Comparison of Functional Groups in Green Tea Kombucha (Camellia sinensis) and Chamomile Flower Kombucha (Matricaria chamomilla) Using Fourier Transform Infrared Spectroscopy (FTIR)

Yolanda Safira Virginia[1], Amanda Ameliya[1], Syafa Putri Nabila[1], Risa Purnamasari[1], Eva Agustina[1], Nova Lusiana[2]*

[1]*Faculty of Science and Technology, Sunan Ampel State Islamic University, Surabaya, Indonesia*
[2]*Faculty of Psychology and Health, Sunan Ampel State Islamic University, Surabaya, Indonesia*
* novalusiana@uinsa.ac.id

Keywords: Kombucha, Chamomile flowers, Green tea, FTIR.

Abstract: Kombucha is a fermented beverage made by SCOBY (*Symbiotic Culture of Bacteria and Yeast*). Substrates in making kombucha drinks can use ingredients that contain high phenolic compounds, such as green tea and chamomile flowers. Green tea is a type of tea that is not fermented or non-fermented and contains more catechins (one of the flavonoid components) and is beneficial for health. Chamomile flower preparations in the form of tea are believed to reduce pain, because they contain flavonoid compounds that can reduce pain, can cure canker sores, overcome sleep problems, digestion, anxiety. This study aims to determine the functional groups contained in chamomile flower kombucha and green tea. The method used is the analysis of functional groups using *Fourier Transform InfraRed*, then comparisons are made on the resulting functional groups. The results showed that the functional groups contained in chamomile flower kombucha are C-O ether, C-N amine, C=C alkene, C≡C alkyne, C=O ester, and O-H alcohol. While the functional groups found in green tea are C-N amine, C=C alkene, C≡C alkyne, and O-H alcohol. Thus, chamomile flower kombucha and green tea are known to contain secondary metabolite compounds of flavonoids, phenolics, saponins, and tannins.

1 INTRODUCTION

Tea is the most popular drink in society, because tea is the type of drink most consumed by adult humans. Drinking tea is often done by some people in the morning before starting activities, drinking tea is very delicious when it is still warm because it can refresh the body and mind (Anggraini et al., 2018). Green Tea is one type of herbal tea originating from China. This plant is widely cultivated in Southeast Asia as a raw material for making traditional medicine (herbal medicine) This is because green tea contains high amounts of polyphenols, namely 30-40%, higher than black tea which contains 3-10% polyphenols (Amalia et al., 2016).

Green tea is a type of tea that is not fermented or non-fermented and contains more catechins (one of the flavonoid components). Green tea is obtained by drying fresh leaves, has several good effects on health, one of which is to reduce the risk of cardiovascular disease such as blood cholesterol levels and blood pressure. Catechin compounds are secondary metabolite compounds naturally produced by plants and belong to the flavonoid group, catechins in tea leaves are very complex compounds consisting of 6 components, namely Epicatechin (EC), Epicatechin 3-Gallate (ECG), Epigallocatechin (EGC), Epigallocatechin 3-Gallate (EGCG), Catechin (C), Gallocatechin (GC). Tea has the main efficacy derived from the polyphenols contained in it (Kusnan, 2022).

Chamomile is native to Europe and Western Asia, but is currently considered a cosmopolitan species. Since its presence in Mexico in the 16th century, this species has been used mainly to treat diseases related to the gastrointestinal system such as diarrhea. In

other countries, the flowers of this species are often used in tea form to treat spasmolytics and as a sedative (Putra & Septa, 2018). Chamomile or also called chamomile is a plant in the Asteraceae family that grows throughout Europe and Asian regions that have 4 seasons. This plant is also widespread because it is cultivated by humans in North America and Australia, which has many benefits in medicinal herbs. The Latin name of the chamomile plant is Matricaria chamomilla L. This plant can grow as high as 15-60 cm with long and small leaves in groups of three leaves on a stalk. The flowers look like daisy flowers, with white petals with a yellow center. Matricaria chamomilla is used as a traditional medicine to treat wounds, mouth ulcers, inflammations, bacterial infections and other diseases. M. chamomilla in the form of water extracts is often used as a mild sedative.4 Some studies say that this M. chamomilla has functions as anti-inflammatory, anticancer, antioxidant, and antibacterial (Alim & Hayuningtyas, 2023).

Chamomile tea has the property of relaxing tense nerves, this plant contains about 120 chemical compounds including flavonoids, 3% glycosides, azolines, apigenin, and methoxycoumarin. The content of chamomile, especially bisabolol and karmasolen, has anti-inflammatory effects, chamomile is also an analgesic, antipyretic, antirheumatic, anti-inflammatory, carminative and sedative properties known to reduce menstrual pain (Yuliyanti, 2021). M. chamomilla has many benefits for medicinal herbs due to its phytochemical content, including flavonoids, alkaloids, saponins, terpenoids, tannins / phenols, steroids, and chamazulene. Flavonoids can be divided into several parts, the first being flavones (apigenin, luteolin), flavonols (quercetin), flavanols (hesperetin), anthocyanins, and proanthocyanidins or tannins. Flavonoids are proven to improve health, prevent disease, and have antioxidant, cardiovascular disease prevention, anti-inflammatory, anticancer activities, besides that they are very safe and have low toxicity. One of the compounds found in chamomile is flavonoid compounds. Flavonoids contained in this plant have a function as anti-inflammatory. Flavonoids can stimulate the immune system by increasing the activity of macrophages and lymphocytes. Most of the anti-inflammatory effects of flavonoids are on the biosynthesis of cytokine proteins that separate the adhesion of circulating leukocytes to the site of injury (Alim & Hayuningtyas, 2023).

A preliminary study found that 82% of people given chamomile extract reported "complete" pain reduction. This plant has a soothing effect on mucous membranes, and ingredients that have a healing effect. Tea from concentrated chamomile can be used as a mouthwash; three or four times a day for the treatment of aphthae (Putri, 2015). Chamomile is widely used in the form of aromatherapy oil or drunk as tea. Chamomile flowers are often used in tea form to treat spasmolytics and as a sedative. Both fresh and dried chamomile flowers have aromatic, flavoring and coloring properties. They are used in a number of commercial products including soaps, detergents, perfumes, lotions, ointments, hair products, alcoholic beverages and herbal teas. The use of chamomile as an herbal remedy dates back to ancient Greek and Roman times. Chamomile has been used as a traditional herbal medicine because of its calming effect (Putra & Septa, 2018).

The mechanism of chamomile therapy in reducing pain is related to the mechanism of anti-inflammatory effects and arome therapy where nerve fibers in the nose carry sensory input in the brain which is the center of instinct, memory, and various vital functions are formed. Chamomile is most commonly used to treat sleep disorders, digestive problems, pain relief, and many others (Putri et al., 2018). Chamomile tea has a soothing effect on mucous membranes, and the ingredients have a healing effect. Tea from concentrated chamomile can be used as a mouthwash; three or four times a day for the treatment of aphthae (Putri, 2015). The mechanism of flavonoids in inhibiting the inflammatory process in burn wounds through various ways, namely inhibiting capillary permeability, inhibiting the release of serotonin and histamine to the site of inflammation, arachidonic acid metabolism by inhibiting the work of cyclogenase, and the secretion of lysosomal enzymes which are inflammatory mediators inhibition of these inflammatory mediators can inhibit the proliferation of the inflammatory process, neutrophil cells, and endothelial cells (Anisa et al., 2019).

The advantage of kombucha tea over regular tea liquid is the content of organic acids and several compounds such as vitamins and amino acids. The results of research conducted by Purwaning (2010) and Rahayu (2005) using kombucha tea on white rats showed a decrease in cholesterol levels. Making kombucha tea made from a variety of leaves, namely bay leaves, guava leaves, betel leaves, soursop leaves, coffee leaves, and tea leaves, states that the best kombucha tea is made from tea leaves, because the tannin contained in tea leaves is the highest so that it affects the antioxidant level of kombucha mushroom growth media (Purnami et al., 2018).

Fourier Transformed Infrared (FTIR) is one of the tools or instruments that can be used to detect functional groups, identify compounds and analyze mixtures of analyzed samples without damaging the sample. The working principle of FTIR spectrophotometry is the interaction between energy and matter. Infrared that passes through the gap to the sample, where the gap serves to control the amount of energy delivered to the sample. Then some infrared is absorbed by the sample and the other is transmitted through the sample surface so that the infrared rays pass to the detector and the measured signal is then sent to the computer, then recorded in the form of peaks from the sample tested (Sari et al., 2018). The advantage of using chemometric techniques for IR spectrum interpretation is its ability to link the spectrum profile with hidden information contained by the plant. The resulting FTIR spectrum is a very complex data information that will thoroughly describe the chemical characteristics of a material. Changes that occur in band position and intensity in the FTIR spectrum will be related to changes in the chemical composition of a material. Therefore, FTIR spectra can be used to distinguish plants from one another even though the composition of the chemical compounds is not known with certainty (Purwakusumah et al., 2014).

2 METHODS

Tools and Materials

The tools used in this research include drop pipettes, tissues, and IR spectroscopy. While the materials used are chamomile Kombucha, green tea Kombucha, and alcohol as a blank.

Preparation of Kombucha

2000 ml of water is boiled until boiling and 200 grams of sugar (10% b/v) is added from the amount of water used and 10 grams of 0.5% (b/v) tea is added. Then filtered and the filtrate is covered with aluminum foil and let stand until the tea has room temperature. After that, 200 ml of kombucha culture starter (10% b/v) was added to the steeping tea and the container was tightly closed. Propagation of kombucha culture starter was left for 14 days.

pH test

Before conducting the test, the pH meter must be calibrated using Buffer 4.0 and 7.0 solutions before continuing the measurement. After that, the sample is measured by attaching the pH meter electrode to it and waiting for a while until a stable value is read.

Function Group Analysis

Chamomile kombucha and green tea that have been made are analyzed using IR spectroscopy.

3 RESULTS and DISCUSSIONS

The results of qualitative tests of compounds present in green tea kombucha samples using an Infrared spectrophotometer (FTIR) are shown in Figure 1. From the spectrum, it can be seen that there are several peaks showing absorption at wavelengths of 1136.83804 cm-1, 1638.16498 cm-1, 2118.99156 cm-1 and 3267.01161 cm-1. This wavelength data was then compared with the frequency data available in the literature. The functional groups identified from this comparison for each wavelength are presented in.

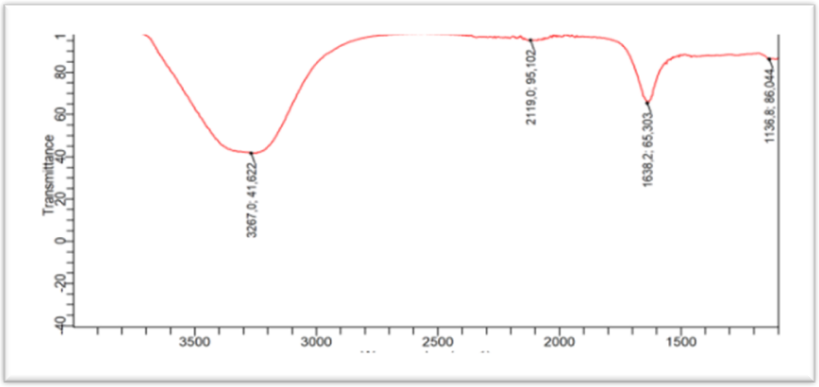

Figure 1: Apperarance of Chamomile Flower Kombucha & Green Tea Kombucha

Table 1: FTIR Data of Green Tea Kombucha

Wave number value obtained (cm)-1	Literature	Bond type
1136,83804	1050-1300	C-O (Alcohol, ethers, carboxylic acids, esters)
1638,16498	1610-1680	C=C (alkenes)
2118,99156	2100-2260	C≡C (Alkynes)
3267,01161	3200-3600	O-H (Hydrogen-bonded, alcohols, phenols)

Based on the data analysis of the FTIR spectrum of the chamomile flower kombucha sample, it shows several wave numbers associated with various types of bonds. At wave number 1628.84663 cm-1, the C=C bond was detected. Strain vibrations from alcohol-bound C-O groups at wave numbers 1140.56538 cm-1 and 1267.29486. Strain vibrations of C≡C bonds at wavelengths of 2154.40127 cm-1, 2173.03796 cm-1, and 2249.44838. As well as absorption that shows the stretching vibrations of the O-H group at a wave number of 3229.73824 cm-1.

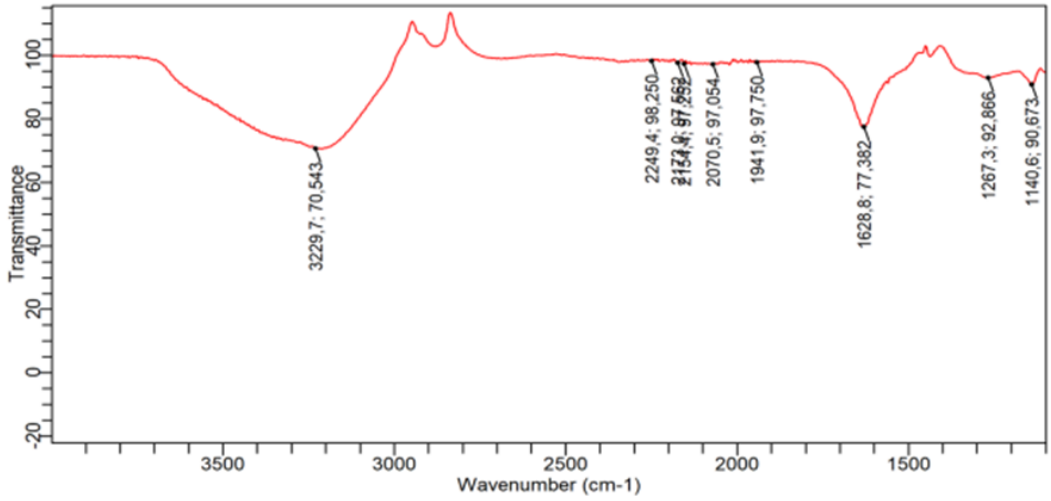

Figure 2. FTIR spectrum of chamomile flower kombucha

Table 2: FTIR Data of Chamomile Flower Kombucha

Wave number value obtained (cm)-1	Literature	Bond type
1140,56538	1050-1300	C-O (Alcohols, ethers, carboxylic acids, esters)
1267,29486	1050-1300	C-O (Alcohols, ethers, carboxylic acids, esters)
1628,84663	1610-1680	C=C (alkenes)
2154,40127	2100-2260	C≡C (Alkynes)
2173,03796	2100-2260	C≡C (Alkynes)
2249,44838	2100-2260	C≡C (Alkynes)
3229,73824	3200-3600	O-H (Hydrogen-bonded, alcohols, phenols)

Phytochemical compounds contained in green tea based on table 1. include flavonoids, tannins, saponins, and phenolic compounds. According to research by (Kusmiyati et al., 2015) green tea has high flavonoid levels, which are around 20-30% of dry weight, especially in the catechin group. It was also stated by Lindawati and Anggraini, 2020 that green tea contains polyphenolic compounds including flavanols, flavonoids, flavandiols, and phenolic acids where the total amount can reach 30% of the weight of dry tea. Apart from the four antioxidant compounds, green tea also contains tannins, alkaloids, triterpenoids, and saponins. The content is detected in the form of functional groups C-O (Alcohol, ethers, carboxylic acids, esters), C=C (Alkenes), C≡C (Alkynes), and O-H (Hydrogen-bonded, alcohols, phenols). The presence of aromatic rings is indicated by the absorption in the wave number region 1638,16498 cm-1 which is the absorption of the aromatic C=C ring stretch as a typical chromophore group of flavonoids in conjugated bond systems. Tannin compounds were detected by the presence of O-H, C=C, and C-O groups which are characteristic functional groups in tannin and saponin compounds.

The functional groups that can be detected in chamomile kombucha are alcohol or ether groups (C-O), alkenes (C=C), alkynes (C≡C), and hydroxyls (O-H). Therefore, the content of Chamomile flowers is not much different from the content in green tea. In the study said that the content of secondary metabolites in chamomile includes flavonoids, alkaloids, saponins, terpenoids, tannins / phenols, steroids, and chamazulene. Flavonoids can be divided into several parts, the first being flavones (apigenin, luteolin), flavonols (quercetin), flavanols (hesperetin), anthocyanins, and proanthocyanidins or tannins. Flavonoids are proven to improve health, prevent disease, and have antioxidant, cardiovascular disease prevention, anti-inflammatory, anticancer activities, besides that they are very safe and have low toxicity. One of the compounds found in chamomile is flavonoid compounds. Flavonoids contained in this plant have a function as anti-inflammatory. Flavonoids can stimulate the immune system by increasing the activity of macrophages and lymphocytes. Most of the anti-inflammatory effects of flavonoids are on the biosynthesis of cytokine proteins that separate the adhesion of circulating leukocytes to the site of injury (Alim & Hayuningtyas, 2023).

Chamomile flowers have long been used as an ingredient in traditional medicine, especially in traditional Iranian medicine where it is often used as an anti-inflammatory, antioxidant, remedy for skin problems, remedy for respiratory problems, sedative tranquilizer, and so on. Previous studies have shown that chamomile tea has anti-inflammatory and woundhealing properties. Ingredients such as bisabolol in chamomile can help reduce inflammation and speed up the healing process of burn wounds. In addition, flavonoids contained in chamomile extract have an important role in plant biochemistry and physiology, which functions as an antioxidant, antibacterial and anti-inflammatory. Flavonoids can accelerate the wound healing process by increasing the rate of wound contraction, decreasing the epithelialization period, increasing collagen deposition, and forming granulation tissue (Hakim et al., 2021).

The difference in the content of secondary metabolites in plants is influenced by the age of the sample and the environmental conditions in which the plant grows, although qualitatively the content of secondary metabolites is almost the same. The content of secondary metabolites in plants can vary depending on environmental factors and factors in the plant itself. The age and maturity level of a plant affects the content of secondary metabolites that are maximally active in the plant. Strengthened by the statement The growth of a biota is influenced by external and inner factors. External factors are territory, season, water temperature, type of food available and other environmental factors, while internal factors are age, size, and other biological factors (Supriatna et al., 2019).

Table 3: pH Test Results

Types of kombucha	pH
Green Tea Kombucha	2.78
Chamomile kombucha	3.32

After analyzing the functional groups on the graph, the pH test was continued to determine the acidity level of each kombucha. The pH test on chamomile kombucha and green tea kombucha is shown in Table 3. It can be observed that the difference is quite far, namely 2.78 in chamomile kombucha and 3.32 in green tea kombucha. pH shows the success rate of the fermentation process in making kombucha tea. pH is one of the most important environmental parameters that affect kombucha fermentation due to the formation of several acids formed such as acetic and gluconic acids. It is also closely related to microbial growth and changes in the structure of phytochemical compounds that can affect antioxidant activity (Hafsari et al., 2021).

4 CONCLUSIONS

The study demonstrates that Fourier Transform Infrared Spectroscopy (FTIR) effectively identifies functional groups in both green tea kombucha and chamomile flower kombucha. The results indicate that the fermentation process in both kombucha types influences the chemical composition, leading to similar functional groups despite the different sources. This suggests that the fermentation process plays a pivotal role in defining the chemical properties of kombucha, potentially affecting its health benefits and flavor profiles. Future research could explore the impact of varying fermentation durations on these properties.

5 REFERENCES

Alim, F. G., & Hayuningtyas, R. A. (2023). Potensi chamomile sebagai agen antiinflamasi oral. *Jurnal Kedokteran Gigi Terpadu*, *5*(1).

Amalia, F. (2016). *Pengaruh Grade Teh Hijau dan Konsentrasi Gula Stevia (Stevia rebaudiana bertoni M.) Terhadap Karakteristik Sirup Teh Hijau (Green Tea) (Doctoral dissertation, Fakultas Teknik Unpas).*

Anggraini, L. D., Rohadi, R., & Putri, A. S. (2018). Komparasi sifat antioksidatif seduhan teh hijau, teh hitam, teh oolong dan teh putih produksi PT Perkebunan Nusantara IX. *Jurnal Teknologi Pangan dan Hasil Pertanian*, 13(2), 10-21.

Anisa, N., Amaliah, N. A., Al Haq, P. M., & Arifin, A. N. (2019). Efektifitas Anti Inflamasi Daun Mangga (*Mangifera indica*) Terhadap Luka Bakar Derajat Dua. *Sainsmat: Jurnal Ilmiah Ilmu Pengetahuan Alam*, 8(1), 1.

Hafsari, AR, & Farida, WN (2021, Desember). Karakteristik pH Kultur Teh Hitam Kombucha dengan Berbagai Jenis Gula dalam Fermentasi Kultur Batch. Dalam Seri Konferensi Gunung Djati (Vol. 6, hlm. 228-232).

Kusmiyati, M., Sudaryat, Y., Lutfiah, I. A., Rustamsyah, A., & Rohdiana, D. (2015). Aktivitas antioksidan, kadar fenol total, dan flavonoid total dalam teh hijau (Camellia sinensis (L.) O. Kuntze) asal tiga perkebunan Jawa Barat. *Jurnal Penelitian Teh dan Kina*, *18*(2), 101-106.

Kusnan, A. (2022). Pengaruh Teh Hijau Dalam Menurunkan Tekanan Darah: Systematic Review. *Nursing Update: Jurnal Ilmiah Ilmu Keperawatan* P-Issn: 2085-5931 E-Issn: 2623-2871, 13(1), 69-79.

Lindawati, N. Y., & Anggraini, R. (2020). Pemanfaatan Ekstrak Etanol Teh Hijau (Camellia sinensis L.) sebagai Chelating Agent Logam Berat Cu dengan Metode SSA. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)(e-Journal)*, *6*(2).

Purnami, K. I., Jambe, A. A., & Wisaniyasa, N. W. (2018). Pengaruh jenis teh terhadap karakteristik teh kombucha. *Jurnal Ilmu dan Teknologi Pangan*, 7(2).

Purwakusumah, ED, Rafi, M., Safitri, UD, Nurcholis, W., & Adzkiya, MAZ (2014). Identifikasi dan autentikasi jahe merah menggunakan kombinasi spektroskopi FTIR dan kemometri. *Agritech* , 34 (1), 82-87.

Putra, I. L., & Septa, T. (2018). Efek Pemberian Teh Chamomile (*Matricaria recutita*) Terhadap Pasien GAD (Generalized Anxiety Disorders). *Majority*, 7(3), 296-300.

Sari, N. W., Fajri, M. Y., & Wilapangga, A. (2018). Analisis Fitokimia dan Gugus Fungsi Dari Ekstrak Etanol Pisang Goroho Merah (*Musa acuminate* L.). *Indonesian Journal of Biotechnology and Biodiversity*, 2(1), 30-34.

Supriatna, D., Mulyani, Y., Rostini, I., & Agung, M. U. K. (2019). Aktivitas antioksidan, kadar total flavonoid dan fenol ekstrak metanol kulit batang mangrove berdasarkan stadia pertumbuhannya. *Jurnal Perikanan Kelautan*, *10*(2).

Yuliyanti, D. D. (2021). *Pengaruh Chamomile Tea Terhadap Kecemasan Saat Pembelajaran Daring Dan Skripsi Pada Mahasiswa Universitas Kusuma Husada Surakarta Di Masa Pandemi Covid-19 (Doctoral dissertation, Universitas Kusuma Husada Surakarta).*