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# RESEARCH ON THE EFFECT OF CULTURE TIME ON THE KOMBUCHA TEA BEVERAGE'S ANTIRADICAL CAPACITY AND SENSORY VALUE

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## ABSTRACT

**Background.** Recent consumption trends shows high consumer acceptability and growing medicinal interest in the biological value of kombucha tea. This tea is a sweetened tea leaf brew fermented with a layer containing mainly acetic acid bacteria, yeast and lactic acid bacteria. The main antioxidants in tea leaves are polyphenols, the consumption of which is proven to be beneficial for human health, e.g. protecting from reactive oxygen species (ROS). The aim of the present research was to evaluate antiradical activity, total polyphenol content (TPC) and sensory value of kombucha tea brews.
**Material and methods.** In the present study, Kombucha tea beverages were analyzed for TPC content, DPPH radical scavenging method and sensory value.
**Results.** The highest TPC content and DPPH radical scavenging capacity values were evaluated in yellow tea samples, both unfermented and kombucha, which did not differ within the storage time. The results of sensory evaluations of kombucha tea brews depend on the tea leaf variety used for preparing the drink.
**Conclusions.** Research indicates that the fermentation process of tea brews with kombucha microbiota does not affect significantly its polyphenol content and antiradical capacity, and retains its components' biological activity.

**Key words:** tea, *Camellia sinensis*, kombucha, antioxidant, radical scavenging activity, polyphenols, sensory value

## INTRODUCTION

The latest research shows a growing interest in microbial fermented tea, which has become a very popular drink in some European and American countries. Although kombucha, a natural microbial fermentation product, has a long history in China, scientific research into it started late. Traditionally, kombucha tea is a mixture of a tea leaf brew, sugar, living microorganisms and their metabolites (Blanc, 1996). The kombucha tea beverage is a sparkling, sour and refreshing drink similar to apple cider. The kombucha fermentation strain is composed of acetic acid bacteria, yeast and lactic acid bacteria in different combinatoins. However, yeast and acetic acid bacteria represent a mutually beneficial symbiotic relationship in the kombucha drink. At the beginning of the kombucha fermentation stage, acetic acid bacteria can not use sucrose directly or do so at a very low rate. The yeast, however, will degrade sucrose into glucose

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and fructose, then ferment further to produce ethanol (Sievers et al., 1996). Acetic acid bacteria in the glucose and fructose abundant culture medium start to grow and reproduce. Following the production of ethanol by yeast, acetic acid is metabolized from the glucose. Data show that yeast produce ethanol and stimulate the growth of acetic acid bacteria, producing a cellulose acetate membrane. Bacteria producing acetic acid will further stimulate yeast to produce ethanol, whose presence protects the yeast and bacteria from contamination by other microorganisms (Liu et al., 1996). Research shows that the kombucha fermentation broth strongly inhibits pathogenic bacteria (*Epidericha coli, Salmonella enteritidis, Shigella dysenteriae, Listeria monocytogenes, Pseudomonas fluorescens* and *Staphylococcus aureus*). It was found that *Lactobacillus* is able to secrete bacteriocin – plantaricin, a thermally stabile small peptide, which significantly inhibits gram-positive and gram-negative bacteria under acidic conditions (Holo et al., 2001; Sreeramulu et al., 2000). Furthermore, the kombucha broth exhibited strong antiradical activity in scavenging the hydroxyl radical, DPPH and superoxide anions (35%, 60.07% and 3.80%, respectively). Traditionally, the substrate for kombucha fermentation substrate are green and black tea leaves brew sweetened with sucrose, although other plants like mulberry and herbs, or fruit juices are also reported to be used in the manufacturing of kombucha beverage with satisfying quality (Velicanski et al., 2013; Yavari et al., 2010).

Kombucha contains glucuronic acid, gluconic acid, acetic acid, alcohol, lactic acid, amino acids, proteins, folic acid, and a variety of B vitamins (Chen and Liu, 2000). Analysis of two kinds of kombucha from Taiwan showed that it contains glycerine, acetic acid and ethanol (Liu et al., 1996). A kombucha tea leaf brew also contains a variety of polyphenols, mainly catechins such as EGCG, GCG, EGC, ECG, EC, although EGCG (18%) and ECG (23%) are subjected to degradation throughout the kombucha fermentation process (Jayabalan et al., 2007). A reduction in EGCG and ECG degradation was noticed in green as compared to black tea kombucha, but it was found to be converted to corresponding catechins: EGC and EC. Other components of tea, like thearubigins and theaflavin, were consistently degraded. Kombucha drinks contain trace elements beneficial to the human body: e.g. zinc, copper, iron, manganese, nickel and cobalt, the content of which is higher than in pure tea brews, despite the content of harmful elements, such as lead and chromium is lower in kombucha tea (Bauer-Petrovska and Petrushevska-Tozi, 2000).

The mechanism of action related to kombucha's benefits for human health has not yet been completely revealed. It is known that kombucha tea is a detoxyfing and energizing drink, exhibiting hypoglycemic, hypocholesterolemic, and antioxidative activity (Bhattacharya et al. 2013; Shenoy, 2000; Yang et al. 2009). Kombucha is a source of antimicrobials, and can prevent hepatotoxicity and cancer (Afsharmanesh and Sadaghi, 2014; Battikh et al., 2012; Jayabalan et al., 2011). Kombucha contains tea leaves brewing, which health effect and antioxidants composition is greatly acknowledged (Gramza-Michałowska, 2014; Gramza-Michałowska et al., 2007; Kujawska et al., 2016a; 2016b; Mika et al., 2015).

In this experiment, four kinds of kombucha tea brews were evaluated for antiradical capacity and total polyphenol content, with the aim being to check the hypothesis that using kombucha microbiota enhances the antiradical activity of the tea beverage.

## MATERIAL AND METHODS

The research was conducted on kombucha beverages based on tea leaves brews, and control samples of pure tea brews. For the research four kinds of dried tea leaves were selected: white (Pai Mu Tan – Fujian, China), green (China Lung Ching – Zhejiang, China), yellow (China Kekecha – Guangzhou, China), black (Yunan Golden Leaf – Yunnan, China), supplied from the local tea store (Dom Herbaty, Poznań). The kombucha layer was supplied by the Yangjunshijia company (China) and analyzed.

Microbial analysis of the kombucha layer was conducted in accordance with the ISO method (PN-EN ISO 6887-1:2000). Microbial analysis included: the total number of bacteria (broth with 2% of agar, temp. 30°C, 48–72 h); mould and yeast (YGC medium, temp. 20°C, 120 h); lactic acid bacteria (MRS-agar medium, 37°C, 48–72 h); and *Escherichia coli* (Macconkey medium, temp. 37°C, 24–48 h). The microorganism number was presented as an arithmetic mean of the total colony-forming units [CFU] in 1 g of the product.

The kombucha fermentation process was conducted according to Hoon et al.'s method (2014) with slight modifications. Four grams of tea leaves were added to 1 L of boiling distilled water and allowed to infuse for 10 min, after which the infusion was filtered through a sterile sieve, following the addition of sucrose (80 g). The infusion was left to cool (30 ±2°C), and was then poured into sterile jars with lids protected from sunlight, inoculated with a kombucha layer and incubated for 11 days of fermentation at 28 ±2°C. The control samples were subjected to the same procedure except for kombucha layer inoculation (Fig. 1).

To evaluate antioxidant activity changes during storage, samples of kombucha beverages were stored for eleven days, in accordance with the approximate time that the consumer could store the product without any loss of quality (traditional recipes advise a fermentation process of 5–11 days). No signs of changing the direction of fermentation were noticed (no negative change in aroma and colour). Prior to the evaluation of total phenol content (TPC) and DPPH radical scavenging assay, the samples were collected every 4th day of the fermentation process, filtered with syringe filters (Membrane Solutions PTFE 0.45 μm) then centrifuged (5 min, 2000 rpm) and the supernatant was stored for further analysis. The pH scale of kombucha tea was evaluated using a pH-meter (Mettler Toledo).


**Fig. 1.** Pure tea and kombucha tea with microbiota layer appearance

The kombucha samples were evaluated according to TPC using the Folin-Ciocalteu reagent colorimetric method (Shahidi and Naczk, 1995). The absorbance of a sample was measured spectrophotometrically at a wavelength $\lambda = 725$ nm (Metertech). Results were evaluated on the basis of a standard curve for $y = 4.5251x - 0.0172$ ($R^2 = 0.9904$) and presented as mg of gallic acid equivalents (GAE) per 200 ml of infusion.

The DPPH procedure described by Sánchez-Moreno et al. (1998) based on the absorbance decrease of DPPH solution (2,2-diphenyl-1-picrylhydrazyl) at $\lambda = 515$ nm in the presence of antioxidants. The DPPH radical scavenging activity was evaluated on the basis of a standard curve for $y = 83.8x - 0.0172$ ($R^2 = 0.9718$), and presented as mg of Trolox equivalent (TE) per 200 ml of the beverage.

The dry matter of kombucha beverage samples was determined by drying at 103 ±2°C until a constant weight is reached (ISO 1572:1980).

Sensory evaluation was carried out in a sensory analysis room on kombucha and control tea beverages, using 10 ml of the drink for a selected and trained sensory panel of 14 testers. Each beverage sample was subsequently coded with letters and evaluated in triplicate for overall acceptability, consistency, clarity, color, taste and aroma. The panelists rated each of four kombucha tea samples using an unstructured hedonic scale with border variants for each attribute ranging from 1 (strongly dislike), 5 (neither like or dislike) to 10 (like strongly) for overall acceptability, consistency and clarity (ISO 4121:2003). The color, taste and aroma was evaluated with border variants for each attribute ranging from 1 (noticeable) to 10 (intense). The results of sensory analysis ratings were calculated for all the attributes of each beverage and session separately.

Data representing the mean values of three independent experiment repetitions were analyzed using one-way analysis of variance (ANOVA), which was used to determine the samples' value differences, and as well as Tukey's multiple-range test ($p < 0.05$). All statistical analyses were performed using *Statistica* software (StatSoft).

## RESULTS AND DISCUSSION

The kombucha layer applied for the tea brews production was primarily analyzed according to microbiota content. The results of microbiological analysis showed that acetic acid bacteria and yeast are the dominant ones in the kombucha layer (Table 1). Standard acetic acid bacteria work well with yeast in the culture media, and tolerate the alcohol produced, which usually inhibits bacteria growth (McDonnell and Russell, 1999). The analysis showed no lactic acid bacteria and *Escherichia coli* present in the cultured kombucha layer.

**Table 1.** Microbiota levels in the kombucha layer sample

| Microbiota | Microbiota number log 10 CFU/1 g of sample |
|---|---|
| Total number of bacteria | 2.96 ±0.03 |
| Acetic acid bacteria | 2.73 ±0.10 |
| Yeast | 2.90 ±0.06 |
| Lactic acid bacteria (MRS) | absent |
| *Escherichia coli* (MAC) | absent |

The pH scale of kombucha tea was evaluated every 4th day of the fermentation process to ascertain whether the process is conducted correctly. Initially, the tea brews showed pH = 7.8, then after the addition of the kombucha layer, this decreased to 4.2 (4$^{th}$ day), and 3.4 (7$^{th}$ day), reaching 2.9 on the 11$^{th}$ day of the fermentation process. Total polyphenol content in the samples tested was found to be affected by the kind of tea leaf used for the brewing process. The highest TPC was identified in samples of yellow tea (15.92 mg GAE/200 ml), then in green tea (9.89 mg GAE/200 ml), and the lowest content was identified in samples of white (5.93 mg GAE/200 ml) and black tea (5.79 mg GAE/200 ml). The samples' polyphenol content did not change significantly during the 11 days of storage, and was similar for pure tea and kombucha tea brews (Fig. 2). Chu and Chen (2006) identified up to 7.8 mM of gallic acid equivalent. The total phenol content increased up to 98%, suggesting thearubigin biodegradation during fermentation, resulting in the release of smaller molecules with higher antioxidant activities. The results of the present research did not confirm these findings.

A DPPH radical scavenging assay is used to characterize the substance antioxidant capacity in the presence of free radicals, usually expressed as an inhibition. A higher DPPH radical scavenging value indicates stronger antiradical capacity of the substance examined. Scientific research shows that tea polyphenols are major antioxidants, characterized by strong DPPH radical scavenging capacity, which is up to 100 times higher than ascorbic acid. Tea polyphenols as antioxidants, effectively remove free radicals playing a preventive effect in oxidative changes of the substrate (Gramza-Michałowska et al., 2015).

The evaluation of antiradical activity varied with the tea variety used for brewing (Fig. 2). The highest activity was evaluated for yellow tea through pure brewing and kombucha. The brews were ranked from highest to lowest DPPH radical scavenging potential as follows: yellow tea > yellow tea kombucha > green tea > green tea kombucha > white tea ≥ white tea kombucha > black tea ≥ black tea kombucha. The antiradical activity of yellow tea brews was three times higher than white tea and four times higher than black tea brews' activity. Results showed that a significantly higher DPPH radical capacity in partially fermented tea leaf samples and slightly lower in unfermented green tea, both pure and kombucha brews. The results confirmed previous findings by Gramza-Michałowska et al. (2015), showing significantly higher activity of yellow tea extracts in comparison to other tea samples, which differed within the fermentation process. There was no significant influence of storage time on the DPPH radical scavenging capacity of white, green and black teas, neither pure and kombucha. However, differences were found in samples of yellow tea, where the antiradical capacity decreased by 20% in comparison with the first day of storage. Kombucha yellow tea samples were significantly

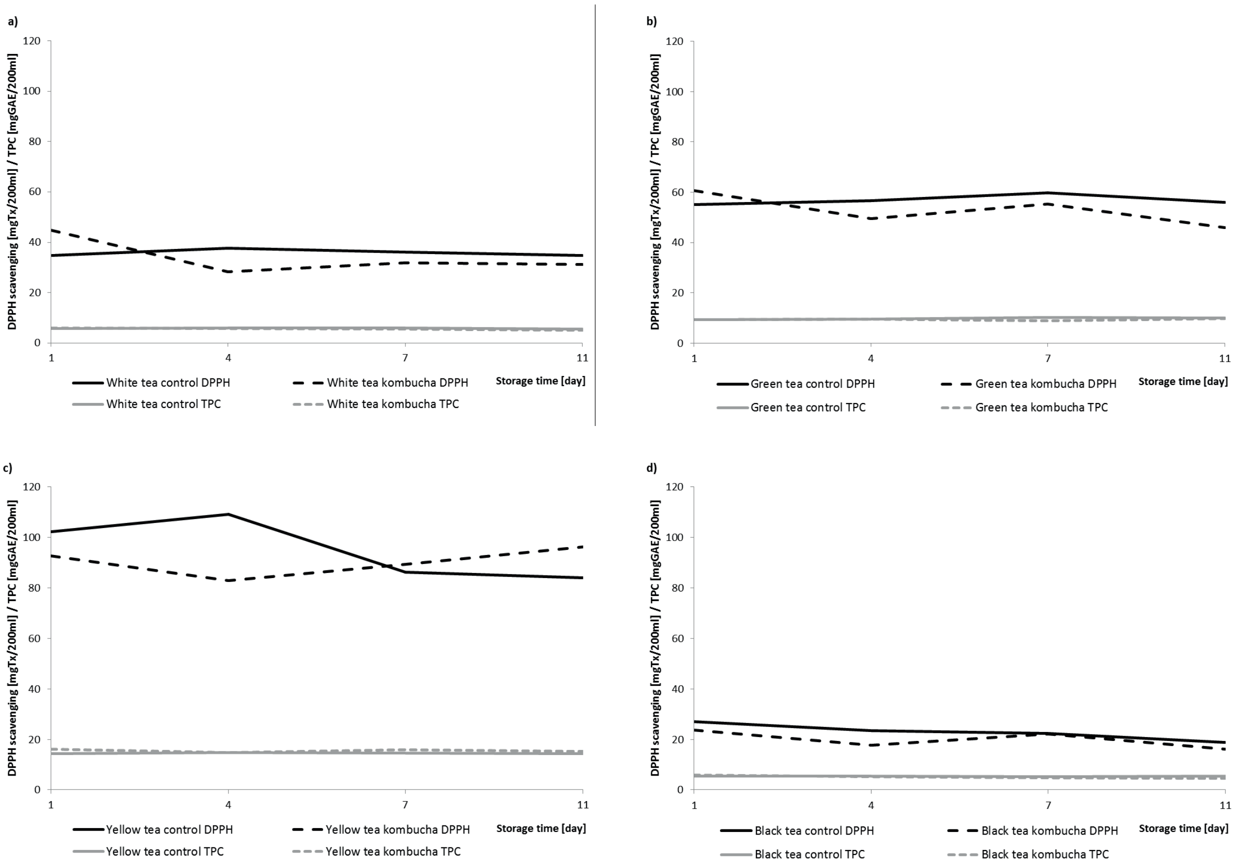


**Fig. 2.** Total polyphenol content (TPC) and DPPH radical scavenging activity of stored kombucha tea leaf brews: a) white, b) green, c) yellow, d) black

decreased on 4th day of storage and increased on 11th day, reaching the initial capacity. In summary, there were no statistically significant differences between the samples of pure tea and kombucha brews, showing that the addition of microbiota into tea brews does not change their antioxidant potential, nor their polyphenol content. The differences in DPPH radical scavenging capacity were due to the different methods of processing tea and the initial polyphenol content, of which yellow and green tea contains a higher amount of biologically active substances. Jayalaban et al. (2008) suggested that structural modification of tea components is due to enzymes liberated by bacteria and yeast during kombucha fermentation. Confirmation of the above results was found in research by Hoon et al. (2014), who examined different tea leaf kombucha brews and found that the polyphenol content and antioxidant activity increased, while pH decreased gradually. Chu and Chen (2006) evaluated the effects of kombucha origins and fermentation time on their antioxidant properties and found that after 15 days of fermentation, the average antioxidant potential increased to about 70% (DPPH), 40% (ABTS), 49% (linoleic acid peroxidation), while ferrous ion binding ability was inversely diminished by 81%. Our results showed slightly changing stability during fermentation. DPPH radical scavenging capacity reduced at a slow rate. Mester and Tien (2000) suggested that the enzymes produced during the fermentation degraded aromatic hydrocarbons in non-specific, radical-based oxidation, which could result in the appearance of more hydrophilic components. Chu and Chen (2006) concluded that phenol content might not determine the antioxidative potential of kombucha, whereas the metabolites produced play an essential role. The differences in DPPH radical scavenging capacity are due to different processing methods of tea and the initial polyphenol content, of which yellow and green tea contains a higher number of biologically active substances (Jayabalan et al., 2008; Malbaša et al., 2011). Jayabalan et al. (2014) suggested that the antioxidant activity of kombucha tea is due to the presence of tea polyphenols and ascorbic acid. Furthermore, it could be a result of the production of low-molecular-weight component and the structural modification of polyphenols by bacteria and yeast enzymes. Their findings showed that the activity depended upon the tea material used, fermentation time, and microbiota of the kombucha culture. However, prolonging the fermentation process results in the accumulation of organic acids, which could exhibit a harmful effect on humans after consumption (Vijayaraghavan et al., 2000). Mo et al. (2008) suggested that natural antimicrobial agents from kombucha fermented teas may offer innovative and interesting applications as natural and biological preservatives in food products.

### Comparison of different types of kombucha drinks and their effect on dry matter

Results showed a significant decrease in the dry mass of kombucha tea brews in comparison with unfermented samples. The highest decrease in dry mass was evaluated in samples of white and yellow tea kombucha, while green and black tea did not differ during the storage (Fig. 3). It was found that kombucha fermentation product content depends on the kombucha layer microbiota composition, and on the enzymes produced (Chu and Chen, 2006). Other authors suggested that biomass yield changes should be ascribed to the nitrogen-containing compounds present in the system (Malbaša et al., 2008). It was noted that the level of these compounds was higher in the systems with molasses than with sucrose, and correlated positively with the higher yield of kombucha biomass. This activity statement is due to the fact that kombucha microbiota continuously consume sugar following the development of the kombucha strain. Moreover, the loss of kombucha activity could be the reason why many substances produced during the fermentation stage cannot be consumed, or why insufficient amount of sugar is consumed. The occurrence of this phenomenon prevents a regular solution balance from being maintained, leading to the kombucha strain not having enough nutrients, and finally resulting in the death of the strains in the solution.

### Sensory evaluation of different kinds of tea samples

Evaluation of the sensory value of kombucha tea brews showed moderate acceptability and other descriptors. The highest overall acceptability, consistency, clarity and smoothness were evaluated for white and yellow, while the lowest was for black tea kombucha brew samples (Fig. 4). Clarity scores for four samples also registered approx. 5 points, which might have been the result of *Acetobacter xylinum* producing a fibrous structure, leading to the cloudiness of the liquid.

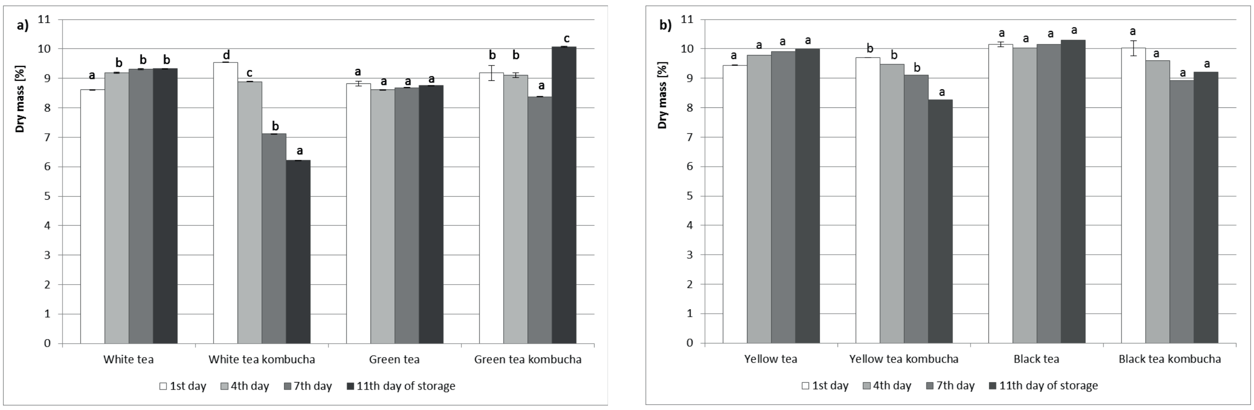


**Fig. 3.** Dry mass changes in different types of pure and kombucha tea brews: **a**) unfermented teas, **b**) fermented teas; a, b, c – mean values with different letters differ statistically according to the beverage sample ($p \leq 0.05$)

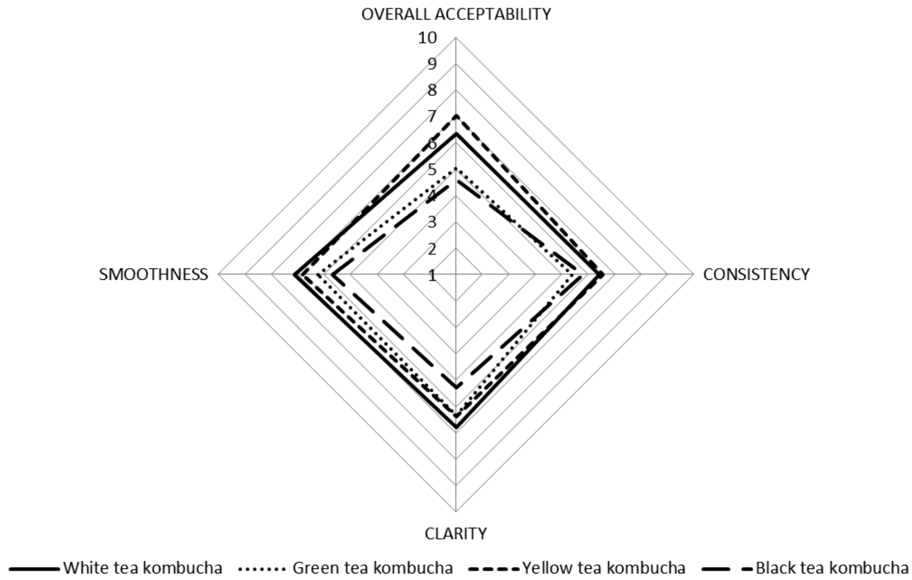


**Fig. 4.** Overall acceptability, consistency, clarity and smoothness evaluation of different tea kombucha brews

Samples of kombucha tea brews differed with regard to color intensity (Fig. 5). It was evaluated that white and green tea kombucha were yellow with a hint of amber and green color. Yellow tea kombucha was a moderately amber and yellow color, whereas black tea kombucha was brown with amber and yellow tones. The taste of kombucha samples was evaluated as depending on the kind of tea leaf used for the kombucha fermentation process (Fig. 6). All samples were characterized by a sweet, sour and citrus taste, although

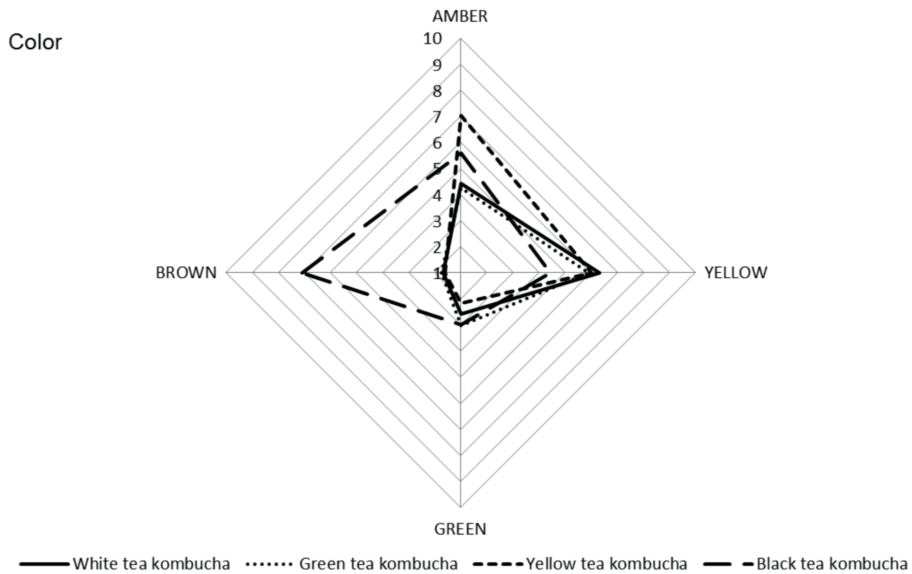


**Fig. 5.** Color evaluation of different tea kombucha brews

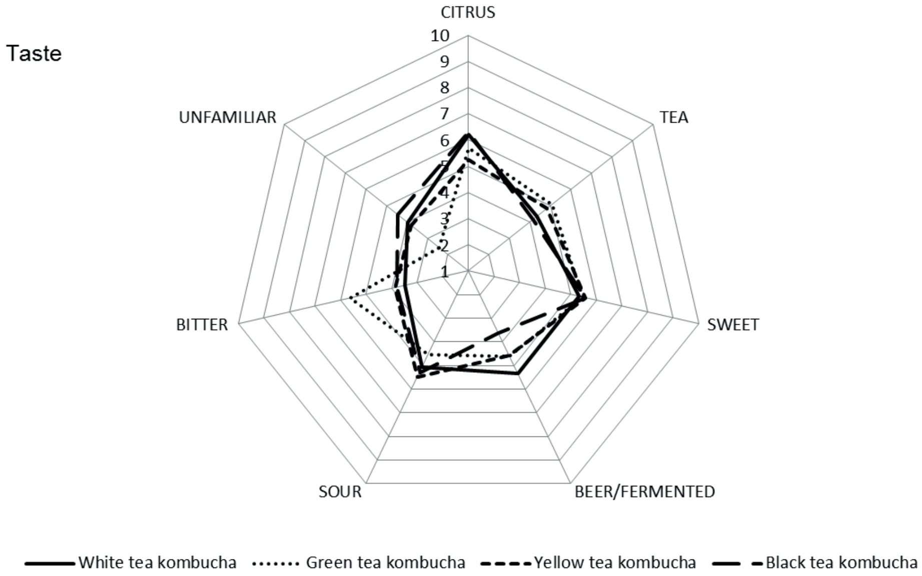


**Fig. 6.** Taste evaluation of different tea kombucha brews

the green tea sample was significantly more bitter in comparison to the other samples. Tea, beer and an unfamiliar taste was slightly noticeable by the panelists. A beer taste is typical for kombucha tea brews, since microbiota fermentation leads to the occurrence of a characteristic taste and aroma, similar to cider.

Acetic acid bacteria produce acid, which is recognized as a citrus taste in brewed tea leaves. Moreover, the tea taste is rather recognized, and kombuchas do not produce an abnormal taste, which has no effect on the original taste of the kombucha tea brew. The bitter taste is reduced by the kombucha layer activity during

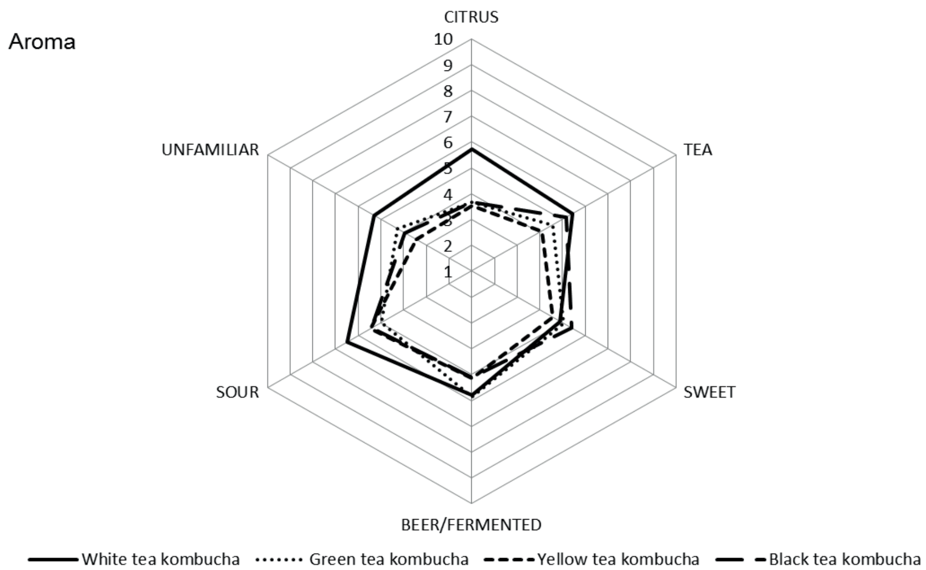


**Fig. 7.** Aroma evaluation of different tea kombucha brews

the fermentation process, since it produces amino acids reducing the bitterness of the tea alkaloids present. Research showed that the acidic substance maintains a balance in kombucha, giving an abundant flavor, graded as sour and beer.

Kombucha tea brews also differed with regard to aroma intensity (Fig. 7). It was evaluated that white tea kombucha was characterized by citrus with an unfamiliar and sour aroma, while other samples were less intense in aromas. All samples were difficult to distinguish, proving that the citrus taste is not intensive and very easy to smell, although acidic substances can volatilize, replacing the citrus flavor with a beer and sour aroma. Kombucha samples were moderately characterized by the tea aroma, which in some cases was slightly noticeable for the consumer panel. Malbaša et al. (2008) also noticed differences in the kombucha samples, and described them as being a light brown colour, sour and sparkling drink. The literature does not show any results of comparisons of kombucha beverages manufactured on the basis of different teas leaf varieties. The findings of a study by Gramza-Michałowska et al. (2016) showed that using yellow tea leaves in other products, e.g. cookies, significantly enhanced their radical scavenging activity, but still did not have a negative influence on the sensory evaluation.

## CONCLUSION

The radical scavenging capacity and total polyphenol content of four kinds of kombucha drinks varied. The highest values were evaluated in samples of yellow tea leaves brews, pure and kombucha, while other samples exhibited significantly lower activity. These results confirmed that the antioxidant activity of tea components was not significantly reduced by kombucha microbiota fermentation processing. Differences in polyphenol content and radical scavenging activity was due to the kind of tea used for the brewing preparation, and was highest in samples of partially fermented yellow tea, and lowest in totally fermented black tea leaves.

The sensory evaluation scores of the four tea brews investigated changed when a kombucha layer was added. The color and taste were most accepted, recording higher scores than those of pure tea brews, although consumers' willingness to accept the overall appearance of the kombucha drink was noticed. From the health point of view, kombucha's radical scavenging activity could possibly influence the antioxidant capacity of the human body, although further research is required to prove the potential health effects of consuming kombucha tea brews.

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