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Original Research

Comparative *in vitro* biological characterization of black and green tea infusions fermented with brewer's yeast and SCOBY with special emphasis on antioxidant activity

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Abstract

This research work was designed for a detailed comparative *in vitro* biochemical characterization of different fermented tea infusions. Green tea, crush-tear-curl (CTC) black tea and orthodox black tea, along with brewer's yeast (*Saccharomyces cerevisiae*) and traditional starter SCOBY (symbiotic colony of bacteria and yeast), were co-cultured for fermentation to produce different tea wines and kombuchas respectively. Primary investigations for qualitative characters on these fermented broths revealed the presence of total phenol, flavonoid, coumarin, tannin, protein, cardiac glycoside, glycerol, terpenoid, steroids and alkaloid. All the broths except kombuchas contained caffeine. Total phenol and flavonoid contents were also quantified, with fermented green tea samples showing a noticeable result. While all the fermented tea samples showed high free-radical scavenging activity, the best results were found in the green tea samples (before and after fermentation). However, fermented CTC samples had the highest fermentation-led increasing antioxidant properties. Physicochemical properties like acidity (pH), Brix, specific gravity and alcohol percentage (ABV) were considered as qualitative parameters to study the fermentation process and judge the acceptability of broths as edible beverages. Interestingly, when correlated with antioxidant property results, fermented CTC tea samples exhibited comparatively higher alterations in physicochemical properties due to fermentation. Comparatively, more

glucose uptake capacity (*in vitro* antidiabetic activity) was found in fermented CTC broths, while inhibition of lipid peroxidation was substantive with yeast-fermented tea samples only. Unlike the yeast-fermented samples, the antibacterial properties exhibited by kombucha samples were promising. The presence of alcohol, biologically active groups of components, antioxidant and antibacterial activity etc. have added value to these fermented tea broths and established their acceptability as edible beverages. The production and *in vitro* biological characterization of different fermented tea broths have allowed a comparative analysis that is much needed for future research on and development of fermented tea-based drinks.

Keywords: Tea, kombucha, tea wine, fermentation, antioxidant, antibacterial

Introduction

Tea has a long and glorious history. It is the most popular beverage after water and its beneficial health effects arouse great scientific interest and inspire research works [1].

After China, India is the second largest producer, exporter and consumer of tea today where mainly two types of tea are consumed i.e. green tea and black tea (orthodox hand-rolled whole leaf tea and crush-tear-curl or CTC tea). Tea is usually consumed as an infusion or decoction with water or milk.

The correct moisture content is an important factor in manufactured tea leaves because exposure to moisture can cause contamination and reduce the quality of the tea. For this reason, it is not recommended to store manufactured tea for a long time unless it is fermented. Due to its medicinal properties, brewed tea can be used as a potential substrate in fermentation technology. This approach has been used globally by applying the ancient and traditional knowledge of kombucha, where tea infusion is fermented with the starter SCOBY (symbiotic colony of bacteria and yeast) [2]. After years of research and development on kombucha, the term 'tea wine' was invented by oenologists. Fermentation of sugared tea broth using wine yeast (*Saccharomyces cerevisiae*) or koji (*Aspergillus oryzae*, a traditional rice wine starter) to produce a low alcoholic 'tea wine' is reported [3]. However, there is very little information available on the production and characterization of tea wine in the scholarly world, unlike kombucha, which often tops food science and food technology trends.

The biological activities and microbiology of kombucha have been studied extensively by various international scientific communities. The symbiotic action of kombucha's yeasts (*Saccharomyces cerevisiae*, *Schizosaccharomy-*

ces pombe, *Saccharomycodes ludwigii*, etc) and acetic acid bacteria (*Acetobacter xylinoides*, *Bacterium gluconicum* etc) [4] is highly praised for producing secondary metabolites, which are associated with a number of health benefits [5] such as antioxidant, antibacterial, anticancer, antidiabetic, gastroprotective (anti-ulcer), neuroprotective, cardioprotective and hepatoprotective (liver detoxification) properties.

In addition to probiotic kombucha, wines are also well recognized for their antioxidants such as flavanoids anthocyanins, flavanols, catechinis, leucoantthocyanins and resveratrol [6]. Like kombucha, tea wine also contains catechins, gallic acid and other strong active antioxidants, proper intake of which may prevent many diseases [3, 7]. Pu-erh tea, dark tea or Chinese Hei Cha tea are examples of traditional fermented teas where physical oxidation and microbial (Aspergillus niger) fermentation play the decisive roles in the generation of aroma compounds [8]. Reported metabolomic changes during fermentation like microbial conversion of tea polyphenols (epicatechin gallate and epigallocatechin gallate) and amino acids; elevated amounts of terpenoids; increase of total phenolic content; changes in alkaloid (mainly caffeine) profile etc. [2, 9–12] have together been of great interest and have stirred up the need for a comparative biochemical analysis.

Research on the development of fermented beverages and tea has intensified globally in recent times where ethnobiological claims always remain at the centre of attraction. Fermentation depends on the type of substrate material and the starter's microflora. Among various innovations and technologies, microbial fermentation is an option that can be utilized to increase biological activities and alter the flavour profile of a typical cup of tea [9]. Recent reports on tea flower wine [13] and production of bioactive formulations like 'tea haria' by using the indigenous starter bakhar [14] also justified the importance of microbial fermentation.

This study focused on a comparative analysis with *in vitro* biochemical tests on kombuchas and wines prepared from three different teas as substrate materials – black tea (orthodox and CTC) and green tea infusions. Qualitative detection of bioactive groups of molecules, quantification of total phenolics and flavonoids, antioxidant and antibacterial tests were chosen as different parameters to characterize the samples and to study the effect of different starters on biochemical changes during the fermentation of different teas.

Materials and methods

Collection of tea samples and starters

Samples of garden-fresh CTC black tea, orthodox black tea and green tea were collected from the Nuxalbari tea factory located in the Terai region of West Bengal, India in the foothills of Darjeeling Himalaya (26°40'00.2'N 88°12'06.4'E). Live SCOBY (manufactured by Ayureshmi Herbal Concentrates, Kerala, India) was purchased through an online trading portal and dry brewer's yeast or *Saccharomyces cerevisiae* (Goodrich, India) was bought from a local market. Other brewing ingredients and equipment i.e. sulphur-free sugar (Uttam sugar, India), synthetic white vinegar (William chemical co., India) and fermentation jars were also bought from a local market.

Preparation of fermentation batches

Fermentation batches of tea were prepared following the methodologies developed for kombucha fermentation [15], tea petal wine [13] and tea wine fermentation using the indigenous starter bakhar [14] with incorporated modifications. Aiming for a comparative biochemical analysis, batches were prepared on the same day, in a similar manner (with a

fixed ratio of each ingredient) and incubated under similar conditions. Ten grams of each tea (1% w/v) were added to one litre of freshly boiled double distilled sterile water (at 98±1°C) and left for fifteen minutes to infuse. The teas prepared were CTC tea infusion (CTCI), orthodox tea infusion (OTI) and green tea infusion (GTI). Each infusion was then filtered through a sterile muslin cloth into a sterile glass jar to which 100 g of sucrose (10% w/v) was added as a nutrient or carbon source for fermenting microbes. The jars were then autoclaved for sterilization. After cooling at room temperature (lowering its temperature up to 20±2°C), starters (100 ml of starter liquid for kombucha and 2 grams of dried yeast for yeast fermenting broths) were added to the infusions. Following the addition of starters, sterile acetic acid or synthetic white vinegar was added (to maintain the pH at 5) to the jars to increase the acidity of the fermentation broth (considered as an ideal fermentation condition). Finally, sterile polythene sheets with pores and/or sterile muslin cloths were used to cover the top of the jars to facilitate gas (CO_2) release. Muslin cloth, glass goods, polythene cover etc. were autoclaved and sterilized with 70% ethanol before using. The jars were incubated for fifteen days under dark conditions in a well-ventilated and airy room following the protocol of Majumder *et al.* [13]. Each control batch with each starter was prepared by inoculating starter and other ingredients in broths where the tea infusion was replaced by double distilled water. A total of eight samples were prepared, including four broths with yeast: 1. control wine (CY), 2. CTC tea wine (CTCY) 3. orthodox black tea wine (OTY) 4. green tea wine (GTY); and four broths with SCOBY: 1. control kombucha (CS), 2. CTC tea kombucha (CTCS) 3. orthodox black tea kombucha (OTS) 4. green tea kombucha (GTS). Following the same procedure, a total of three jars for each sample were prepared and the results of further experiments were expressed as mean of the three replicates ±SD. Healthy batches of fermenting broths (without any sign of mould or contamination) were selected after fifteen days of incubation and used as samples for further experimentation. **Fig. 1** depicts fermentation batches photographed on day zero and day 15 of fermentation.

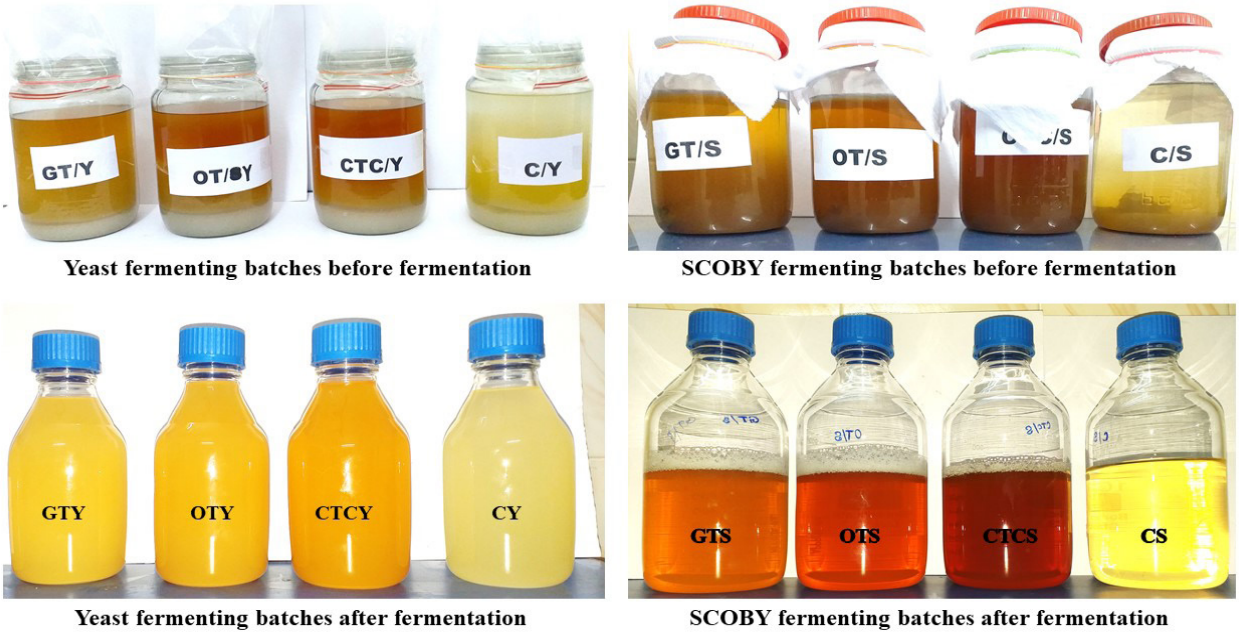

Figure 1 Yeast and SCOBY fermented tea infusion batches before and after fermentation

Preliminary qualitative tests

Qualitative tests for the detection of twelve groups of molecules (total phenol, flavonoid, coumarin, tannin, protein, cardiac glycoside, glycerol, starch, terpenoid, steroids, alkaloid and caffeine) were conducted to determine and compare their presence in all samples following the protocol of Ghosh *et al.* [16] and Majumder *et al.* [14]. The same tests were also performed on each tea infusion (CTCI, OTI and GTI) to allow comparison and to study the changing effects or alteration of biochemical profiles due to fermentation.

Quantification of total phenol and flavonoid content

Quantification of total phenolic content (TPC) was done following the Folin–Ciocalteu method described by Majumder *et al.* [14].
TPC was measured against the gallic acid standard curve (R^2 = 0.9975; y = 0.0043x - 0.1672) and results were expressed as gallic acid equivalent (mg GAE/100 ml). Total flavonoid content was determined by the aluminium chloride method demonstrated by Ghosh *et al.* [16]. A standard curve of quercetin was used as reference (R^2 = 0.962; y = 0.207x - 0.204) for this test and the result was expressed as quercetin equivalent (mg QE/100 ml). Experiments were carried out in triplicates. Results were expressed as the mean of three replicates ± standard deviation.

Analysis of physicochemical properties

Monitoring of important physicochemical parameters like estimation of pH (for acidity), specific gravity (SG), percentage brix (% Bx) and alcohol by volume (% ABV) of the fermented samples followed the protocols developed by Majumder *et al.* [14] to study the fermentation driven alteration in those properties and analyse the fermented samples in a comparative way.

In vitro antioxidant activity (DPPH assay)

Samples were tested for *in vitro* antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay following the protocol of Majumder *et al.* [13]. Results were expressed as the percentage of DPPH inhibition (% inhibition) occurring due to exposure of samples. Any increases in antioxidant activity in the tea infusions after fermentation were also calculated from this experiment. The ascorbic acid standard curve was used as reference (R^2 = 0.9904; y = 0.9088x + 12.331). Results of this assay were expressed as a mean of three replications. In addition to the traditional experiment i.e. quantification of antioxidant value, DPPH assay was utilized to observe the time-dependant interaction which was termed as 'DPPH inhibition kinetics' by Chakraborty *et al.* [17]. 0.2 ml of each sample was added to 3 ml of 0.2 mM DPPH solution and the absorbance was recorded for 30 minutes using the kinetics function of Cary-60 UV-vis spectrophotometer (Agilent). The aim of this experiment was to observe and differentiate the power of antioxidants present in each sample, how fast a sample can scavenge the DPPH and to determine the duration needed for a sample to complete the reaction.

In vitro antidiabetic activity (determination of glucose uptake capacity by yeast cells)

Glucose uptake assay using yeast cells was used to determine *in vitro* antidiabetic activity following the protocols developed by Shettar *et al* [18]. Metronidazole was used as the standard drug (R^2 = 0.9862; y = 0.0119x – 0.0003) and results were expressed as mg MetE/ml (mg Metronidazole Equivalent per millilitre).

In vitro lipid peroxidation inhibition assay

The *in vitro* lipid peroxidation inhibition assay (also called hepatoprotective assay) was determined following the protocol developed by Rahman *et al.* [19]. The value of estimated lipid peroxidation inhibition activity was measured from a standard curve (R^2 = 0.9973; y = 0.0077x + 0.0321) prepared using tocopheryl acetate. Results were expressed as mg TAE/ml

(milligram tocopheryl acetate equivalent per millilitre) of each sample.

In vitro antibacterial activity

The well diffusion method described by Ghosh *et al.* [16] was used to assess the antibacterial activity of crude samples. Overnight grown cultures of two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria were used for this experiment. Antibacterial activity was measured from the diameter of the inhibition zones formed around the well containing the samples.

Results and discussion

Preliminary qualitative tests

Qualitative biochemical tests revealed the presence of several bioactive groups of molecules, i.e. phenol, flavonoid, coumarin, tannin, protein, glycoside (cardiac glycoside), glycerol, terpenoid, steroids, alkaloid and caffeine in both tea infusions and fermenting samples. The heatmap (**Fig. 2**) shows the results of this experiment, which revealed that starch was completely absent in all samples. Together, phenol, flavonoids, coumarin and tannin represented the group of phenolic compounds (polyphenols), are well established as tea infusion metabolites (mainly tannin and flavonoid). Green tea batches (GTY and GTS) and infusion (GTI) and orthodox tea kombucha (OTS) exhibited a significant presence of phenol, flavonoids and coumarin, while tannin was found in black tea infusions (CTCI and OTI) at higher levels than expected; tannin is the signature molecule of black tea while green tea is comparatively richer in flavonoids and other polyphenols [20]. Microbial conversion of tea polyphenols (epicatechin gallate and epigallocatechin gallate) and an increase of total phenolic content by yeast [2, 10] were also recorded. The resulting heatmap neatly expresses the validated fermentation-led reduction in tannin and the increase in phenol in fermenting broths. Protein (representing peptides, amides and group of amino acids) was high in yeast fermenting batches of tea (CTCY, OTY and GTY), but not in kombuchas. Glycosides and glycerol are mainly sugar-derived fermented products, however, tea contains cardiac glycosides [21]. It follows that the detection of a high amount of glycoside in kombucha samples and a high glycerol content (a signature yeast metabolite) in yeast fermented samples is justifiable. Terpenoids and steroids are also important compositions of black tea [21]. The results of this experiment indicated high amounts of these molecules in samples of CTC and orthodox tea. The fermentation process of traditional pu-erh (over-fermented black tea) contributes to volatile transformation where components like terpene alcohol increase significantly [8–9]. It also suggests a microbial fermentation-led elevation of terpenoids, which was also reflected in the resulting heatmap. Moreover, caffeine and its derived alkaloids including theobromine and theophylline are reported to be metabolized through the purine catabolic pathway of yeast which leads to the changes of alkaloids in tea [11]. Through chromatographic study, Majumder *et al.* [15] have validated how tea stimulants like caffeine and other alkaloids are completely withdrawn by SCOBY to make the broth of kombucha completely free from such components. This effect is not exclusive to kombucha. Wang *et al.* [9] also reported a reduction of caffeine and other alkaloids by the wine yeast *Saccharomyces cerevisiae* during fermentation of green tea wine. They have reported caffeine as the nitrogen source for the yeast. Results of this research have suggested and validated that same hypothesis as kombucha broths were found to contain zero caffeine and much reduced alkaloid, while yeast fermenting broths

also exhibited a reduced amount of caffeine. In control batches (CY and CS), tested molecules were found to be either absent or very low – except glycerol in CY which again suggests that glycerol is a common metabolite of wine yeast where tea infusions have no role to play.

Figure 2 Heatmap (dark to light = high to low) showing levels of different groups of molecules, tested on tea and control samples

Quantification of total phenol and flavonoid content

Tea is rich in bioactive components such as polyphenols (mainly flavonoids), which gives tea a range of medicinal properties, with the ability to reduce the risk of a variety of diseases [22]. Catechins (epicatechin gallate, epigallocatechin gallate, epicatechin gallate, epicatechin etc.) are major polyphenolic components of green tea while in black tea those components are converted into theaflavins and thearubigins due to a series of oxidation processes. Research findings suggest that these polyphenols have anticancer properties. The possible health benefits of tea polyphenols are being extensively studied and have received a great deal of interest in recent times. Tea polyphenols are also credited with substantial antioxidant, antidiabetic, hepatoprotective, gastroprotective, cardioprotective, antiobesity and antimicrobial properties [22]. Fermented tea-based beverages (kombucha and wines) are also rich in polyphenols and are reported to possess better biological activities [2, 9–10] some of which have also been investigated *in vitro* in this research. Jayabalan *et al.* [2] reported the breakdown of catechins and an increase of total phenolic content in green tea kombucha while the black tea polyphenols (theaflavins and thearubigins) are comparatively stable. Their finding is clearly reflected in the results of this research where fermented green tea samples yielded a huge amount of total phenolic content, i.e. 65.70±1.26 mg in 100 ml sample for GTY and 87.53±0.71 mg for GTS. Green tea kombucha (GTS) had the highest amount of total phenol, surpassing all other samples. However, in the flavonoid quantification test, all samples had similar results (about 70 mg QE/100 ml of each sample) except for CTCS (only 57.32±1.02 mg QE/100 ml). In control batches (CY and CS), a very low amount of phenol was found. **Fig. 3** depicts the graphical representations of results from these two assays.

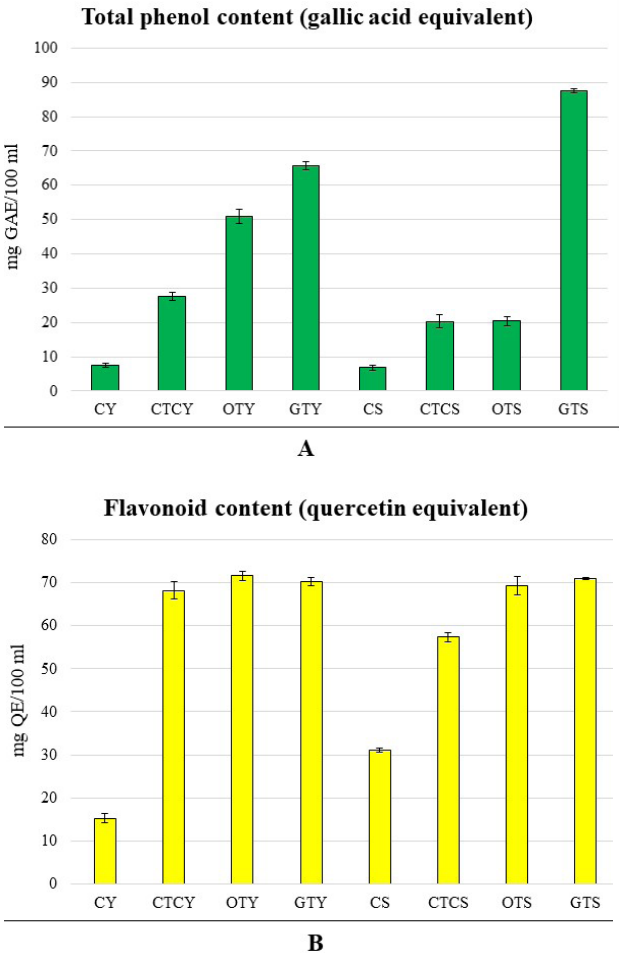

Figure 3 Total phenol content (A) and flavonoid content (B) in different fermented samples

Analysis of physicochemical properties

Determination of acidity (pH), specific gravity, Brix (% Bx) and alcohol by volume (% ABV) are important when judging the physicochemical qualities, fermentation rate and acceptability of a fermented broth as a beverage, especially alcoholic ones, which are incorporated in this research. Following previously standardized research methodologies, changes to these properties in fermenting tea broths were recorded on day zero and day 15 [13–14]. **Fig. 4** depicts the results of physicochemical properties as graphical representations. Acidity increased during fermentation in all samples, shown as a decreasing pH result. The pH of a standard cup of tea infusion is generally in the range of 5.0–6.0 [21]. The pH of substrates used in this research was detected to be 5.36, 6.10 and 5.69 for CTCI, OTI and GTI respectively. These values were adjusted to 5.0 in all samples using synthetic vinegar to provide a similar and controlled acidic environment as mentioned in the methodology. After fifteen days of fermentation, decreasing pH/increased acidity was highest in yeast fermenting CTC tea infusion (from a pH of 5±0.32 to a pH of 2.89±0.15) followed by OTY, GTS, CTCS, GTY and OTS. The control batches (CY and CS) did not respond well, which clearly suggests the effect of tea on fermentation (**Fig. 4A**). Fermented CTC tea samples were found to be most acidic after fermentation compared to other samples for respective starters. Typically, the pH of wine ranges between 3.0 and 4.0; increasing acidity/decreasing pH is a characteristic of the fermentation process in all wines [13]. The pH of all the fermented samples analysed were also found to be within the acceptable range except for the most acidic sample – CTCY (pH 2.89±0.15). Generally, during fermentation, yeast or other fermenting microbes cause acidification through metabolism where the fermenting broths turn acidic (increased amounts of organic acids) as a result. Thus, the results of these experiments demonstrate that the rate of fermentation was at its peak during the first week of fermentation. Decreasing pH, along with increasing antioxidant activity, is a cumulative parameter that

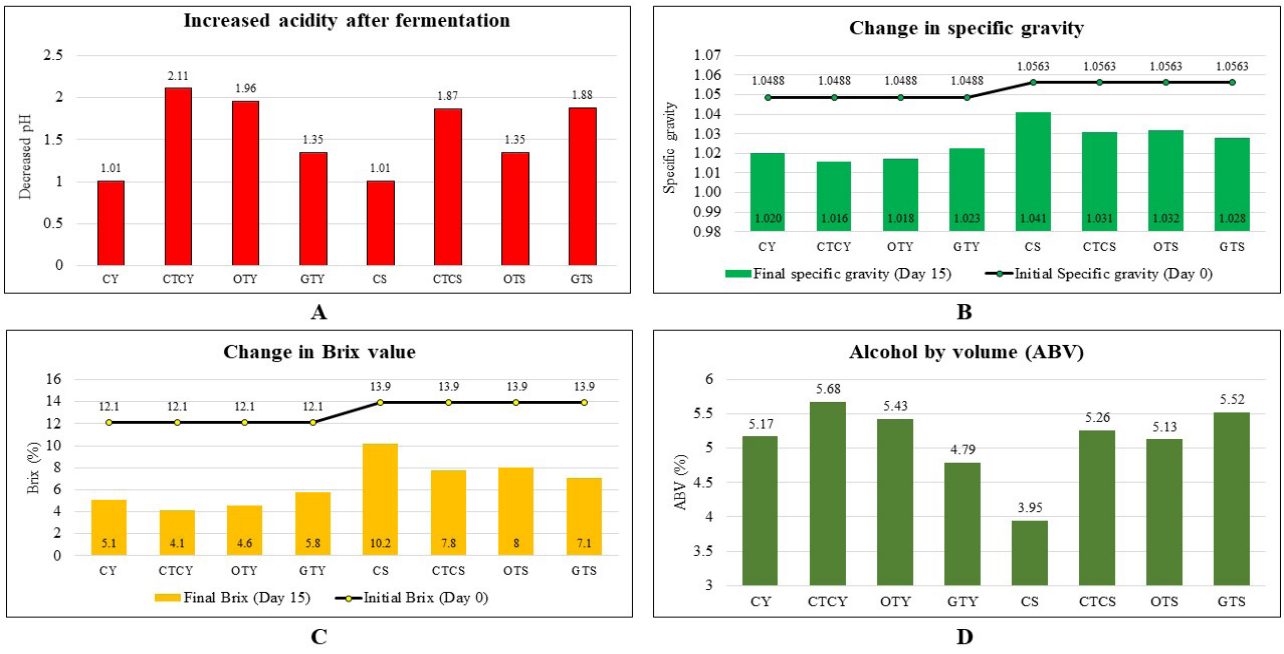

Figure 4 Alteration in physicochemical properties such as, (A) Increased acidity/decreased pH value (B) Specific gravity (C) Changes in Brix % (D) Alcohol percentage (% ABV)

judges the quality of fermented beverages [13–14]. In this research, samples of fermented tea also exhibited this same characteristic to confirm an excellent fermentation, which is further described in the antioxidant assay. As changes in acidity and fermentation are positively correlated with each other, results of alcohol percentage (% ABV), Brix and specific gravity were found to correlate completely with the pH results (**Fig. 4**). Specific gravity and Brix were measured to detect the amount of soluble sugars in broths, the levels of which are important in kombucha and wine. Specific gravity (**Fig. 4B**) and Brix (**Fig. 4C**) were recorded before and after fermentation. The results of which showed a lowering of the values suggesting utilization of sugars and nutrients during fermentation. Previously, Majumder *et al.* [14], stated that a drop in specific gravity and Brix confirms the loss of sugars, which are apparently converted into alcohol and carbon-dioxide due to fermentation. Similarly, like the pH assay, CTCY showed the highest alterations in other physicochemical properties also. Final Brix was recorded comparatively higher in control batches, suggesting comparatively lower rate of fermentation.

In vitro antioxidant activity

Mean results for DPPH scavenging activity were 79.98±0.23%, 84.843±0.12% and 85.838±0.36% for CTCI, OTI and GTI respectively. The aim of this experiment was to analyse the effect of fermentation on antioxidant activity. To achieve this, DPPH inhibition kinetics were carried out alongside orthodox quantification of inhibition percentage. The changing rate of antioxidant activity is equivalent to the rate of microbial metabolism [23]. Therefore, increased antioxidant activity reflects an increased rate of microbial function. All fermented tea samples inhibited more than 90% DPPH from the solution except the control batches prepared without tea infusion (**Fig. 5A**). Fermentation-led antioxidant increase (**Fig. 5B**) was also calculated from the results, which showed fermented CTC samples (CTCY and CTCS) returned the highest results among their respective types. For green tea and orthodox black tea infusions (GTI and OTI), unfermented samples showed good antioxidant properties (approx. 85% of scavenging activity) and further fermentation brought no considerable changes. This suggests that CTC tea is ideal as a fermentation substrate both scientifically and economically (it is the cheapest of the teas).

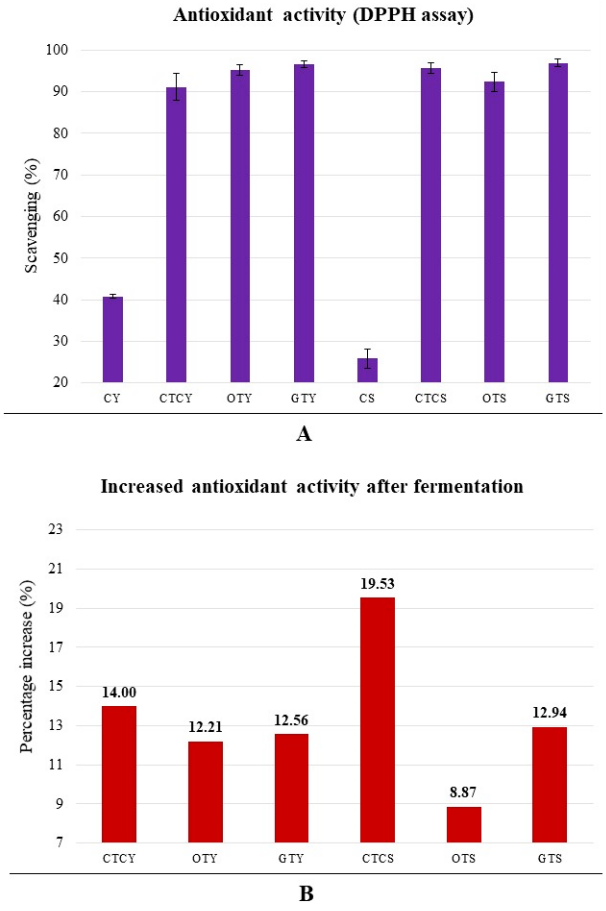

Figure 5 (A) DPPH free radical scavenging activity (%) shown by fermented tea samples
Figure 5 (B) Fermentation-led increase in antioxidant properties

DPPH inhibition kinetics was performed with the fermented samples to observe the nature of inhibition and to differentiate each sample as percentage inhibition was found to be similar (more than 90%) in all fermented tea infusions. A more sharply curved declining

absorbance graph always indicates a higher rate of inhibition. **Fig. 6** illustrates the kinetics of DPPH inhibition for 30 minutes. Graphs of fermented green tea infusions (GTY and GTS) exhibited a sharp decline in the first 10 minutes of incubation before stabilising and running parallel to the x-axis, which confirmed the completion of inhibition during the first one third of incubation period. As expected, CTCS, OTY and CTCY exhibited curved and declining graphs. In contrast, any decline was hard to observe in the control samples (CY and CS) due to low inhibition percentage. Of the others, OTS, which showed the least fermentation-led antioxidant increase, also exhibited the slowest rate of decline in the kinetics graph. Absorbance kinetics were also used to determine the time required for 50% inhibition. The results, in minutes, from lowest to highest were: GTS (1.05), GTY (1.21), CTCS (2.18), OTY (2.52), CTCY (7.76) and OTS (15.65), which clearly indicate that the antioxidant release rate was comparatively fastest in fermented green tea samples and slowest in OTS.

In vitro antidiabetic activity

Glucose uptake capacity provided the parameter to evaluate the antidiabetic activity of samples where metronidazole was used to prepare the standard curve [14]. One millilitre of CTCY resulted a glucose uptake capacity equivalent to the capacity shown by 26.46±0.65 mg metronidazole while 1 ml of CTCS exhibited an antidiabetic activity of 15.9±0.63 mg MetE. These were the highest results among all samples. Khan and Mukhtar [22] and Das *et al.* [21] previously named black tea as the best tea to show anti-hyperglycemic activity; their findings are reflected in the results of this research. Regarding comparison between two starters, yeast fermenting broths possessed better results than kombucha samples (**Fig. 7**).

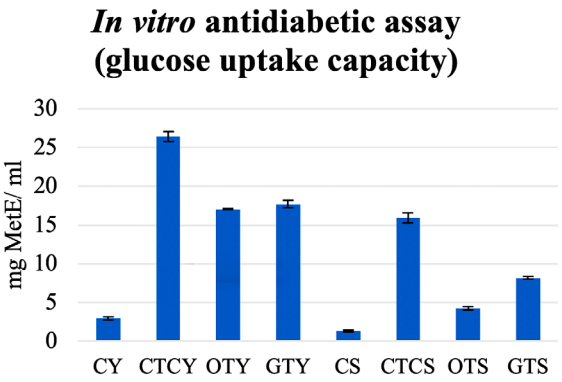

Figure 7 Antidiabetic activity shown by fermented tea samples

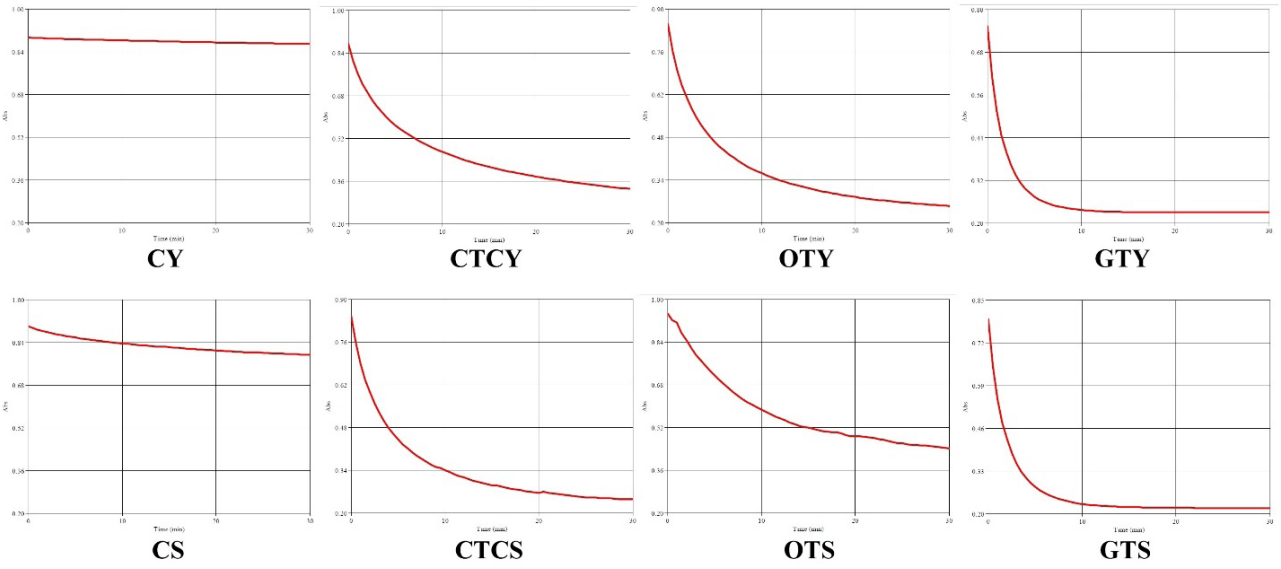

Figure 6 Spectrophotometric DPPH inhibition kinetics produced by different fermented tea samples

In vitro lipid peroxidation inhibition assay

Lipid peroxidation is a chain of reactions of oxidative degradation of lipid molecules. It is the process in which free radicals collect electrons from the lipids in cell membranes, resulting in cell damage. In this assay, liver cells (goat liver homogenate) were used to evaluate *in vitro* lipid peroxidation inhibition activity. Thus, objective of this experiment was to study the liver cells' protective efficacy of fermented tea samples. Activity for yeast fermenting batches were estimated to be 89.8±6.32%, 80.11±3.21% and 95.18±5.49% for CTCY, OTY and GTY respectively while SCOBY fermented tea samples returned comparative low results (**Fig. 8**). Results also suggests that among the different types of tea studied, green tea was the best inhibitor of lipid peroxidation.

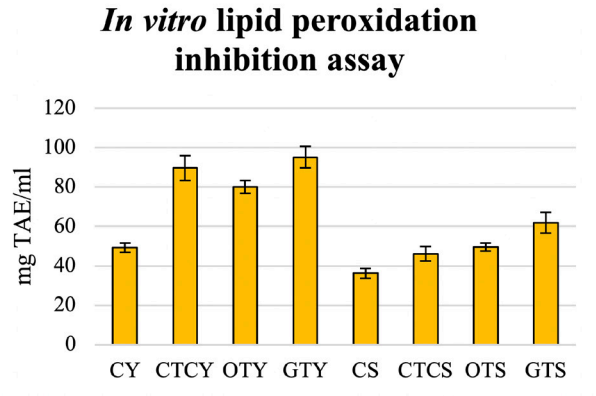

Figure 8 *In vitro* lipid peroxidation inhibition shown by fermented tea infusions

In vitro antibacterial activity

The ability of fermented samples to inhibit bacterial growth was observed and recorded in **Table 1** and represented graphically in **Fig. 9**. Maximum inhibition zones were created by GTS followed by GTY; other samples were not as promising. Green tea is already known to have better antibacterial properties than other types of tea [22]; Battikh *et al.* [24] have reported on the antimicrobial efficacy of green tea kombucha and this was reflected clearly in the results of this research.

Table 1 Inhibition zones (mm) against different bacteria produced by different samples

	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumoniae*
CY	0.25	0	0	0
CTCY	0.25	0.25	0	0.5
OTY	0.25	0.25	0	1
GTY	1	3	2	3
CS	0.25	0	0	0
CTCS	1	0.25	3	0.25
OTS	0.25	0	0	0.25
GTS	7	6	7	3

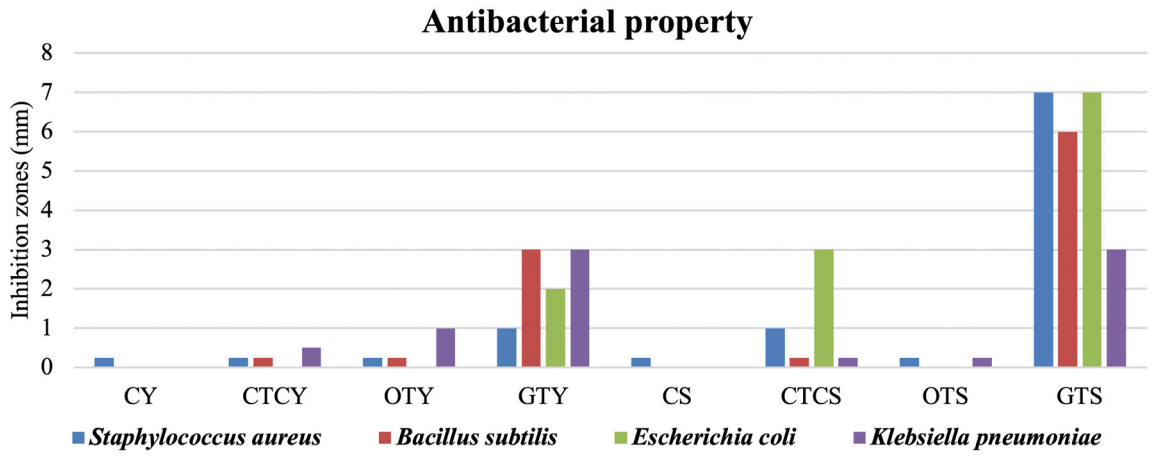

Figure 9 Antibacterial activity (inhibition zones) shown by different fermented tea samples

Conclusion

This research carried out an *in vitro* experimental comparison among fermented CTC, orthodox and green tea infusions in similar conditions with different starters (SCOBY and yeast) and explored biochemical characteristics through a preliminary but detailed level of research. The results of this comparative analysis-based research revealed the effect and potential of fermentation techniques on enhancing the biological activities of tea infusions. Moreover, this research shows that it is not necessary to use high-end orthodox hand-rolled whole-leaf black tea and costly green tea for fermentation-driven quality enhancement as these teas possess high qualities before being fermented. Our results show that fermentation-driven quality enhancement is highest in the less expensive CTC tea infusions. This detailed *in vitro* comparative analysis, through the detection of different groups of bioactive biochemicals, physicochemical properties and *in vitro* biological activities (antioxidant, antidiabetic, hepatoprotective and antibacterial activity) was instrumental in characterizing the fermented tea broths. As a valuable source of antioxidants, fermented teas can be added to our regular diet in addition to green foods, tea and wine.

Conflicts of interest None

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