

# Comparison of physicochemical properties and anti-microbial activities of tea and cocoa-based kombucha

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**Abstract.** Adzadogo RS, Gbewoyo WSK. 2018. Comparison of physicochemical properties and anti-microbial activities of tea and cocoa-based kombucha. *Biotechnologi* 15: 33-37. Kombucha tea is a slightly sweet, slightly acidic refreshing beverage consumed worldwide. Kombucha is a symbiosis of the Genera *Acetobacter* and *Gluconobacter*, with *Acetobacter xylinum* as a characteristic species, and various yeasts such as genera of *Brettanomyces*, *Zygosaccharomyces*, *Saccharomyces* and *Pichia* depending on the source. The tea fungus broth is composed of two portions, a floating cellulosic pellicle layer and a sour liquid broth. Black and green tea are known to be the best substrate for kombucha preparation. Black tea and cocoa powder served as the substrate in this study. The study aimed to compare the physical and chemical as well as the anti-microbial properties of tea and cocoa-based kombucha. The study revealed that tea kombucha is more acidic compared with cocoa kombucha; both tea and cocoa kombucha have similar phytochemicals. Tea and cocoa kombucha were found to inhibit the growth of *Candida albican* and *Shigella* spp., confirming its anti-microbial activity. The presence of phytochemicals and anti-microbial properties support the school of thought that kombucha drink provides a maximum health benefit to humans.

**Keywords:** Anti-microbe, tea, cocoa, kombucha

## INTRODUCTION

Kombucha tea is a fermentation product of microbe symbiosis from the genera *Acetobacter* and *Gluconobacter*, with *Acetobacter xylinum* as a characteristic species, and variety of yeasts such as genera of *Brettanomyces*, *Saccharomyces*, *Zygosaccharomyces*, and *Pichia* depending on the source (Mayser et al. 1995). This tea is consumed worldwide is also known as Tea Fungus, Kargasok Tea, Haipao and Manchurian Mushroom. It has a slightly sweet, slightly acidic, and refreshing flavor.

The yeast ferments the sugar to ethanol in the cultivation medium, which is further oxidized to acetic acid by bacteria. The fermentation process results in a reduced pH of the medium. The final product is a sour, acidic beverage, slightly carbonated, comprised of sugars, tea components, organic acids, vitamins, and minerals. Many flavor compounds, including alcohols, ketones, aldehydes, esters, and amino acids have been identified. The tea fungus consists of two portions, a floating cellulosic pellicle layer, and a sour liquid broth.

The primary metabolites found in the fermented beverage are acetic acid, gluconic acid, lactic acid, glucuronic acid and the alcohol and glycerol (Blanc, 1996; Chen and Liu 2000; Jayabalan et al. 2007). The changes that occur in the beverage are due to the metabolic activity of the bacterial and yeast ('tea fungus') in the medium. Some of the changes that occur include changes in phenols, minerals, total acidity, and vitamin content (Malbasa et al. 2006; Chen and Liu 2000).

Kombucha tea exhibits anti-microbial activity due to the presence of acetic acid (Steinkraus et al. 1996), as well as some antibacterial agents such as bacteriocins produced during the fermentation. This beverage also contains gluconic acid, which is known to be useful in detoxification of the liver and other antioxidant and anti-inflammatory properties such as gluconates and vitamins (Sreeramulu et al. 2001). Earlier studies on kombucha has shown potential phytochemicals having anti-microbial activity against a spectrum of disease caused by microbes. In agreement, concentrates of unfermented tea components had anti-microbial properties.

In Ghana, kombucha was first introduced in the mid-1980s. Its popularity has increased ever since regarding numerous health benefits associated with this beverage. However, there is lack of scientific evidence on the physiochemical and phytochemical properties of kombucha brewed in Ghana. Furthermore, the current development of antibiotic resistance by human pathogens calls for the search into new anti-microbial substances from various sources. Hence, the objectives of this study are: (i) To determine pH and total acid in tea and cocoa kombucha. (ii) To determine phytochemicals content in tea and cocoa kombucha. (iii) To evaluate the anti-microbial activity of tea and cocoa kombucha.

## MATERIALS AND METHODS

### Materials

Black tea (Lipton, Ghana), sucrose (St. Louis refined sugar, France), cocoa powder and starter culture were provided by Rev. Dr. W. S. K. Gbewonyo of the Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon, Accra, Ghana. The organisms used, *S. aureus*, *C. albicans*, and *Shigella* spp. were also obtained from the Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon, Accra, Ghana.

### Methods

#### *Preparation of tea kombucha and cocoa kombucha*

Sweetened black tea was prepared by infusing one bag of black tea (Lipton) and sucrose (37.7g) into boiled distilled water (1 L) for 3 min, then allowed to cool to room temperature. Previous starter culture kombucha was inoculated to the sweetened black tea. The fermentation took place for 14 days, after which the tea kombucha was harvested and kept in a refrigerator. Cocoa kombucha was prepared according to the method described elsewhere (Gbewonyo 2013).

#### *Freeze-drying of samples*

Two hundred milliliters of both tea and cocoa kombucha samples (fermented and unfermented) were freeze-dried to obtain a dried residue of the tea and cocoa samples. The weights of freeze-dried samples were measured. Each sample was reconstituted in distilled water to get a stock solution at a concentration of 1g/ml and was stored in a refrigerator until ready for use. All experiments were performed at the School of Agriculture in the College of Basic and Applied Sciences of the University of Ghana.

#### *Determination of pH and total acid content*

The pH was determined from 50 ml of freshly prepared broth of cocoa and tea kombucha. The titratable acid contents of tea and cocoa kombucha samples were determined by the volumetric method. A standardized NaOH<sub>(aq)</sub> (1.0M) was titrated against 10 ml of each of tea and cocoa kombucha samples. The concentration of total acid present in each sample was then calculated (Greenwalt et al. 1998).

#### *Qualitative analysis of sugars in tea and cocoa kombucha*

The stock samples were applied to Thin-layer Chromatography (TLC) silica gel 60 F<sub>254</sub> plates with fluorescent indicator. A solution of (0.02g/ml) fructose, glucose, and sucrose served as standards. Plates were run using a combination of chloroform, methanol, and acetonitrile (1: 1: 0.5 V/V). The plate was developed using 10% H<sub>2</sub>SO<sub>4</sub> and heated at 110 °C. Retention factor (Rf) was then calculated for both kombucha samples and the standard sugars used.

### Phytochemical analysis

Fermented and unfermented samples were screened for saponins, tannins, alkaloids, terpenoids, and flavonoids.

#### *Test for saponins*

An equal volume of a test sample and distilled water were shaken vigorously for 2 minutes, then observed for persistent foaming, indicative of the presence of saponins (Kokate, 1999).

#### *Test for tannins*

Two drops of FeCl<sub>3</sub> (5% W/V) were added into 2ml of the aqueous test samples; formation of green precipitate confirms the presence of tannins (Trease and Evans, 1996).

#### *Test for alkaloids*

Two milliliters of each test sample were dried using heat, cooled and 5 ml of 2M HCl was then added to each reaction mixture. Upon complete dissolution of all the residues, three drops of Wagner's reagent was added. The formation of reddish-brown flocculation suggested the presence of alkaloids (Geetha and Geetha 2014).

#### *Test for terpenoids.*

One milliliter of chloroform was added to 2 ml of the test samples, diluted 1 in 2 with distilled water. Aliquot of concentrated H<sub>2</sub>SO<sub>4</sub> was added gently to the mixture. If reddish brown precipitate or ring at the junction of the two solutions were observed, this suggests the presence of terpenoids (Rimjhim and Kumari 2014).

#### *Test for flavonoids*

Three milliliters of 1M NH<sub>3</sub> was added to an equal volume of the test samples and allowed to stand for 2 minutes after which 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added. A yellow precipitate formed indicated the presence of flavonoids (Evans, 1997).

#### *Test for reducing sugar*

Three milliliters of Fehling's solution A and B was added to the test samples and heated at 60°C. Formation of a brick-red color suggests the presence of reducing sugar (Geetha and Geetha 2014).

### Anti-microbial activity

Media was made by dissolving an appropriate mass of nutrient broth and nutrient agar in a designated volume of distilled water. The media was sterilized by autoclave, pour into sterile petri dishes, and allowed to cool or set. Indicator strains for anti-microbial activity assay of tea and cocoa kombucha were *C. albicans*, *Shigella* spp., and *S. aureus*. Sterile Whatman filter paper discs (6.0 mm diameter) were impregnated with each of the fermented and unfermented extracts of tea and cocoa. After inoculating the plate with the cultured organisms using cotton swabs, the dried paper disc impregnated with the samples were placed on the plates. Positive control was set up using ceftriaxone and fluconazole; and a negative control using unfermented tea and cocoa. The plates were then incubated at room temperature for 24 hours to allow growth of the microorganisms. The anti-microbial activity of the kombucha samples was evaluated by measuring the diameter of the inhibition zone after 24 hours (Mounyr et al. 2016).

## RESULTS AND DISCUSSION

### Physiochemical properties and phytochemical screening

We recorded a decreased pH in the fermented tea and cocoa kombucha where tea kombucha was shown to be more acidic (Figure 1). This result indicates that tea kombucha produced more organic acids compared with cocoa kombucha as reported that, the pH value of kombucha beverage decreases due to the production of organic acids during fermentation (Dufresne and Farnworth 2000). Accordingly, the total acid content of tea kombucha is higher compared with cocoa kombucha (Table 1).

The phytochemical analysis results revealed the presence of tannins, terpenes, flavonoids, reducing sugar and saponins in tea and cocoa kombucha. Alkaloids were absent in both tea and cocoa kombucha (Table 2). Glucose and fructose were detected in tea and cocoa kombucha by TLC. Sucrose was detected in unfermented tea; fructose was present in fermented tea and unfermented cocoa; glucose was found to be present in fermented cocoa (Table 3).

### Anti-microbial activity of tea and cocoa kombucha

Freeze-dried samples of tea and cocoa kombucha were tested against *C. albicans*, *Shigella* spp and *S. aureus*. Freeze-dried unfermented tea and cocoa served as negative control, whereas the antibiotics fluconazole served as positive control for *C. Albicans*. Ceftriaxone served as positive control for *Shigella* spp and *S. aureus*. Figure 2.A-B shows the anti-microbial activity of cocoa kombucha and tea kombucha towards *C. albicans*. Figure 3.A-B shows the anti-microbial activity of cocoa kombucha and tea kombucha towards *Shigella* spp. Figure 4.A-B shows the anti-microbial activity of cocoa kombucha and tea kombucha towards *S. aureus*. Figure 5 shows the quantification of zone inhibition by the test samples towards the tested pathogenic microorganisms. Although the positive control showed a high level of inhibition against *S. aureus*, neither fermented or unfermented tea and cocoa showed inhibition toward *S. aureus*.

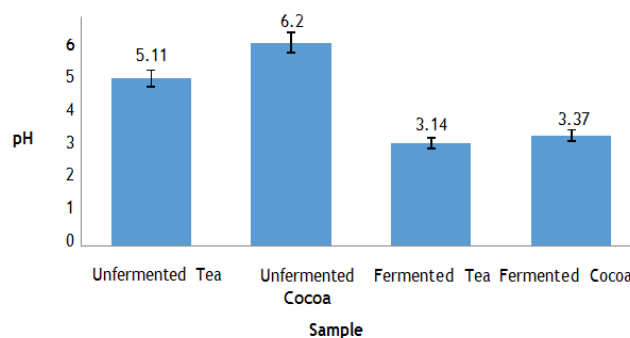


Figure 1. The pH of tea and cocoa kombucha.

Table 1. Total acid content of tea and cocoa kombucha.

Sample	Total acid concentration (g/L)
Tea kombucha	0.126
Cocoa kombucha	0.087

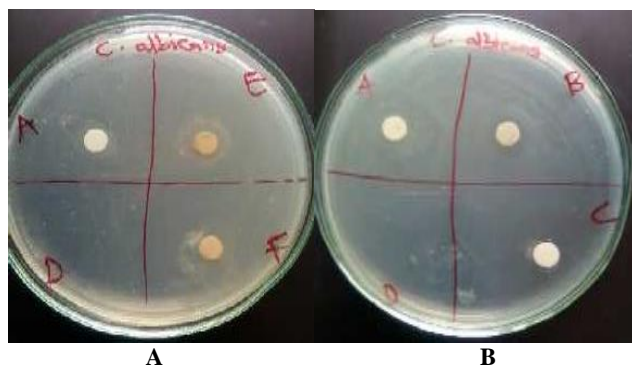
Table 3. TLC analysis of sugars present in tea and cocoa kombucha.

Samples	Retention factor of sugars		
	Sucrose	Fructose	Glucose
<b>Unfermented</b>			
Tea	0.36	-	-
Cocoa	-	0.50	-
<b>Fermented</b>			
Tea	-	0.52	-
Cocoa	-	-	0.58
<b>Standards</b>	0.36	0.50	0.58

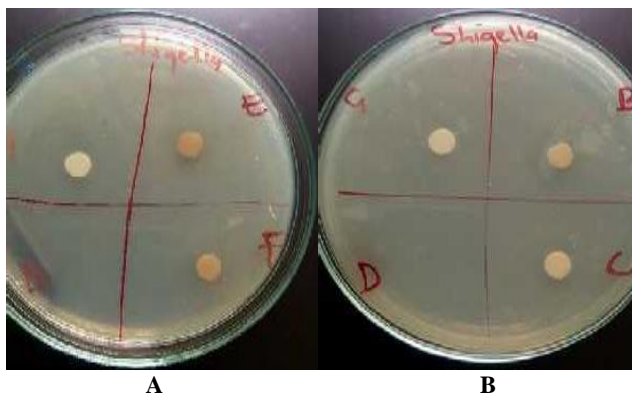
Table 2. Qualitative phytochemical analysis of tea and cocoa kombucha.

Test	Observation	Inference			
		Unfermented Tea	Unfermented Cocoa	Fermented Tea	Fermented Cocoa
Tannins	Formation of a green ppt.	+	+	+	+
Terpenoids	Reddish-brown ppt formed at the interphase	+	+	+	+
Saponins	Forming persist after shaking vigorously.	+	+	+	+
Flavonoids	Yellow solution formed, No ppt	+	+	+	+
Phenol	Red coloration formed	+	+	+	+
Reducing sugars	Brick-red color formed	+	+	+	+
Alkaloids	Brown color of solution persist	-	-	-	-

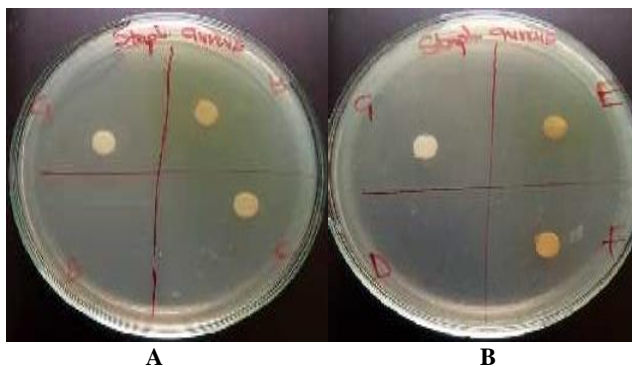
Note: + : Presence, - : Not present, ppt : Precipitate



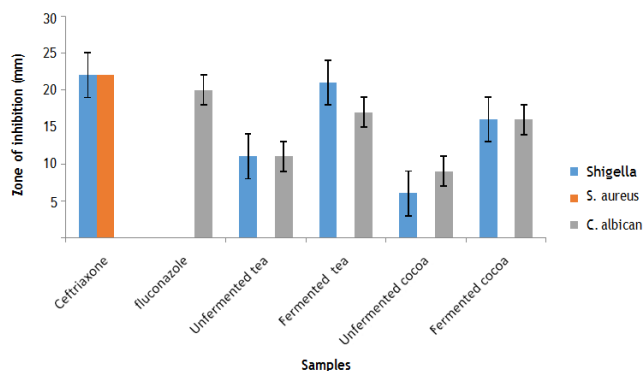
**Figure 2.** Anti-microbial activity of cocoa kombucha (A) and tea kombucha (B) towards *C. albicans*. Note: A: fluconazole, B: fermented cocoa, C: unfermented cocoa, D: untreated, E: fermented tea, F: unfermented tea.



**Figure 3.** Anti-microbial activity of cocoa kombucha (A) and tea kombucha (B) towards *Shigella*. Note: B: fermented cocoa, C: unfermented cocoa, D: untreated, E: fermented tea, F: unfermented tea, G: ceftriaxone.



**Figure 4.** Anti-microbial activity of cocoa kombucha (A) and tea kombucha (B) towards *S. aureus*. Note: B: fermented cocoa, C: unfermented cocoa, D: untreated, E: fermented tea, F: unfermented tea, G: ceftriaxone.



**Figure 5.** Zone of inhibition of tea and cocoa kombucha against indicator strains.

### Discussion

Kombucha is a symbiotic culture of bacteria and yeast. Yeast cells hydrolyze sucrose to glucose and fructose. Meanwhile, acetic acid bacterial convert glucose and fructose into gluconic acid and acetic acid during the fermentation process. These organic acids produced throughout the fermentation of the tea and cocoa kombucha resulted in a corresponding decrease in pH value (Dufresne and Farnworth 2000) that prevents the symbiotic culture from becoming contaminated by undesirable microorganisms not contained in the tea fungus. In our study, a decrease in pH was also recorded in both the fermented tea and cocoa kombucha confirming the result of the previous report (Greenwalt et al. 1998; Dufresne and Farnworth 2000; Jayabalan et al. 2007).

Phytochemicals screening in the tea and cocoa kombucha showed that they contain compounds commonly found in tea and cocoa (Table 2). A study by Subhashini et al. (2010) revealed that cocoa contains tannins, saponins, terpenoids, and flavonoids. Tea is also known for its richness in tannins, phenols, flavonoids, terpenoids, etc. These phytochemicals were detected in both tea and cocoa samples (fermented and unfermented) in this study.

Phytochemicals are known to offer protection against human diseases. The consumption of flavanol-rich cocoa improves cerebral blood flow, which is critical for optimal brain function and reduced dementia. The presence of these phytochemicals in both fermented and unfermented tea and cocoa-based kombucha, indicates that preparation of kombucha does not compromise the potential health benefits derived from its consumption. Thus, the health benefit of kombucha beverage reported by Dufresne and Farnworth (2000) and Teoh et al. (2004) may be attributed to the presence of these phytochemicals.

Glucose and fructose, the hydrolyzed product by yeast, were detected in tea and cocoa kombucha by TLC. Table 3 shows that tea kombucha contains fructose and cocoa kombucha contains glucose. Loncar et al. (2006) described that during the fermentation process yeast cells hydrolyze sucrose into glucose and fructose. Glucose produced during the fermentation is used by the yeast to produce ethanol and carbon dioxide as metabolites. The bacteria then oxidize ethanol in the presence of air to acetaldehyde, then to acetic acid (Reiss 1994; Loncar et al. 2006). The primary

metabolites of ethanol and acetic acid act as catalyzing agents yeasts are stimulated to produce ethanol by acetic acid. Whereas, ethanol stimulates the growth of acetic acid bacteria and their production of acetic acid (Liu et al. 1996). The acetic acid bacteria also utilize some of the glucose to produce gluconic acid. Fructose is used to a lesser extent, and some remain after the fermentation process (Greenwalt et al. 1998). The presence of the unmetabolized glucose, together with the remaining fructose, is responsible for sweetness associated with the beverage.

Kombucha beverages exert anti-microbial properties against a spectrum of known human pathogens (Greenwalt et al. 1998; Sreeramulu et al. 2001) perhaps due to its acetic acid content (Steinkraus et al. 1996). Fermented cocoa kombucha showed anti-microbial activity towards *C. albicans* with a zone of inhibition of 16 mm, higher than that of the unfermented cocoa kombucha. Also, fermented tea showed anti-microbial activity on *C. albicans* with an inhibition zone of 17 mm as compared to its unfermented form which gave an inhibition zone of 13 mm. Kombucha beverages reduced the growth of *C. albicans*, albeit still lower than the positive controls. Based on the size of inhibiting zone, tea has a higher anti-microbial activity than cocoa against *Shigella* (Figure 5). The result might be attributed to lower pH of tea than cocoa kombucha (Figure 1). Furthermore, because anti-microbial activity against pathogenic microorganisms is attributable primarily to acetic acid (Greenwalt et al. 1998), we speculate there might be more acetic acid in tea than cocoa kombucha. Sreeramulu et al. (2000) reported that the anti-microbial activity of kombucha tea is determined by the presence of organic acids, mainly acetic acid, large proteins, and catechins.

Nevertheless, unfermented tea did inhibit the growth of the indicator strains used in this study. Many reports reveal that the polyphenols or tannins extracted from tea inhibit a broad spectrum of Gram-positive and gram-negative bacteria (Zhu et al. 2000). Toda et al. (1991) reported that unfermented tea at high concentrations (using 20% dry tea) inhibit *Staphylococcus epidermidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigelladysenteriae*, *Shigella flexneri*, and *Vibrio* spp.

### Conclusion

The results suggest that tea kombucha is more acidic compared with cocoa kombucha as demonstrated by their pH and total acid content. The presence of phytochemicals in the tea and cocoa kombucha, especially phenols which are known for their anti-microbial activity, might be associated with their significant inhibitory action against the growth of *Shigella* and *C. Albicans*. However, phenol was also detectable in the unfermented tea and cocoa; thus, it could be the reason why the unfermented samples display

anti-microbial activity although in a lesser extent as compared to the fermented ones. The study highlights tea and cocoa-based Kombucha beverage as potential anti-microbial agents.

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