Microprocessor – based pH/mV/°C, which is done according to the potentiometric pH determination methodology. This method is standardized and widely applied in laboratory practice for the analysis of both food and beverages. The potentiometric method is based on an electrochemical principle, when pH is measured using glass with the help of the pH electrode and the reference electrode pair. The pH meter determines the potential difference caused by the activity of hydrogen ions in the solution. An electronic pH meter was used, calibrated at pH 4.0, 7.0 and 10.0 according to the manufacturer's instructions. The samples were measured three times.

The sugar content was measured using a refractometer Digital Handheld Refractometers DR101-60/DR201-95 (manufacturer – A. KRÜSS Optronic GmbH, country of origin – Germany) according to the manufacturer's instructions.

Each measurement was repeated three times, and the article presents average values. The mean square error of the direct individual measurement was evaluated by the formula:

$$\sigma_x = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{(n-1)}},$$

where: $\sigma_x$ – the mean square error of an individual measurement, $x_i$ – the result of the i-th measurement, $\bar{x}$ – the arithmetic mean of the measurements, $n$ – the number of measurements.

The sensory properties (taste, smell) of the fermented tea beverage are evaluated after six days using a descriptive assessment method involving ten evaluators with no respiratory or taste disorders. The general requirements for assessors are regulated by LST ISO 6658 [22]. In addition to these requirements, assessors participating in odor detection and identification tests must not take part in any other tests related to the detection or evaluation of odors or aromatic substances for 20 minutes prior to the start of the test. Before the sensory test, assessors must not eat or drink any strong-tasting products for at least 1 hour. Samples for sensory evaluation are presented in 50 ml portions, in identical containers randomly. Smell is evaluated before tasting. The characteristics are evaluated on a scale from 1 to 5, where 1 means the worst rating and 5 means the best score.

## Results and discussion

The following conditions are favorable for the manufacturing process of the fermented tea beverage, using tea fungus (a symbiotic culture of acetic acid bacteria and yeast): suitable pH level, initial pH approximately 4.5; temperature – optimal 24 ÷ 29°C, nutrients for yeast and bacteria – i.e., sugar content.

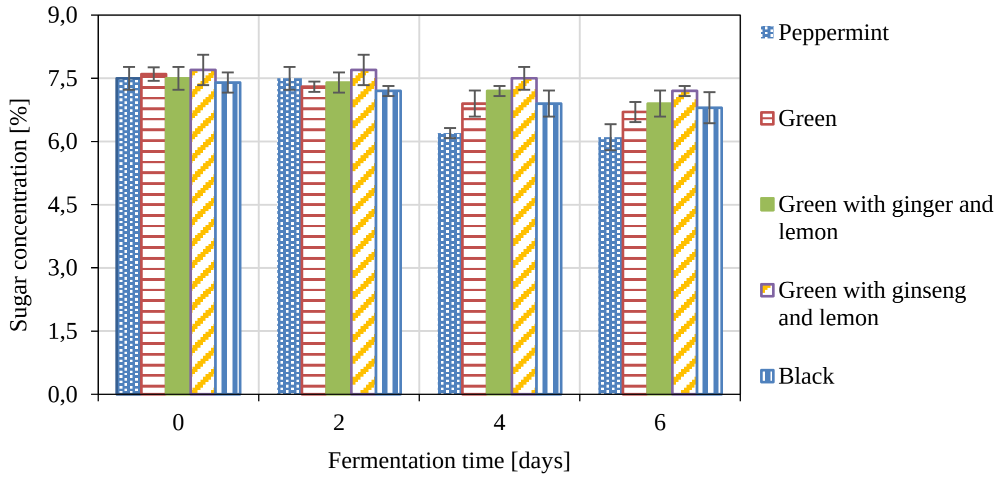


Figure 1. Changes in sugar content in the tea beverage samples at different fermentation times

In order to evaluate the chemical properties of the fermented tea beverage, such as the pH and sugar content, changes in different types of the sweetened tea drink during fermentation and the dependence of these properties on sample temperature were studied, using five samples, made from different types of tea and teas with additives. In Figure 1, the data of the research of changes in sugar content in tea samples at different fermentation times are presented.

The initial average sugar concentration (0 days) in the tea samples was equal to 7.54 ± 0.26 %, the samples had a sweet taste, and the additives in the tea (ginger with lemon and ginseng with lemon) gave it a pleasant aftertaste. After two days of fermentation, the average sugar content in the tea samples decreased slightly to 7.42 ± 0.22 %, and the samples had noticeable sweetness and slight sourness. The sugar content was still high, indicating that there were sufficient nutrients for yeast and fungi in the tea fungus to thrive. After four days of fermentation, the average sugar content in the tea samples decreased to 6.94 ± 0.23 %, and the samples had characteristic sweetness and noticeable acidity. After six days of fermentation, the average sugar content in the tea samples decreased to 6.74 ± 0.27 %. When evaluating the change in sugar content in different tea samples, it was observed that the average sugar content was reduced by 10.6 % on the sixth day of fermentation. When analyzed according to the tea leaves used, it was found that the highest decrease in sugar content on the sixth day of fermentation was when using peppermint – 18.7 %, and the lowest – when using green teas with additives (ginger and lemon – 8.0 %; ginseng and lemon – 6.5 %) and black tea (8.1 %). Based on this, we can assume that the additives used in green tea have a negative effect on the fermentation processes, performed by yeast. The sensory properties of the tea samples varied greatly, and their average assessment scores are presented in Table 1.

Table 1. Tea samples taste, smell assessment results after the six-day fermentation

| Sample | Smell | | | Taste | | |
|---|---|---|---|---|---|---|
| | Intensity, 1 - slight, 5 - strong | Pleasure, 1 - unpleasant, 5 – highly pleasant | Specific smells | Sweetness, 1 - unsweet, 5 – highly sweet | Acidity, 1 – with little acidity, 5 – highly acid | Pleasure, 1 - unpleasant, 5 – highly pleasant |
| 1. Peppermint leaves tea | 4.2 | 3.1 | Peppermint felt | 1.2 | 3.7 | 1.1 |
| 2. Green tea | 3.9 | 4.2 | - | 1.5 | 4.1 | 2.6 |
| 3. Green tea with ginger and lemon | 4.3 | 4.1 | Peach flavor is felt | 2.7 | 2.5 | 4.0 |
| 4. Green tea with ginseng and lemon | 2.9 | 4.9 | Ginseng after-smell | 2.4 | 2.8 | 4.0 |
| 5. Black tea | 4.4 | 2.5 | Acetic acid after-smell is felt | 2.3 | 3.9 | 3.7 |

The samples without additives (peppermint, green tea, black tea) had a strong sour taste. The assessors rated the green tea samples without additives as having an unpleasant taste (1.1 for peppermint, 2.6 for green tea), while the black tea sample was rated at 3.7 points. When evaluating the aroma of tea samples without additives, they were rated on average at 3.1 and 4.2 points, while the aroma of the black tea sample was unpleasant and was rated at 2.5 points. The sugar concentration in the above samples after six days of fermentation was the lowest: 6.1 ± 0.31 % (peppermint), 6.7 ± 0.24 % (green tea), 6.8 ± 0,37 % (black tea).

Evaluating the taste of the tea samples, it was noticed that black tea gives kombucha a rich, slightly caramel flavor and a darker color, while green tea gives it a fresher, milder, slightly grassy flavor and often has a lighter acidity. Herbal teas (e.g. peppermint) impart distinctive aromas; however, they should be used with caution, as some herbs can affect the fermentation process. In the case under analysis, the researchers indicated that the highest acidity was found in the fermented tea samples made using tea without additives: peppermint – 3.7 points, green tea – 4.1 points, black tea – 3.9 points. It is interesting that the concentration of hydrogen ions in the green tea samples without additives was found to be higher than average (3.13 ± 0.15) in the tea samples fermented for six days – 3.20 ±0.12 for peppermint and 3.23 ± 0.15 for green tea, while the pH of the black tea sample was 3.0 ± 0.2, which was lower than that of all the samples tested (Fig. 2).

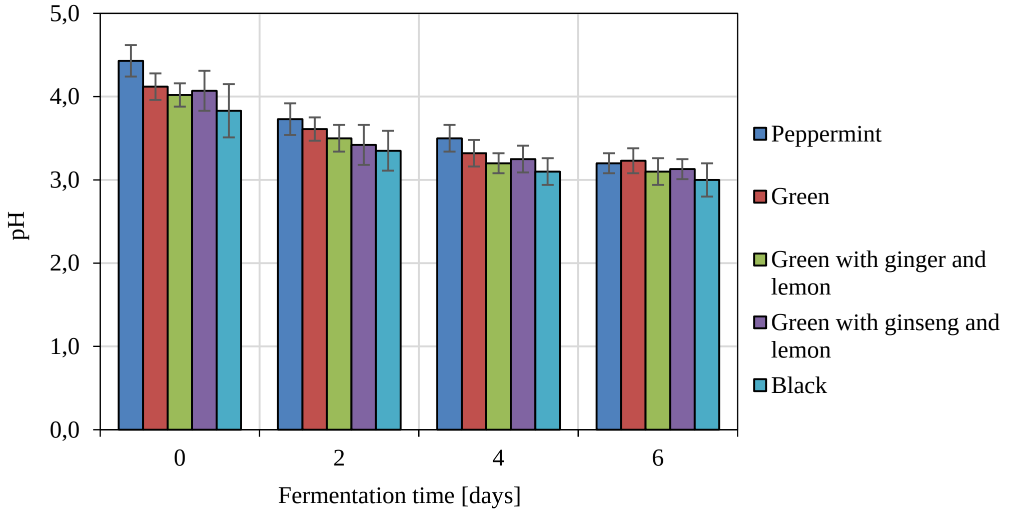


Figure 2. Hydrogen ion concentration changes at the different time of the tea fermentation

At the initial time point, the average hydrogen ion concentration in the tea samples was 4.09 ± 0.21, after two days it decreased down to 3.52 ± 0.19, after four days it amounted 3.27 ± 0.15, and after six days it was reduced to 3.13 ± 0.15. The assessors of sensory properties indicated that the samples with green tea with ginger and lemon and the samples with green tea with ginseng and lemon were less acidic (acidity was rated at 2.5 and 2.8 points respectively, i.e. moderately acidic), the hydrogen ion concentration in these samples was found to be close or equal to average in tea samples, 3.1 ± 0.16 and 3.13 ± 0.12 respectively. Based on these results, we assume that it is not possible to link the acidity of fermented tea to the hydrogen ion concentration of the fermented beverage directly; a more accurate correlation would be possible after a quantitative assessment of the organic acids (acetic, gluconic, lactic), formed during the fermentation process.

The pH, sugar content changes indicate the efficiency of the fermentation process. The ambient temperature (in this case, the environment is the fermented tea beverage) is a very important factor for the vital functions of yeast and microorganisms, and thus for the fermentation process. During the research, the correlation between the chemical properties of fermented tea (pH, sugar content) and the ambient temperature is evaluated. In Figures 3, 4, 5, the findings of the correlation analysis are presented.

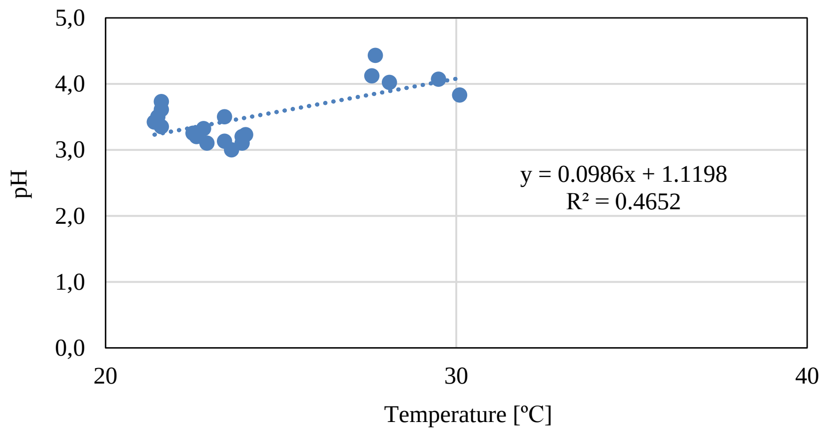


Figure 3. Fermented tea pH – temperature research data correlation analysis results

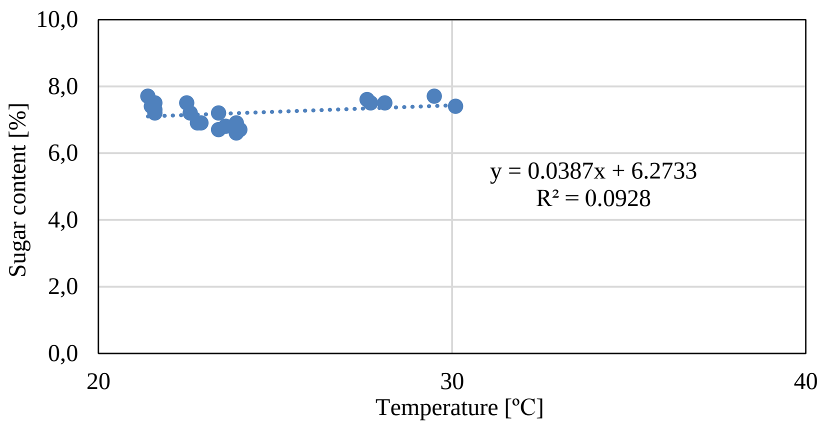


Figure 4. Fermented tea sugar content – temperature research data correlation analysis results

A correlation analysis of the chemical properties (pH, sugar content) and temperature data of fermented tea showed that in both cases there is a characteristic positive correlation, however, it varies in strength:

– the relationship between the pH and temperature is of medium strength ($r = 0.682$). This demonstrates that higher temperatures are consistently associated with higher pH values, confirming a systematic and coherent variation between these parameters.
– the relationship between the sugar content and temperature is weak ($r = 0.305$). This shows that higher temperatures are consistently associated with higher sugar content, confirming a systematic and coherent variation between these parameters.

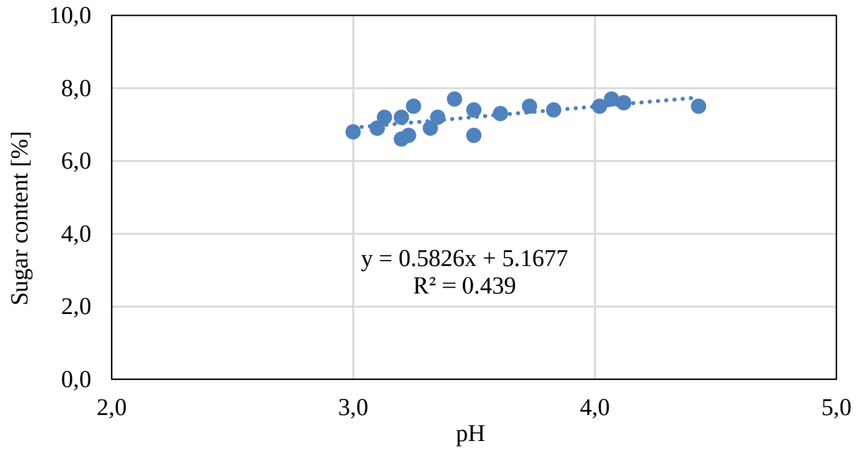


Figure 5. Fermented tea sugar content – pH research data correlation analysis results

This shows that both the pH and sugar content of fermented tea are directly dependent on temperature, but the relationship between pH and temperature is more reliable than the relationship between sugar content and temperature, which may be influenced by factors other than temperature, such as the chemical composition of the tea leaves and the fermentation time.

When analyzing changes in the chemical properties of fermented tea (pH, sugar content) (c), it can be observed that the above-mentioned factors also have a positive correlation, and the relationship between pH and sugar content can be described as having moderate strength ($r = 0.663$).

When evaluating the data in Figures 3 and 4, it is observed that the data is distributed across two data clouds. The presence of two distinct data clusters is most likely related to differences in sample preparation. One group of measurements was taken from freshly prepared kombucha samples (higher data cloud 3 and 4 figures cases), while the other group represents samples that had already undergone partial fermentation (bottom data cloud 3 and 4 figures cases). Fresh samples typically show higher initial pH values and different physicochemical characteristics, which results in a systematic shift in the data. The distribution of the lower data clouds in figure 3, 4 is characterized by a negative correlation. The linear regression (according to figure 4 bottom data cloud) analysis indicates a moderate negative correlation between temperature and pH ($R^2 = 0.4941$). This suggests that pH decreases as temperature increases, although temperature explains only about half of the observed variability. The remaining variation is likely influenced by additional chemical or measurement factors. The linear regression (according to figure 4 bottom data cloud) analysis revealed a strong negative correlation between temperature and sugar content ($R^2 = 0.7103$). This indicates that the variation in sugar content can be explained by temperature changes. The negative slope shows that sugar content decreases as temperature increases, suggesting a clear temperature-dependent effect on the system. This separation therefore reflects inherent differences in sample state rather than random measurement variability.

## Conclusions

1. It was found that, on average, the sugar content decreased by 10.6 % on the sixth fermentation day. The largest decrease in sugar content on the sixth fermentation day was found in the peppermint tea sample (18.7 %), while the lowest decrease was found in green tea with additives (ginger and lemon – 8.0 %; ginseng and lemon – 6.5 %) and black tea (8.1 %) – the assumption is made that the additives used in green tea have a negative effect on the fermentation (bio-degradation) processes, performed by yeast.
2. The average pH value in tea samples after six days of the fermentation process was 3.13 ± 0.15, i.e., lower than the level of 4.2, recommended in scientific literature references as the point at which the fermentation process is recommended to be stopped, given the possible negative effects of the beverage on health.
3. The concentration of hydrogen ions in the green tea samples without additives, which was fermented for six days, was found to be higher than average (3.13 ± 0.15) – 3.20 ± 0.2 (peppermint), 3.23 ± 0.15 – green tea. Meanwhile, the pH of the black tea sample was 3.0 ± 0.2, which was lower than that of all the samples tested.
4. After performing a correlation analysis between the chemical properties of fermented tea (pH, sugar content) and ambient temperature, it can be stated that in both cases there is a characteristic positive correlation, however, it varies in strength: the relationship between pH and temperature is of moderate strength ($r = 0.682$); the relationship between the sugar content and temperature is weak ($r = 0.305$).

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## WPŁYW SPOSOBU PRZYGOTOWANIA FERMENTOWANEGO NAPOJU HERBACIANEGO NA WYBRANE WŁAŚCIWOŚCI FIZYKOCHEMICZNE I SENSORYCZNE

S t r e s z c z e n i e

**Wprowadzenie.** W artykule przeanalizowano zmiany właściwości chemicznych (pH, zawartość cukru) fermentowanego napoju herbacianego podczas procesu fermentacji oraz wpływ tych właściwości na cechy sensoryczne produktu końcowego.

**Wyniki i wnioski.** Średnia zawartość cukru zmniejszyła się o 10,6 % w szóstym dniu fermentacji; największy spadek zawartości cukru w tym dniu odnotowano w próbce herbaty miętowej (18,7 %), natomiast najmniejszy – w zielonej herbacie z dodatkami (imbir i cytryna – 8,0 %; żeń‑szeń i cytryna – 6,5 %) oraz w czarnej herbacie (8,1 %). Średnia wartość pH wyniosła 3,13 ± 0.15 – czyli poniżej poziomu 4,2, po którego osiągnięciu literatura naukowa zaleca zakończenie fermentacji ze względu na możliwy negatywny wpływ napoju na zdrowie. pH próbek zielonej herbaty bez dodatków było wyższe niż średnia (3,13 ± 0.15) – 3,20 ± 0.12 (mięta), 3,23 ± 0.15 (zielona herbata), natomiast pH próbki czarnej herbaty wyniosło 3,0 ± 0.2– było więc najniższe spośród wszystkich badanych próbek. Wykazano dodatnią korelację między zawartością cukru, zmianą pH a temperaturą, jednak o różnej sile: zależność między pH a temperaturą była średnia (r = 0,682), natomiast między zawartością cukru a temperaturą – niska (r = 0,305).

**Słowa kluczowe:** fermentowany napój herbaciany, pH, zawartość cukru, smak, zapach.