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I. Introduction

Kombucha is a type of refreshing beverage that is commercially produced by many companies and produced homemade. It is widely consumed in the world. It has a delicious taste, antioxidant properties of tea extracts, and beneficial effects of fermentative bacteria, which have antioxidant properties. Because of these, its consumption is increasing day by day. Phenolic substances found in the leaves of tea used in kombucha production also have antimicrobial effects on microorganisms such as *Aeromonas, Bacillus, Clostridium botulinum, Clostridium perfringens, Enterobacter, Klebsiella, Proteus, Pseudomonas, Shigella, Staphylococcus aureus, Streptococcus* [1,2].

Bacteria and yeasts lead a symbiotic life in the cellulosic film layer, where the microorganisms that play a role in the fermentation of kombucha are found. Gram-negative aerobic bacilli, yeasts, and lactic acid bacteria in the *Acetobacteraceae* family were identified in this film layer [3,4,5,6]. This cellulosic structure, which increases in thickness as fermentation progresses, provides the necessary oxygen for microorganisms [3,6]. These microorganisms create free phenolics using phenolic compounds in tea leaves, and they create useful fermentation products such as various organic acids, vitamins, and minerals for the health and also contribute to the sensory properties of the product. The metabolites formed depend on the variety of tea leaves, the variety of microorganisms in symbiotic culture and fermentation conditions [3,6,7,8,9,10].

Kombucha is usually prepared from black tea and green tea. This research was carried out by considering it would be beneficial to prepare kombucha by sage, linden, and mint teas, which are highly valuable for health. In sage, flavones such as apigenin, luteolin, kaempferol, and quercetin were determined.

Carnosol and carnosic acids are also antioxidant phenolic compounds found in sage. Mint *(Mentha piperita L.)* is one of the commonly consumed herbal teas. The leaves of mint contain phenolic components rosmarinic acid and various flavonoids such as eriocitrin, luteolin, and hesperidin. Peppermint has significant antimicrobial and antiviral activities, strong antioxidant and antitumor activities, and some antiallergenic potential [11]. Some of the beneficial effects of kombucha are anti- microbial, antioxidant, anti-carcinogenic, and anti-diabetic properties that have positive effects on stomach ulcers and high cholesterol. It is also recommended for use in the treatment of different diseases such as AIDS, arthritis, atherosclerosis, cancer, constipation, gallbladder diseases, hemorrhoids, hair growth, hypertension, and indigestion. It has also been shown to be effective in immune responses and liver detoxification [9]. Kombucha regulates intestinal flora, strengthens cells, helps the body maintain acid-alkaline balance, and acts as a natural antibiotic [12]. Great attention should be paid in home-made kombucha since pathogenic microorganisms are likely to contaminate the tea. Some people consuming homemade tea have dizziness, headache, nausea, and allergic reactions. For this reason, it can lead to contraindications in pregnant and lactating women and should be consumed with caution [8]. However, as a result of tests carried out by the U.S.

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Food and Drug Administration, kombucha has been reported to be safe for human consumption [10].

In this study, black tea, green tea, sage, mint, and linden teas were used in making the kombucha. The fermentation period was kept for 14 days in making kombucha.

II. Material and Methods

a) Materials

Black tea, green tea, mint, linden, sage, used for making kombucha in the research were obtained from local markets in Tekirdag. Kombucha mushrooms were purchased from the online shopping site via the internet.

b) Preparation and Fermentation of Herbal Teas

In order to extract the teas, 10 g of each tea sample, which is used as a bacterial medium for fermentation, was left in 1 L of boiled water for 15 minutes [13]. After extraction, the suitable medium was prepared by adding 70 g/L of sucrose. The prepared tea samples were transferred to glass jars, which were sterilized at 121°C for 20 minutes (Fig. 1). After we waited until these samples reached room temperature, all samples were inoculated with 7 g/L kombucha mushroom culture. After glass jars were covered with cheesecloth, left to fermentation at 24 ± 1°C and in the dark for 14 days. Tea samples were taken from the incubator at 3-day intervals, and analyzes were performed.

Figure 1: Black, green, mint, linden and sage teas before fermentation

c) Microbiological Analysis

Serial dilutions of kombucha samples were prepared in aseptic conditions using sterile saline containing 0.89% NaCl. MRS Agar (de Man Rogosa Sharpe, Merck) was used for counting lactobacilli, Petri plates were incubated for 72 hours at 30°C [14]. Lactococci were counted after 48 hours incubation at 30°C in M17 Agar (Oxoid) [15]. PDA (potato dextrose agar) was used for total yeast count, and Petri plates were incubated for three days at 25°C [16]. For total acetic acid bacteria count, a medium containing 1% glucose, 1% yeast extract, 2% $CaCO_3$, and 1.8% agar (Calcium Carbonate Agar) was prepared and sterilized, and then 3% ethanol (95%) (Merck 100983) was added. Then the Petri dishes were incubated for three days at 30°C [17]. Colonies were evaluated for all microorganisms at the end of the incubation, and the results were expressed as "log CFU/mL".

d) Statistical analysis

To test the differences between the parameters analyzed before and after fermentation in tea samples, two ways analysis of variance (ANOVA) was performed. Significant differences between means were determined by Duncan's multiple range tests at $P<0.05$ significance level.

III. Results and Discussion

Kombucha is a fermented beverage. Ambient temperature, pH, oxygen content, dissolved CO_2, process conditions, and composition of the medium affect fermentation [18]. As a result of this, the nutritional quality, the organoleptic properties, and other physicochemical properties of the product are affected. The different plant varieties, sugar concentrations, fermentation time, and the composition of the tea mushroom also affect fermentation and the properties of the fermented product [10,19].

Kombucha is created with the help of a symbiotic culture. This symbiotic culture consists of acetic acid bacteria (*Gluconobacter* and *Acetobacter* sp., *Komagataeibacter*) [20] lactic acid bacteria (*Lactobacillus*, *Lactococcus*) [18]. And yeasts *(Saccharomyces cerevisiae, Saccharomycodes ludwigii*,

Schizosaccharomyces pombe, Zygosaccharomyces bailii, Kloeckera apiculata, Torulaspora delbrueckii, Brettanomyces bruxellensis [21], *Candida, Pichia, Lachancea,* and *Kluyveromyces* [9,18,21].

Kombucha fermentation includes alcohol, lactic acid and acetic acid fermentation. In the fermentation of Kombucha, fermented tea and biofilm are formed as a result of the activity of different yeasts and bacteria species. First, in fermentation, sucrose breaks down into glucose and fructose as a result of yeast hydrolysis. Alcohol occurs as a result of fermentation of glucose and fructose. Then acetic acid bacteria convert alcohol into acetic acid. In addition to acetic acid, gluconic and glucuronic also occur [10].

Kombucha tea is usually made from black and green tea. In this study, different herbal teas known to be beneficial for health were also used. The aim is to make the benefit of the kombucha tea even more useful with these herbs. The number of *Lactobacillus*, *Lactococcus*, yeast, and acetic acid bacteria determined during fermentation were shown in Table 1.

Table 1: Changes of some microbiological properties of Kombucha samples during fermentation (log CFU/mL)

Samples	Days	*Lactobacillus* log CFU/mL	*Lactococcus* log CFU/mL	Yeast log CFU/mL	Acetic Acid Bacteria log CFU/mL
Black Tea	3	5,53 A*a**	6,47 ABb	6,45 Bab	6,05 Cd
	7	6,04 Ab	5,37 ABb	6,30 Bc	6,01 Cc
	10	5,90 Aab	5,09 ABb	6,21 Bb	4,44 Cb
	14	5,56 Aa	5,00 Aba	5,39 Ba	2,00 Ca
Green Tea	3	6,07 Ba	4,74 Bb	4,86 Bab	4,44 Ad
	7	6,65 Bb	6,30 Bb	6,41 Bc	4,49 Ac
	10	6,25 Bab	6,34 Bb	6,23 Bb	5,38 Ab
	14	6,26 Ba	5,03 Ba	6,23 Ba	4,82 Aa
Mint Tea	3	5.12 Aa	6,32 ABb	4,67 Aab	5,90 Bd
	7	5,98 Ab	5,70 ABb	5,77 Ac	5,77 Bc
	10	5,19 Aab	5,05 ABb	5,01 Ab	4,95 Bb
	14	5,37 Aa	4,97 Aba	4,97 Aa	2,98 Ba
Linden Tea	3	6,31 Ba	5,48 Ab	6,31 Cab	5,07 Ad
	7	6,50 Bb	5,61 Ab	6,90 Cc	4,79 Ac
	10	6,56 Bab	6,14 Ab	6,29 Cb	3,33 Ab
	14	6,12 Ba	4,01 Aa	5,86 Ca	2,22 Aa
Sage Tea	3	5,64 Aa	5,27 Bb	5,88 Bab	6,30 Dd
	7	6,22 Ab	6,01 Bb	6,19 Bc	6,13 Dc
	10	6,27 Aab	6,18 Bb	6,62 Bb	5,84 Db
	14	5,56 Aa	6,05 Ba	6,04 Ba	5,73 Da

* Capital letter refers to the difference between tea varieties.
** Lower case refers to the difference between fermentation days.

Lactobacillus number of black, green, and mint teas reached the highest value on the 7th day of fermentation. *Lactobacillus* number of linden, and sage teas reached the highest number on the 10th day of fermentation (Fig. 2). As a result of statistical analysis, the difference between the tea varieties in terms of lactobacilli numbers was found significant at the level of $P<0.01$ ($F=7,716^{**}$). The difference between fermentation days was also found significant at $P<0.01$ ($F=4,498^{**}$). Degirmencioglu et al. [6] prepared the teas from white, green, black, oolong and pu'er tea leaves (100g / L sugar and 6g / L tea leaf, 100 mL / L fermented kombucha and SCOBY) at 30°C after 21 days fermentation time. In their study, there was a rapid increase in lactic acid bacteria numbers until the 3rd day of fermentation, and this increase continued until the 12th day of fermentation. The highest value in LAB numbers was determined as 5.80 log CFU/mL in green tea. Their results are similar to those of this study.

The *Lactococcus* number increased rapidly in all samples until the 3rd day of fermentation. *Lactococcus numbers* decreased in the kombucha samples prepared from black tea and mint tea after the 3rd day of fermentation and in the others after the 10th day of fermentation (Fig. 3). As a result of statistical analysis, the difference between the tea varieties in terms of *Lactococcus* numbers was found to be significant at the level of $P < 0.05$ ($F = 3.194^{*}$) and the difference between the fermentation days at the level of $P < 0.01$ ($F = 11.643^{**}$). Due to lactic acid they produce, Lactic streptococci and lactobacilli can lower the pH to 4.5-4.3 and 3.5-3.2, respectively [22]. At low pH, lactic acid is in toxic form for most bacteria and yeasts. Highly produced acetic acid by the lactic acid bacteria interacts with the cell membrane. It can affect intracellular acidity and cause protein denaturation. The acidity constant of acetic acid is higher than that of lactic acid (lactic acid 3.08, acetic acid 4.75). Lactic acid increases the effect of acetic acid by lowering the pH of the medium [23]. Kılıç [24] stated that acetic acid is effective on *Saccharomyces* sp.

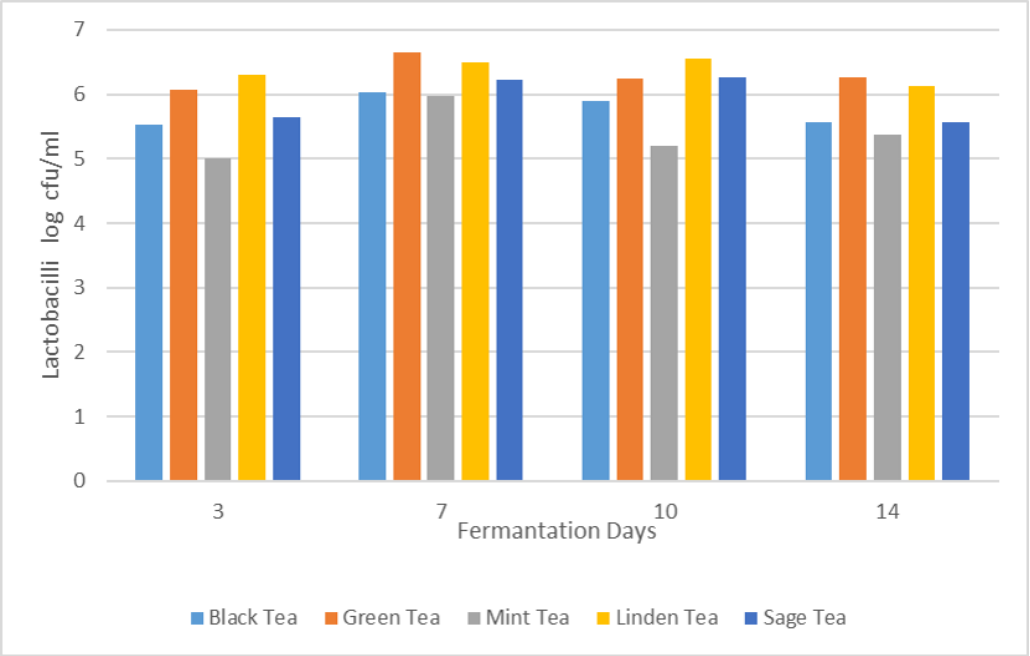

Figure 2: Changes of *Lactobacillus* counts in Kombucha samples during fermentation

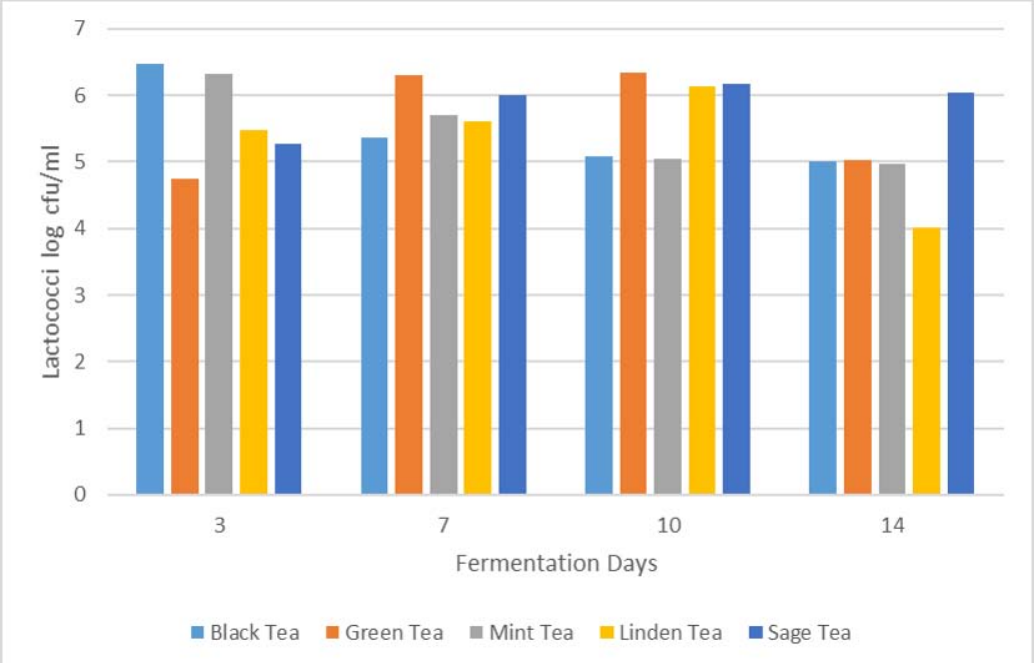

Figure 3: Changes of *Lactococcus* counts in kombucha samples during fermentation

The number of yeasts increased rapidly in all samples until the 3rd day of fermentation. Yeast numbers decreased after the 3rd day of fermentation in the sample prepared from black tea, and after the 7th day of fermentation in other tea samples (Fig. 4). As a result of statistical analysis, the difference between tea varieties and days in terms of yeast numbers was found to be significant at the level of $P<0.01$. ($F=9,715^{**}$; $F=9,890^{**}$). In the study of Degirmencioglu et al. [6], the number of yeast in the green tea sample increased until the 12th day of fermentation, the number of yeast in the black tea sample was almost unchanged and decreased after the 12th day of fermentation. The yeast numbers in this study are similar to the yeast numbers in their study. The decrease in yeast numbers during fermentation may be due to a decrease in pH value. pH values decreased rapidly starting from the first days. These pH values are far below the pH value of yeasts that are effective in fermentation. However, it has shown that bacteria and yeasts in symbiotic culture can continue their activities. Yıkmış and Tuğgüm [25] produced kombucha from black tea leaves (10g / L tea leaves, 10% sugar, and 10% pre-prepared compost tea) after a fermentation that lasted ten days at 24°C and stored at +4°C. The yeast count of this sample was 2.51 log CFU/mL on the 1st day of storage. That value is considerably lower in our study than the number of yeasts on the 10th day of fermentation of kombucha produced from black tea leaves. Jayabalan et al. [26] prepared kombucha from black tea leaves (1.2% black tea leaves, 10% sugar, 3% SCOBY, 10% fermented kombucha) at the end of fermentation (at 24°C) for 21 days. While the number of yeast was around $45x10^7$ in 7 days of fermentation, it reached $47.5x10^7$ in the 14th day of fermentation. Yeast number is higher than determined in our study. The reason for this difference may be the microflora in the cultures used. The number of yeasts in the kombucha produced from mint leaf was lower than that of the others on all days of fermentation. The reason for this may be an inhibitory effect of peppermint against certain yeasts. Schelz et al. [27] found that peppermint oil was very effective against S. cerevisiae 0425 52C and *S. cerevisiae* 0425 ϕ / 1 strains in their study.

Microbial interactions between *Saccharomyces* and non-*Saccharomyces* yeasts seem to be an advantageous option in mixed fermentation processing, having several benefits like avoiding the risks of a stuck fermentation, the addition of flavors and aromas allows the modification of undesired parameters, between others [28]. The interaction of yeasts in Kombucha causes the formation of the desired properties in the fermented product [30].

The dominant bacteria of Kombucha tea culture are acetic acid bacteria [29]. In this study, the number of acetic acid bacteria increased rapidly until the 3rd day of fermentation in all tea samples, and after the 3rd day of fermentation in samples produced from black, peppermint, linden, and sage tea showed a regular decrease. In the sample produced from green tea, it increased until the 10th day of fermentation and then decreased (Fig. 5). As a result of statistical analysis, the difference between the tea varieties in terms of the number of acetic acid bacteria was found to be significant at the level of $P<0.01$ ($F = 214,793^{**}$). Differences among fermentation days were also significant at the level of $P<0.01$ ($F = 146.318^{**}$) In the study of Degirmencioglu et al. [6], the number of acetic acid bacteria increased rapidly until the 3rd day of fermentation, and the increase was slower until the 12th day. It was faster from the 12th to the 21st day of fermentation. In a study of Jayabalan et al. [26], while the number of acetic acid bacteria of the samples was around $41x10^4$ on the 7th day of fermentation, it increased to around $45.5x10^4$ on the 14th day of fermentation. In our study, the number of acetic acid bacteria decreased. Acetic acid bacteria show the best growth in the range of 25-30 °C. [30]. The optimum pH range for the development of acetic acid bacteria is 5.5-6.5. They can develop even at low pH depending on the presence of oxygen in the environment. Acetophilic ones can develop at pH 3.5, acetotolerant ones can develop between pH 3.5-6.5 [6]. In this study, since the pH fell below three after the 3rd day of fermentation, the number of acetic acid bacteria may decrease. The difference between our study and other researchers study may be the difference of the culture (SCOBY) used, the culture rate, the amount of sugar, the amount of tea leaf, the infusion time of the tea, and the fermentation conditions. Although other researchers added fermented kombucha in addition to SCOBY to the teas that will be produced, fermented kombucha was not used as a culture in this research.

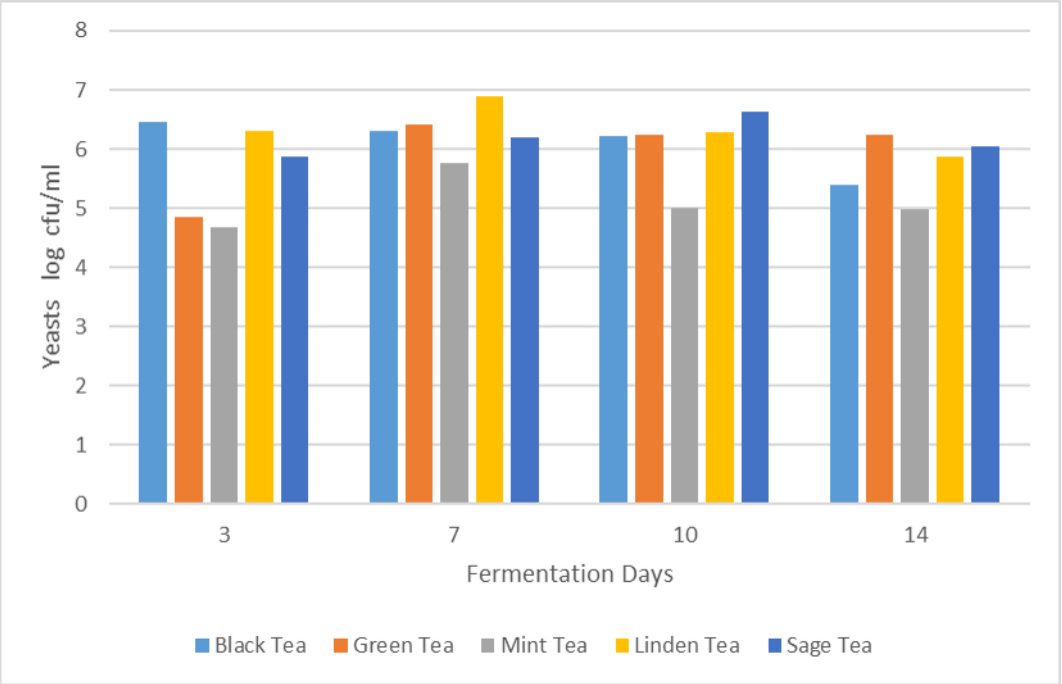

Figure 4: Changes of yeast counts in kombucha samples during fermentation

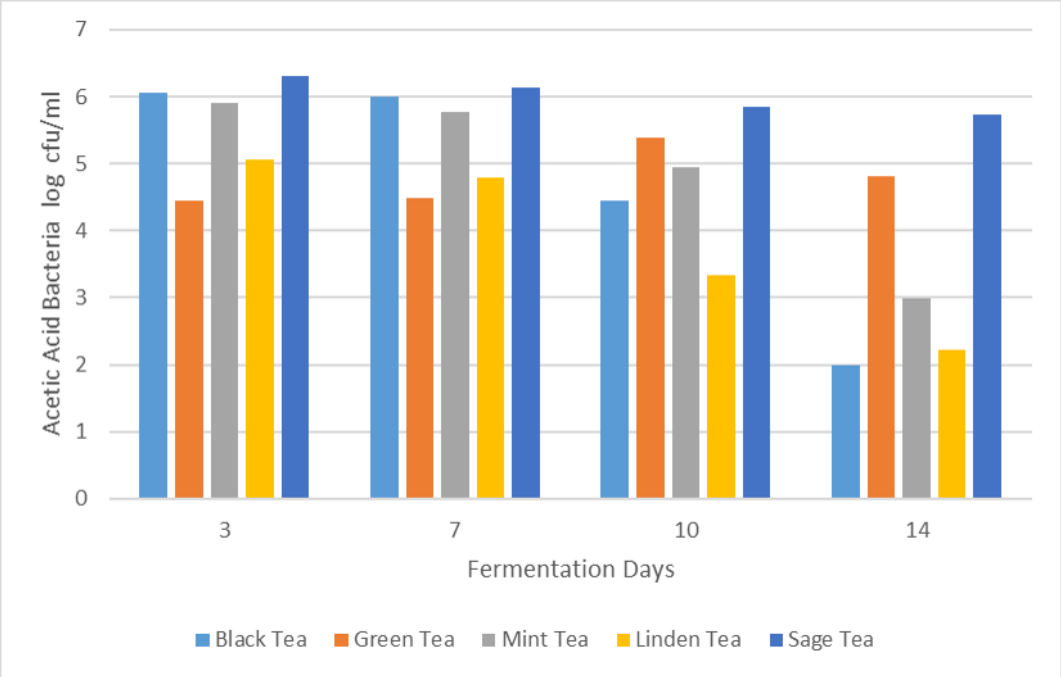

Figure 5: Changes of acetic acid bacteria counts in kombucha samples during fermentation

The kombucha fermentation process leads to the formation of a floating biofilm on the surface of the growth medium due to the activity of certain strains of acetic acid bacteria [31] (*Acetobacter aceti, Acetobacter pasteurianus Acetobacter xylinoides, Bacterium gluconicum*, and *Gluconobacter oxydans*) [8]. It has been reported that the variety in the composition of yeast composition of Kombucha may be due to geographic, climatic and cultural conditions as well as local cross-contamination of yeasts and fungi or possible cross-contamination between cultures [7,32].

In this study, the masses of biofilms formed in the kombuchas produced from black, green, mint, linden, and sage teas were determined as 43.85, 20.70, 7.44, 4.64, and 3.90g at the end of fermentation, respectively (Figure 6,7). The effect of peppermint,

linden, and sage on the growth and activity of some microorganisms may have affected biomass formation. The weight of the biomass formed in the kombuchas obtained from these plants is less than the weight of the biomass in the kombuchas obtained from black and green teas.

Figure 6: Biofilms formed in the kombuchas and kombuchas produced from black, green, mint, linden and sage teas at the end of fermentation

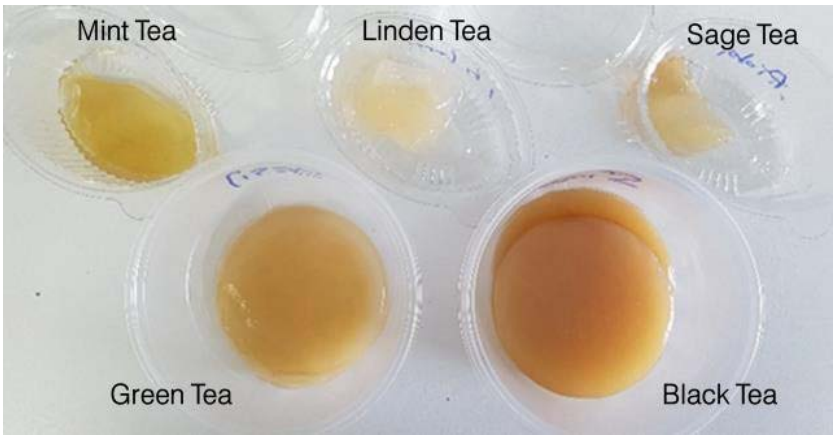

Figure 7: Biofilms formed in the kombuchas at the end of fermentation

IV. Conclusion

The number of Lactobacillus in the kombucha produced from green tea and linden was higher than that of other samples in all days of fermentation. While *Lactococcus* numbers in the kombucha samples produced from black tea and mint continuously decrease during fermentation, *Lactococcus* numbers in the other tea samples increased until the 10th day of fermentation and then decreased. While the yeast number of the kombucha produced from green tea, mint and linden was highest on the 7th day of fermentation, it was the highest on the 10th day of fermentation in the kombucha produced from sage. Acetic acid bacteria increased in only the green tea until the 10th day of fermentation and then decreased. Their number in other tea samples decreased from the 3rd to the 14th day of fermentation. Different herbal teas can be used to produce kombucha with enhanced functional properties. Researching the functional properties of these teas and publishing the results will enlighten the producers and consumers.

References Références Referencias

1. Chung, K. T., Wei, C. I., & Johnson, M. G. (1998). Are tannins a double-edged sword in biology and health? Trends in Food Science and Technology, (4), 168–175. https://doi.org/10.1016/S0924-2244(98)00028-4.

2. Coşkun, F. (2006). Gıdalarda Bulunan Doğal Koruyucular. Gıda Teknolojileri Elektronik Dergisi, 2006(2), 27–33.
3. Leal, J. M., Suárez, L. V., Jayabalan, R., Oros, J. H., & Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites. CYTA - Journal of Food, 16(1), 390–399. https://doi.org/10.1080/19476337.2017.1410499
4. Kurtzman, C. P., Robnett, C. J., & Basehoar-Powers, E. (2001). *Zygosaccharomyces kombuchaensis*, a new ascosporogenous yeast from "Kombucha tea." FEMS Yeast Research, 1(2), 133–138. https://doi.org/10.1016/S1567- 1356(01)00021-6
5. Velićanski, A. S., Dragoljub, D., Markov, C. S. L., Tumbas Šaponjac, V. T., & Vulić, J. J. (2014). Antioxidant and antibacterial activity of the beverage obtained by fermentation of sweetened lemon balm (*Melissa officinalis L.*) tea with symbiotic consortium of bacteria and yeasts. Food Technology and Biotechnology, 52(4), 420–429.
6. Degirmencioglu, N., Yıldız, E., Sahan, Y., Güldas, M., & Gürbüz, O. (2019). Fermentasyon Süresinin Kombu Çayı Mikrobiyotası ve Canlılık Oranları Üzerine Etkileri. Akademik Gıda, 17(2), 200–211. https://doi.org/10.24323/akademikgida.6135 67
7. İleri-Büyükoğlu, T., Taşçı, F., & Şahindokuyucu, F. (2010). Kombucha ve Sağlık Üzerine Etkileri. Uludag Univ. J. Fac. Vet. Med., 29(1), 69–76.
8. Jayabalan, R., Malbaša, R. V., Lon čar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Comprehensive Reviews in Food Science and Food Safety, 13(4), 538–550. https://doi.org/10.1111/1541-4337.12073
9. Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. International Journal of Food Microbiology, 220, 63–72. https://doi.org/10.1016/j. ijfoodmicro.2015.12.015
10. Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., & Taillandier, P. (2018). Understanding Kombucha Tea Fermentation: A Review. Journal of Food Science, 83(3), 580–588. https://doi.org/10.1111/1750-3841.14068
11. McKay, D. L., & Blumberg, J. B. (2006, August). A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytotherapy Research, Vol. 20, pp. 619–633. https://doi.org/10.1002/ptr.1936
12. Kaufmann K (Ed). (2013). Kombucha Rediscovered!: the medicinal benefits of an ancient healing tea. The United States of America.
13. Battikh, H., Bakhrouf, A., & Ammar, E. (2012). Antimicrobial effect of Kombucha analogues. Food Science and Technology, 47(1), 71–77.
14. De Man J C, Rogosa M & Sharpe M E (1960). A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology 23: 130-135
15. Harrigan WF. 1998. Laboratory methods in food microbiology. Calif.: Academic Press, pp 100.
16. Anonymous (2001). Yeasts, Molds and Mycotoxins, FDA Bacteriological Analytical Manual, Chap. 18
17. Fu, L., Zhang, F., & Zhang, B. (2013). Isolation and Identification of Acetic Acid Bacteria. International Proceedings of Chemical, Biological & Environmental Engineering, 50(24), 114–119.
18. Marsh, A. J., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Fermented beverages with health promoting potential: Past and future perspectives. Trends in Food Science & Technology, 38(2), 113–124. https://doi.org/10.1016/j.tifs.2014.05.002
19. Wolfe, B. E., & Dutton, R. J. (2015). Fermented foods as experimentally tractable microbial ecosystems. Cell, 161(1), 49–55. https://doi.org/10.1016/j.cell.2015.02.034
20. Roos, J. D., & Vuyst, L. D. (2018). Acetic acid bacteria in fermented foods and beverages. Current Opinion in Biotechnology, 49, 115– 119. https://doi.org/10.1016/j.copbio.2017.08.007
21. Coton, M., Pawtowski, A., Taminiau, B., Burgaud, G., Deniel, F., Coulloumme- Labarthe, L., & Coton, E. (2017). Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods. Fems Microbiology Ecology, 93(5), 1–16. https://doi.org/10.1093/femsec/fix048
22. Yüksekdağ, Z. N., & Beyatlı, Y. (2003). Kefir Mikroflorası ile Laktik Asit Bakterilerinin Metabolik, Antimikrobiyal ve Genetik Özellikleri. Orlab On-Line Mikrobiyoloji Dergisi, 1(2), 49–69.
23. Akpınar, D., & Kılıç, G. B. (2012). Antifungal Components Produced From Lactic Acid Bacteria. GIDA, 37(1), 47–54.
24. Kılıç S. 2001. Süt Endüstrisinde Laktik Asit Bakterileri. Ege Üniversitesi Ziraat Fakültesi Yayınları No: 542, İzmir, Türkiye, 451 s.
25. Yıkmış, S., & Tuğgüm, S. (2019). Evaluation of Microbiological, Physicochemical and Sensorial Properties of Purple Basil Kombucha Beverage. Turkish Journal of Agriculture-Food Science and Technology, 7(9), 1321. https://doi.org/10.24925/turjaf.v7i9.1321- 1327.2550.
26. Jayabalan, R., Malini, K., Sathishkumar, M., Swaminathan, K., & Yun, S. E. (2010). Biochemical characteristics of tea fungus produced during kombucha fermentation. Food Science and Biotechnology, 19(3), 843–847. https://doi.org/10.1007/s10068- 010-0119-6.

27. Schelz, Z., Molnar, J., & Hohmann, J. (2006). Antimicrobial and antiplasmid activities of essential oils. Fitoterapia, 77(4), 279–285. https://doi.org/10.1016/j.fitote.2006.03.013.
28. Sun, S. Y., Gong, H. S., Jiang, X. M., & Zhao, Y. P. (2014). Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae* on alcoholic fermentation behaviour and wine aroma of cherry wines. Food Microbiology, 44(5), 15–23. https://doi.org/10.1016/j.fm.2014.05.007.
29. Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during Kombucha tea fermentation. Food Chemistry, 102(1), 392–398. https://doi.org/10.1016/j.food chem. 2006.05. 032
30. Gladysheva, E. K., Skiba, E. A., Zolotukhin, V. N., & Sakovich, G. V. (2018). Study of the Conditions for the Biosynthesis of Bacterial Cellulose by the Producer Medusomyces gisevii Sa-12. Applied Biochemistry and Microbiology, 54(2), 179–187. https://doi.org/10.1134/S0003683818020035.
31. Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2016). Enhancement of the antioxidant and starch hydrolase inhibitory activities of king coconut water (Cocos nucifera var. aurantiaca) by fermentation with Kombucha "tea fungus." International Journal of Food Science & Technology, 51(2), 490–498. https://doi.org/10.1111/ijfs.13006
32. Teoh A.L., Heard G., Cox J., 2004. Yeast ecology of Kombucha fermentation. Int J Food Microbiol, 95, 119-126.