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A comparative study on kombucha tea and black tea

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Abstract: *Around the world, kombucha tea (KT), one of the numerous traditional fermented foods, is a well-liked health beverage. Before introducing KT culture mass for fermentation, various white sugar concentrations, ranging from 2 to 10%, were added to the black tea. In a similar vein, attempts were made to prepare KT using varying concentrations of tea dhool (1–5%). The results showed that the addition of different amounts of sugar and tea dhool increased the dry matter production of KT. It was discovered that the ideal concentrations of sugar and tea dhool were 6-8% and 3-4%, respectively. The biochemical components of black tea brews and KT were examined and contrasted. It was shown that practically all of the biochemical components of black tea brews and KT did not differ significantly. When KT was compared to black tea, it was discovered that it had more volatile flavourings including arubigins and flavonoids, proteins, polyphenols, and catechins, as well as caffeine. Furthermore, an endeavour was undertaken to examine the antibacterial efficacy of KT against certain human pathogenic microorganisms. KT demonstrated potent effects on Aeromonas hydrophila, Salmonella typhimurium, and Escherichia coli. This suggests that KT contains antibacterial substances. According to the current study, KT might be a healthy beverage for people.*

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Introduction

As the most widely consumed hot beverage worldwide, tea (Camellia sinensis (L.) O.Kuntz) is also one of the most significant plantation crops. The most popular stimulant in the world is tea, which is made from the dried and meticulously processed leaves of the tea plant. The interplay of intricate metabolic processes that take place in the leaves during processing is primarily responsible for variations in the taste components and quality of tea decoction. While it's generally believed that the type of black tea affects the scent and quality differences, preparation and manufacturing methods have also been found to play a significant role (Pauline et al., 2001).

Of the various tea varieties—black tea (CTC, orthodox), green tea, oolong tea, lemon/zinger/barley tea, instant tea, and kombucha tea—kombucha tea (KT) is particularly significant in the treatment of various human ailments because it secretes an extensive range of secondary metabolites (Yang et al., 2002). In a zoogleal mat that must be cultivated in sugared black tea, acetic acid bacteria (Acetobacter xylinum or Acetobacter xylinoides) and osmophilic yeasts (Saccharomyces ludwigii, Zygosaccharomyces rouxii or Schizosaccharomyces pombe, etc.) grow symbiotically. Gluconic, glucoronic, lactic, acetic, malic, tartaric, malonic, citric, oxalic, succinic, pyruvic, usnic acid, ethanol, purines, lipids, amino acids, water-soluble vitamins, monosaccharides, proteins, vitamins, and antibiotically active substances are just a few of the many metabolites that the KT culture can produce as it grows (Loncar et al., 2000, Qin et al., 2000, Petrovic and Loncar, 1996).

According to Sreeramulu et al. (2000), the International Agency for Research on Cancer working group concluded in 1989 that there is sufficient data to support anticarcinogenicity in humans. KT is a well-liked health drink that is employed as an alternate form of treatment. It is said to offer both preventative and therapeutic effects, and it is ingested in many parts of the world (Blanc, 1996). Antioxidant compounds such as epigallo catechin gallate (EGCG), epigallo catechin (EGC), epicatechin gallate

regulate appetite, control blood sugar in diabetics, and relieve bronchitis and asthma (Dufresne and Faenwork, 2000). A study was conducted to examine the biochemical components and tea quality parameters of black tea decoction and KT brew. Furthermore, an inventory of the microbial consortiums was attempted, and the antibacterial efficacy of KT against a few human pathogenic pathogens was investigated.

Supplies and Procedures- Gathering and preserving the culture of Kombucha tea (KT)

The Tiruppur, Tamil Nadu, India locals provided the kombucha tea culture, which was then kept alive on black tea.

KT preparation

After adding 1.2% (w/v) of clonal black tea made from clone UPASI-9 to boiling water and letting it steep for five minutes, the mixture was filtered through a sterile sieve. After dissolving white sugar (5% w/v) in the heated black tea infusion, the mixture was allowed to cool to room temperature. 200 millilitres of black tea brew were transferred into a 500 millilitre glass jar that had been sterilised. A known weight fresh kombucha culture and 2% (v/v) of previously fermented KT brew were added aseptically to the jar. By bringing the brew's pH down, the addition of previously fermented KT drink inhibits the growth of undesirable microbes. The black tea infusion will begin to ferment, releasing a distinct aroma and releasing carbon dioxide. To keep flies out of the glass jars, the jars were covered with clean fabric. For 14 days, the preparation was kept at 24±3ºC in a dark incubator. Periodically, samples were taken to estimate the levels of caffeine, TF, TR, and total sugar.

Examination of the biochemical components in black tea and KT

A variety of biochemical constituents were estimated, including total sugars (Dubois et al., 1956), reducing sugars (Miller, 1959), non-reducing sugars (Mahadevan and Sridhar, 1996), protein (Lowry et al., 1951), amino acids (Moore and Stein, 1948), nitrogen (AOAC, 1990), lipid (Folch et al., 1957), polyphenols (Bray and Thorpe, 1954), and catechin (Swain and Hillis, 1959), as well as tea quality parameters like flavons and arubigins (Takeo and Oosawa, 1976) and tea alkaloid compounds like caffeine (Newton,
1969). Black tea brew (3.0% w/v) was used to estimate all of the previously mentioned parameters since it was chosen based on its optimal pH concentration and highest generation of dry eight KT biomass.

Effects of varying sugar and tea dhool concentrations on pH and mat formation

An attempt was made to investigate the effects of varying sugar and tea dhool concentrations on pH and mat formation in KT. 200 mL of the mixture was dispensed into a 500 mL sterilised glass jar along with varying concentrations of white sugar (2-
10% w/v) and tea dhool (1-2% w/v). The kombucha culture was injected, and the incubation process was followed as previously mentioned. Following the fermentation period, samples of the mat and KT liquid were obtained for the analysis of pH and dry weight, respectively. A pH metre that is electronic was used to measure the tea liquor's pH (Elico model). On the fourteenth day of fermentation, the KT culture mat was removed in order to measure the wet weight using an electronic scale. at determine the dry weight, the mat was placed in a hot air oven set at 80°C for the entire night.

Microbial consortia counting in KT brew

The kombucha tea was made according to the previous instructions, and tea fluid was utilised to count the microorganisms. Periodically, samples were taken out to count the number of bacteria and yeast. Serially diluted liquid tea extract samples were plated on nutrient agar (peptone -5.0g, NaCl -5.0g, beef extract -3.0g, yeast extract -2.0g, agar -
20.0g, and distilled water -1000 ml) medium containing 4mg of cycloheximide to
do total yeast counting, the diluted samples were plated on OGYA medium, which contains 4 mg of oxytetracycline to inhibit bacterial growth. The medium also contains glucose (10.0g), yeast extract (3.0g), agar (20.0g), and distilled water (1000 ml). For acetic acid-producing bacteria, the plates were incubated at 30°C, and for yeasts, at
25°C.

KT Brew's antimicrobial properties

KT's antibacterial activity against a few human diseases was investigated using the methodology outlined by Evans et al. (1989) and Bauer et al. (1996). For the investigation, pathogenic cultures including Shigella species, Candida albicans, Salmonella typhi, Enterobacter aerogenes, Pseudomonas aeroginosa, and Escherichia coli were chosen.

Using Muller-Hinton agar (meat infusion: 2.0 g, caesin hydrolysate: 17.5 g, starch: 1.5 g, agar: 13.5 g, and distilled water: 1000 ml), a bacterial lawn was created by inoculating the medium with several pathogenic organisms using a sterilised L-shaped glass rod. Prior to being placed on the lawn and cultured at 37°C for 24 to 48 hours, sterilised filter sheets (6 mm in size) containing bioactive components were dipped in KT brew (brew was obtained on the 14th day of fermentation). It was noted and observed that an inhibition zone was forming surrounding the disc. In a similar vein, a control setup was kept in which black tea brew was used to dip sterilised filter papers.

The acquired data were analysed using analysis of variance (ANOVA), and the important means were divided at different significance levels using critical difference (CD) (Gomez and Gomez, 1984).

Findings and Discussion

Comparing the biochemical components of KT and black tea

There was no discernible difference between the KT and black tea brews, according to the biochemical components of both. On the other hand, KT was shown to
have lower total sugars, reducing sugars, and non-reducing sugars than black tea brews. However, when KT was compared to black teas, it was shown that it had higher levels of total polyphenols, catechins, and proteins (Table 1). Bradford (1976) examined the protein concentrations of three liquids: chicken soup, skim milk, and KT. The results showed that KT had almost half the protein content of skim milk. According to reports by Blanc (1996) and Tsubouchi et al. (1985), it was proposed that this protein was mostly made up of extra-cellular enzymes released by the microbes in the tea to break down macromolecules that were too big to fit through the cell walls.
The results on the components of tea quality showed that kombucha and black teas differed significantly in these substances. Caffeine, theaflavins, and arubigins were found to be more prevalent in KT than in black teas. Table 1 shows that the caffeine concentration of the black tea brew and the KT was 0.41% and 0.49%, respectively. At a concentration of 12 grammes of sucrose, the theaflavin and thearubigin levels in KT were 1.08% and 11.89%, respectively (Fig. 1). Up to the 12th day of the fermentation period, all three compounds showed an increasing trend in KT; after that, a reduction was seen while the concentration of sucrose increased. Numerous authorities on kombucha claim that the fermentation process converts up to 99.5% of the sugar used. According to calculations, each serving of KT contains about 5 mg of caffeine (Yang et al., 2002). Those who are sensitive to caffeine have not had any negative effects from KT, and this is the lowest level of caffeine encountered compared to other caffeinated beverages.
KT is mostly linked to liquor's briskness, which is strongly correlated with its flavin concentration. It was also demonstrated that the characteristics of spirits, such as colour, brightness, power, and briskness, are linked to the flavonoids and arubigins. During fermentation, KT's colour shift was another significant observation. After being infected, the solution gradually turned pale brown. It had been dark brown before. According to Qin et al. (2000), this is what makes the KT civilizations unique.

Effects of sugar and tea dhool concentrations on KT pH and mat formation
The addition of various sugar concentrations increased the fresh and dry weight of the KT culture, according to the results. However, the weight of the KT culture was significantly lower with a concentration of 10g of sugar. At a sugar concentration of
10g, the dry matter content of the KT culture was determined to be 17.65%. Consequently, it was discovered that a 6-8% sugar concentration was ideal (Table 2). The outcome matched the findings of Blanc's (1996) report, in which KT was made using different tea dusts and sucrose concentrations ranging from 0 to 6% and 1% to 6% of tea, respectively. He said tea dhool was highly tasty and was considered a nutritious beverage, with sugar concentrations of 3–4 and 6-8%, respectively. According to Loncar et al. (2000), glucuronic acid was quantitatively demonstrated to be present in KT beverages at various white sugar concentrations. A metabolised fructose poorly compared to glucose. xylinim and as a result, it gathered in the mixture. It was shown that 3-4% was the ideal concentration of tea dhool (Table 3). At 5% tea dhool, the dry weight of the KT culture and pH both decreased.
It is noteworthy that 50–70g sugar/L gave the optimal concentrations of ethanol and lactic acid, and precisely this sugar concentration has been used in traditional recipes for the preparation of KT for a long time. The taste of the KT changes during fermentation from a pleasant fruit sour-like lightly sparkling flavour after a few days to a mild vinegar-like taste with prolonged incubation (Reiss, 1994).
Different degrees of pH drop were caused by an increase in sugar concentration in KT. When the fermentation period was extended, the pH of the KT brew gradually decreased from its starting pH of 5.2 on zero day. Ultimately, on the fourteenth day of incubation, the brew's pH

was 2.6 (Table 2). ApH decrease suggested that sugar was fermented during the incubation period (Tsubouchi et al., 1985), and it was caused by the formation of certain organic acids (Qin et al., 2000).At room temperature (25±2oC), the KT culture mat development was found to be optimal and homogenous. Over time, it was discovered that a rise in the solution's sucrose concentration inhibited the growth of mats. Without the addition of sucrose to the solution, the mat formation and fungal growth were normal. It was found that the sucrose concentration dropped until it was at least 0.2 grammes per 100 millilitres of KT solution (Loncar et al. 2000; Hartmann et al. 2000; Malbasa et al. 2001).

KT Brew's overall sugar concentration changed throughout the fermentation process. From the first to the fourteenth day of incubation, there was a discernible drop in sugar content (Fig. 2). The initial sugar level was 10.8 g/100 ml, and on the fourteenth day of the incubation period, it was discovered to be 3.1 g/100 ml. Additionally, from the first to the sixth day, there was a marked drop in total sugars, followed by a modest decline. The duration of fermentation and the total amount of sugar were clearly correlated. According to reports, the majority of the sugar in KT ferments to become alcohol (Pauline et al., 2001).

Number of yeast and bacteria cells in KT brew

The overall bacterial and yeast counts in KT did not correlate with the length of the fermentation process (Fig. 3). But starting on the first day and continuing through the sixth, there was a marked decline in the number of bacterial and yeast colonies. On the first day of brew, there were 8.0x104 bacterial colonies per millilitre. After 14 days of incubation, the brew measured 15.6x104/ml and 32.0 on the sixth day. In a similar vein, 5.3x104 yeast colonies/ml of brew were present on the first day. The brew was 19.6x104/ml on the fourteenth day of incubation, and 39.5 on the eighth. Low pH and insufficient nutrients in the brew were the causes of the drop in the viable cell count (Sinisa et al., 2001). Additionally, the inadequate growth and development of bacteria in KT Brew was caused by the carbon dioxide produced by yeasts fermenting alcohol.

While the added sugar acts as a source of carbon, the tea infusions provide nitrogen and encourage the growth of microbes (Hartmann et al., 2000). The fact that tea components are consumed in KT culture is an observed fact, even though we are unable to determine the prevailing mechanism. More research is needed to determine the mechanism by which this is accomplished (Dufresne and Faenwork, 2000).

Ethanol was used by bacteria to thrive and make acetic acid. Furthermore, the yeasts may be encouraged to create ethanol by the presence of acetic acid. In a spontaneous fermentation of reconstituted orange juice, a similar symbiotic relationship was also noted between the bacteria Gluconobacter oxydans and the yeast Saccharomyces cerevisiae (Chen and Liu, 2000). While both glucose and fructose can be used by yeasts through the process of glycolysis to make ethanol and glycerol, bacteria have a distinct way of using these sugars. Using yeast invertase, yeast cells hydrolyze sucrose into glucose and fructose. Glycolysis then produces ethanol, with fructose serving as the preferred substrate. According to Reiss (1994), acetic bacteria use ethanol to make acetic acid and glucose to produce gluconic acid.

KT Brew's antimicrobial properties

It was determined whether KT had any antibacterial activity against a variety of harmful microbes. According to the findings, KT was found to be susceptible to Salmonella typhi, Pseudomonas aeurginosa, Escherichia coli, Klebsiella species, and Listeria monocytogenes. Aeromonas hydrophila and Staphylococcus aureus were also discovered to be somewhat sensitive. E was the target of KT's strongest effects. coli, Aeromonas hydrophila, and Salmonella typhimurium in contrast to black tea. However, in terms of growth inhibition, Shigella sonnei and Bacillus amyloliguefaciens were not susceptible to KT. This suggests that KT contains antibacterial substances besides acetic acid and big proteins (Table 4). According to Yang et al. (2002), there was a substantial antibacterial activity against both gramme positive and gramme negative pathogenic organisms in the fermented samples with a total acid concentration of 33 g/L. However, after kombucha has taken root, it is safe to eat for humans.

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