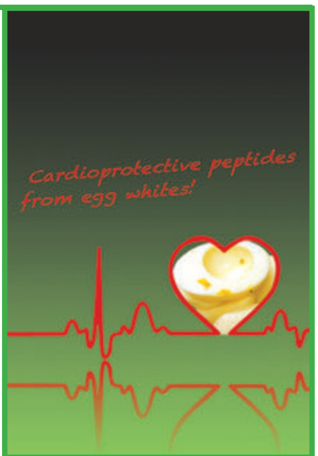
Original Research

Nutrafoods (2016) 15:111-121
DOI 10.17470/NF-016-1030-2



Process optimization for the preparation of apple tea wine with analysis of its sensory and physico-chemical characteristics and antimicrobial activity against food-borne pathogens

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Keywords:
Apple, Tea, Apple tea wine, *Saccharomyces cerevisiae*, Natural fermentation, Functional properties

Abstract

Method for preparing apple tea wine using different types of tea at different concentrations were optimized and the physico-chemical, antimicrobial and sensory characteristics of the wine analysed. All characteristics were found to be directly proportional to the concentration of tea fermented naturally or with *Saccharomyces cerevisiae* var. *ellipsoideus*. In both types of fermentation, CTC (crush, tear and curl) tea-based apple tea wine received significantly higher quality scores ($p \leq 0.05$). Better results in terms of ethanol, higher alcohol concentrations and antimicrobial activity were found with 4 g tea/100 ml apple juice than with other concentrations, particularly 5 g tea/100 ml apple juice. All apple tea wines showed antimicrobial activity (inhibition zone >7 mm) against *Escherichia coli* (IGMC), *Enterococcus faecalis* (MTCC 2729), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MRSA 252) and *Bacillus cereus* (CRI). Apple tea wine fermented with *S. cerevisiae* var. *ellipsoideus* had higher sensory scores for most attributes than naturally fermented apple tea wine. Different treatments were clustered based on tea concentration as a function of fermentation with *S. cerevisiae* var. *ellipsoideus* unlike the natural fermentation where no clear clustering trend was observed. The type of fermentation influenced the quality of the wine as separate clusters for the different fermentations were observed during combined cluster analysis of the different types of wine. Our results demonstrated that the best apple tea wine was made with 4 g CTC tea/100 ml apple juice and fermented with *S. cerevisiae* var. *ellipsoideus*, and showed potential as a functional product which also demonstrates the medicinal properties of tea.

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Introduction

As economic status has improved, growing interest in health and well-being has resulted in increased intake of natural foods. Tea, a non-alcoholic beverage consumed worldwide and prepared from tea leaves (*Camellia sinensis* L.) is gaining popularity as an important 'health drink' and is consumed by up to two thirds of the world's population. Among the 700 chemical constituents present in tea leaves, flavonoids, amino acids, vitamins (C, E, K), caffeine and polysaccharides are considered

Received: September 13, 2015 / Accepted: December 28, 2015

important for human health. The caffeine (3% by weight) in tea provides a stimulating effect [1]. Polyphenols in tea are the most important constituents and act as antioxidants, known to play a significant role in human health. Black tea is considered to be a good fermentation medium because its infusion contains proteins, amino acids, volatile compounds, lipids, enzymes and, more importantly, polyphenols [2].

Wine consumed in moderation is considered a safe and healthy drink and is an important adjunct to the diet [3]. Different studies have shown the beneficial effects of wine consumption due to the presence of phenolics and alcohol, which protect the human body from attack by free radicals and increase HDL levels, respectively [4, 5]. Phenolic compounds also play a major role in enology as they are responsible for the colour and flavour of red and white wine. In addition to their bactericidal role, phenolics are responsible for the 'French paradox' whereby antioxidants and vitamins are apparently protective against cardiovascular disease [6].

Apple juice has been fermented in Eastern Mediterranean areas for more than 2000 years to obtain a pleasant alcoholic beverage [7]. It is also fermented to produce cider, a sparkling and refreshing fruit-flavoured drink consumed in many countries [5, 8], as well as wine and brandy. *Kombucha* or tea cider (a traditional fermented tea product) is often drunk for medicinal purposes due to the acetic acid formed during fermentation and has been used in Russia for several centuries [9]. Alcohol is also produced during fermentation of *kombucha* [10].

The increasing antimicrobial resistance of pathogens isolated from humans and animals, combined with the increasing consumer awareness of chemical food preservatives, necessitates research for more efficient antimicrobials with fewer side-effects on human health. Different plant extracts were examined for antimicrobial activity against pathogens by Papadopoulou *et al.* [11]. Tea extracts have exhibited antioxidant [12], antimutagenic [13, 14], anticarcinogenic [15], antibacterial [16], antiviral [17], antifungal [18] and antitumor activity [19, 20]. Natural antibacterial agents have been increasingly applied for the biological preservation of food in recent years [21]. The antimicrobial activities of fermented tea have been less studied than its beneficial health properties. These antimicrobial activities develop during the natural microbial fermentation process with tea leaves as substrates. The antimicrobial components produced during the fermentation process have shown inhibitory effects against several food-borne and pathogenic bacteria as reported by Sreeramulu *et al.* [22]. Its acidity and low alcohol content allow *kombucha* to resist contamination by most airborne moulds and bacterial spores. The beverage is brewed at home so preparation conditions are generally not sterile. However, most tests indicated a low rate of contamination from spoilage and pathogenic microorganisms, suggesting that *kombucha* has antimicrobial properties against pathogenic and other harmful microorganisms [23]. Some studies have reported that the antimicrobial activity of *kombucha* made with a low concentration of tea (4.4 g/l) was attributable to the acetic acid content [10]. Indeed, the systematic investigation of Sreeramulu *et al.* [22] demonstrated that the antimicrobial activity of *kombucha* was not due to organic acids, ethanol, proteins or tannins present in tea or their derivates.

The chemical constituents of tea and the phytochemical potential of apple juice can both be used to improve the quality of wine. Siby and Joshi [24] supplemented apple wine with spice extracts which increased the polyphenolic content and also enhanced antimicrobial activity. In light of the health benefits of tea, the importance of apples in the wine industry and the role of different microflora in wine fermentation, the aim of this study was to improve the physico-chemical, antimicrobial and sensory properties of apple tea wine produced by fermentation with *Saccharomyces cerevisiae* var. *ellipsoideus* and by natural fermentation. The results of our study are presented below.

Materials and methods

Materials

Apples (Golden variety) were procured from the local market in Solan (Himachal Pradesh, India). Orthodox tea (Dhauladhar, natural organic orthodox Kangra tea) was procured from HPKV, Palampur (Himachal Pradesh, India), herbal tea

(Himalayan brew, Kangra special herbal tea) was procured from the local market in Palampur, and CTC tea (Tajmahal) was procured from the local market in Solan. The yeast strain *S. cerevisiae* var. *ellipsoideus* (UCD 595) was procured from the Indian Institute of Horticulture Research, Bangalore (Karnataka, India). Sugar was procured from the local market. Pectin esterase was procured from M/S Triton Chemical, Mysore (India) and used at a concentration of 0.5%.

Methods

Infusions of tea leaves with apple juice were prepared by boiling different concentrations of tea in apple juice for 3 min at 100°C. The concentrations (2, 3, 4 and 5 g per 100 ml apple juice) and types of tea (CTC, orthodox and herbal) varied, resulting in 12 different combinations. Infusions were filtered through sieves and used as fermentation media. To each infusion, 0.1% diammonium hydrogen phosphate (DAHP) as a nitrogen source and 0.5% pectinesterase for clarification were added, respectively. The total soluble solids (TSS) value was raised to 20°B by adding sugar, and sulphur dioxide (100 ppm) was added to kill wild microorganisms. After 2 h, each must was inoculated with 5% second generation 24-hour-old *S. cerevisiae* var. *ellipsoideus* and left to ferment at room temperature. Combinations were fermented three times at the same conditions so that the effect of multiple replicate fermentation processes on the quality of the wine could be studied.

Fermentation was considered complete when a stable TSS had been reached. Air locks were fitted to the mouths of the glass bottles close to the end of fermentation. After fermentation was complete, the wines were racked, filtered and poured into 200 ml bottles with 2.5 cm head-space, followed by crown corking and mild pasteurization, and used for the analysis of physico-chemical, antimicrobial and sensory properties. Wines fermented naturally at room temperature using the same methods but without inoculation were also produced and bottled.

Analysis

Falls in TSS (°B) were monitored at appropriate time points during fermentation. After bottling, wines were analysed for different physico-chemical properties: TSS, titratable acidity (as malic acid), pH, sugars according to standard methods [25], and ethanol measured colourimetrically by the potassium dichromate method [26]. Volatile acidity was estimated by the titration methods described by Amerine *et al.* [27]. The content of higher alcohols was estimated using the method of Guymon *et al.* [28].

The antimicrobial activity of apple juice, apple tea wine and tea cider was determined against all the test microorganisms, that is, *Escherichia coli* (IGMC), *Enterococcus faecalis* (MTCC 2729), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MRSA 252) and *Bacillus cereus* (CRI), by the well diffusion method [29] under aerobic conditions.

A semi-trained panel of judges analysed the sensory characteristics of different apple tea wines using composite scoring and the hedonic rating test as described by Amerine *et al.* [27] and Joshi [30].

Statistical analysis

Data on the physico-chemical and functional properties of the apple tea wine were subjected to analysis of variance using a completely randomized design (CRD) and the means with critical differences reported. Cluster analyses of data using SPSS 16.0 software was carried out. The results were plotted as dendrograms. Statistical analysis of data obtained from sensory evaluation of the wine was carried out using a randomized block design (RBD) as described by Cockrane and Cox [31]. Results are reported in the tables.

Results and discussion

Fermentation with *S. cerevisiae* var. *ellipsoideus*

Effect of different types of tea

Different types (orthodox, herbal and CTC) of tea were used in order to study their effect on the quality of apple tea wine. The rate of fermentation was higher in wine made with herbal tea (1.11) than the other tea types, possibly due to the lower total phenol content in herbal tea [32]. The highest TSS (7.05°B), reducing sugar content (396 mg/100 ml) and total sugar content (1.20%) were observed in wine made from CTC tea due to the low fermentability of the musts because of their high total phenolic content. The highest titratable acidity (0.75%) was observed in wine made with CTC tea and the lowest in wine made from orthodox tea (0.68%). Volatile acidity did not differ significantly ($p \leq 0.05$) among the wines prepared from different types of tea. However, significantly higher ($p \leq 0.05$) ethanol content (8.98%) was observed in wine made with herbal tea, which might be due to the high rate of fermentation and low total phenolic content of herbal tea extracts, both important in fermentation as described earlier. The lowest alcohol content was observed in wine made with orthodox tea and herbal tea (125 mg/l), while the highest was found in wine made with CTC tea (163 mg/l) (Table 1), possibly due to the composition of CTC tea which is reported to be rich in oxidized products such as theaflavins and thearubigins [33], which might have contributed to the formation of higher alcohols. Higher alcohols are synthesized during fermentation from oxo-acids that are derived as by-products from amino acid and glucose metabolism [34]. The highest levels of total phenols, epicatechin, caffeine, protein content and amino acids were observed in wine made with CTC tea and the lowest were in wine made with orthodox tea as reported earlier [32]. There was a non-significant difference in antioxidant activity among the different tea-based apple tea wines as reported earlier [32].

Effect of different concentrations of tea

The concentration of each tea was varied from 2 to 5 g/100 ml apple juice to determine the effect of concentration on the quality of the apple tea wine. An increase in tea concentration enhanced the rate of fermentation (Table 2), likely due to the high content of amino acids (2–4% dry basis) which are an essential nutrient for yeast growth, accelerating fermentation. Nitrogen is used by the yeast for synthesizing structural proteins, enzymes, nucleic acids and pyrimidine nucleotides [35]. Assimilable nitrogen has been shown to increase sugar fermentation and ethanol formation [35, 36]. However, the results of the present study are contrary to the findings of Jayasundara *et al.* [37] who reported the use of higher concentrations of tea to produce alcoholic beverages led to a decrease in the Brix drop rate, although changes in fermentation efficiency were marginal. A significant ($p \leq 0.05$) increase in the content of reducing sugars (from 275 to 352 mg/100 ml) and total sugars (from 1.10% to 1.14%) (Table 2) was observed with an increase in tea concentration from 2 to 4 g/100 ml apple juice, which might be due to the inhibiting effect of total phenols on alcoholic fermentation which increased as tea concentration increased, as reported earlier [32]. Amerine *et al.* [27] found that the natural phenols present in grapes and wine including tannins, total phenols and pigment polymers, are inhibitory to yeast and bacteria. In addition, a non-significant effect of different concentrations of tea was also observed on the TSS, titratable acidity and volatile acidity of apple tea wine.

The difference in alcohol content among the different concentrations of tea is likely related to differences in the fermentability of the must. Results revealed that alcohol content ranged from 8.36% to 8.82% (v/v) in wine prepared from the different concentrations of tea, which demonstrates that fermentation was completed almost to dryness. Since table wine has an alcohol content of 7–14% [27], all wines prepared in the present study fall into this category. The content of higher alcohols increased with increasing tea concentration: wine made from 5 g tea/100 ml apple juice was found to have 159 mg/l higher alcohols. Higher alcohols are synthesized during fermentation from oxo-acids that are derived as by-products from amino acid and glucose metabolism [34], as mentioned earlier. An increase in concentration of tea from 2 to 5 g was accompanied by a significant ($p \leq 0.05$) increase in total phenols, epicatechin, proteins, amino acids and caffeine in apple tea wine, as reported earlier [32]. A non-significant increase in antioxidant activity was observed with increased tea concentrations, as reported earlier [32].

Natural fermentation

Effect of different types of tea

Different types of tea (orthodox, herbal and CTC) were used in order to study their effects on the physico-chemical and functional properties of apple tea wine. Wine made with herbal tea had the highest rate of fermentation (1.20), while that

Type of tea	Rate of fermentation (°B) 24 h	TSS (°B)	Reducing sugars (mg/100 ml)	Total sugars (%)	Titratable acidity (% malic acid)	Volatile acidity (% acetic acid)	Ethanol (% v/v)	Higher alcohols (mg/l)
Orthodox	1.06	6.75	265	1.11	0.68	0.027	8.23	125
Herbal	1.11	6.75	360	1.06	0.69	0.023	8.98	125
CTC	0.99	7.05	396	1.20	0.75	0.029	8.36	163
CD (*p*>0.05)	0.05	NS	12	0.01	0.03	NS	0.26	2

CD critical difference, *CTC* crush, tear and curl, *NS* not significant, *TSS* total soluble solids

Table 1 - Changes in the physico-chemical characteristics of apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* as affected by different types of tea

Concentration of tea (per 100 ml apple juice)	Rate of fermentation (°B) 24 h	TSS (°B)	Reducing sugars (mg/100 ml)	Total sugars (%)	Titratable acidity (% malic acid)	Volatile acidity (% acetic acid)	Ethanol (% v/v)	Higher alcohols (mg/l)
2 g	0.98	6.60	275	1.10	0.71	0.026	8.44	112
3 g	1.06	6.87	324	1.13	0.69	0.025	8.47	136
4 g	1.07	7.00	352	1.14	0.71	0.027	8.82	142
5 g	1.10	6.93	344	1.13	0.70	0.028	8.36	159
CD (*p*>0.05)	0.06	NS	13	0.01	NS	NS	0.26	2

CD critical difference, *CTC* crush, tear and curl, *NS* not significant, *TSS* total soluble solids

Table 2 - Changes in the physico-chemical characteristics of apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* as affected by different concentrations of tea

made with CTC tea had the lowest (0.79), possibly due to the low polyphenolic content of the CTC tea-based must, which had an inhibitory effect on alcoholic fermentation [27]. The highest TSS (7.80°B) and reducing sugar content (367 mg/100 ml) were observed in wine made with CTC tea and the lowest in wine made with herbal tea owing to the fermentability of these different tea-based musts, as discussed earlier. The lowest total sugar content (0.79%) was observed in wine made with herbal tea and the highest (1.94%) in wine made with orthodox tea. There were non-significant differences in titratable acidity among the different types of tea (Table 3). The highest volatile acidity (0.041%) was observed in wine made with CTC tea and the lowest (0.029%) in wine made with herbal tea due to the rates of fermentation of the two treatments. The highest ethanol content (8.31%) was observed in wine made with herbal tea and the lowest (7.63%) in wine made with orthodox tea (Table 3). The lowest content of higher alcohols (191 mg/l) was observed in wine made with orthodox tea and the highest (304 mg/l) in wine made with CTC tea (Table 3), possibly due to the composition of CTC tea, as discussed earlier. The highest concentrations of total phenols, epicatechin, protein content and amino acids were observed in wine made with CTC tea and the lowest in wine made with herbal tea, as reported earlier [32]. The highest caffeine was observed in wine made with CTC tea and the lowest in wine made with orthodox tea, which was possibly due to the contribution of tea leaves (w/w), as reported earlier [32].

Type of tea	Rate of fermentation (°B) 24 h	TSS (°B)	Reducing sugars (mg/100 ml)	Total sugars (%)	Titratable acidity (% malic acid)	Volatile acidity (% acetic acid)	Ethanol (% v/v)	Higher alcohols (mg/l)
Orthodox	1.11	7.30	237	1.94	0.76	0.034	7.63	191
Herbal	1.20	6.90	99	0.79	0.81	0.029	8.31	199
CTC	0.79	7.80	367	1.85	0.81	0.041	7.91	304
CD (p>0.05)	0.08	0.32	11	0.02	NS	0.007	0.19	2

CD critical difference, *CTC* crush, tear and curl; *NS* not significant, *TSS* total soluble solids

Table 3 - Changes in the physico-chemical characteristics of apple tea wine naturally fermented as affected by different types of tea

Effect of different concentrations of tea

The concentration of each tea was varied from 2 to 5 g/100 ml apple juice to study the effect on the physico-chemical and functional properties of apple tea wine. Must made with 5 g tea/100 ml apple juice had a higher rate of fermentation (1.15), closely followed by must made with 4 g tea/100 ml apple juice (1.13), possibly due to the presence of a high quantity of amino acids which increased with increased tea concentration (Table 4), as discussed earlier. The data revealed that the difference in TSS was non-significant among the different tea concentrations, while varying the concentration of tea from 2 to 4 g resulted in a significant ($p \leq 0.05$) decrease in the content of reducing sugars (from 293 to 177 mg/100 ml) and total sugars (from 2.02% to 1.16%); however, slightly higher values for both sugars were observed for 5 g tea/100 ml apple juice (Table 4). The highest titratable acidity (0.83%) and highest volatile acidity (0.046%) were observed in wine made with 5 g tea/100 ml apple juice as compared to the other concentrations studied. The highest ethanol production was recorded in wine made with 2 g tea/100 ml apple juice (8.19%) (Table 4), likely due to the low total polyphenolic content of the tea and the presence of natural yeast identified as *S. cerevisiae*, which might have boosted alcoholic fermentation [38]. The data show that an increase in tea concentration from 2 to 5 g/100 ml apple juice resulted in an increased content of higher alcohols (from 190 to 277 mg/l) (Table 4). An increase in total phenols, epicatechin, protein content, amino acids, caffeine content and antioxidant activity of the wine was also observed with increased tea concentration, as reported earlier [32].

Effect of type of fermentation on the physico-chemical and functional properties of apple tea wine

The rate of fermentation was lower in naturally fermented apple tea wines compared to apple tea wine fermented with *S. cerevisiae* var. *ellipsoideus*, possibly due to mixed microbial fermentation during natural fermentation, where both yeast and bacteria could have played a role. In addition, secretion of a killer factor by the wild yeast found in the early stage of must fermentation could have inhibited the process and thereby lowered the rate of fermentation as reported by Farris *et al.* [39]. Higher TSS and greater content of reducing and total sugars were observed in naturally fermented wines compared to those fermented with *S. cerevisiae* var. *ellipsoideus* due to differences in fermentability. The high acidity and volatile acidity of naturally fermented wine (Tables 3 and 4) might be due to the presence of natural microflora including both yeast and bacteria. The higher volatile acidity of wine naturally fermented is due to the presence of mixed microflora other than *Saccharomyces* as reported by Caridi *et al.* [40]. These authors described the high volatile acidity of wine fermented with *Hanseniaspora quilliermondii* whose acetogenic property made the yeast unsuitable for wine production as it may encourage the growth of acetic acid bacteria during fermentation (lesser growth increases acetic acid concentrations and consequently volatile acidity).

Equal amounts of ethanol were produced by both fermentation processes, possibly due to the dominance of *S. cerevisiae* var. *ellipsoideus* over natural microflora during the initial stages of natural fermentation. Heard and Fleet [41] reported that *S. cerevisiae* dominated wine fermentation but that there was significant growth of other yeast species

Concentration of tea (per 100 ml apple juice)	Rate of fermentation (°B) 24 h	TSS (°B)	Reducing sugars (mg/100 ml)	Total sugars (%)	Titratable acidity (% malic acid)	Volatile acidity (% acetic acid)	Ethanol (% v/v)	Higher alcohols (mg/l)
2 g	0.84	7.33	293	2.02	0.77	0.032	8.19	190
3 g	0.99	7.27	263	1.69	0.77	0.031	8.09	212
4 g	1.13	7.27	177	1.16	0.80	0.031	7.61	246
5 g	1.15	7.47	205	1.24	0.83	0.046	7.91	277
CD (p>0.05)	0.09	NS	12	0.02	0.04	0.008	0.22	3

CD critical difference, *NS* not significant, *TSS* total soluble solids

Table 4 - Changes in the physico-chemical characteristics of apple tea wine naturally fermented as affected by different concentrations of tea

such as *Kloeckera apiculata*, *Candida stellata*, *Candida colliculosa*, *Candida pulcherrima* and *Hansenula anomala*. These species are susceptible to increasing alcohol levels and are not as alcohol tolerant as *S. cerevisiae* [42]. There was a greater content of higher alcohols in the naturally fermented wine compared to those fermented with *S. cerevisiae* var. *ellipsoideus* due to the presence of mixed microflora during natural fermentation. The highest values for total phenols, epicatechin, proteins, amino acids and caffeine were recorded in naturally fermented wine and were higher than in wines fermented with *S. cerevisiae* var. *ellipsoideus*, as reported earlier [32]. This may be due to the low fermentability of naturally fermented wines which might have resulted in less degradation of caffeine than in wines fermented with *S. cerevisiae* var. *ellipsoideus*. Our results are in line with the findings of Malbasa *et al.* [43] who reported that during kombucha fermentation, caffeine content decreases continuously and is independent of tea concentration.

Antimicrobial activity

Effects of different concentrations of polyphenolics, acid and ethyl alcohol on antimicrobial activity

Analysis of the antimicrobial activity of the different components of apple tea wine (polyphenolics, alcohol and citric acid) produced using different concentrations of tea (Fig. 1) showed that an increase in tea concentration (from 2% to 5% with water) resulted in a slight increase in antimicrobial activity against all tested microorganisms, possibly due to an increased concentration of phenols. Phenolic compounds may affect the growth and metabolism of bacteria, and have an activating or inhibiting effect on microbial growth depending on composition and concentration [44]. The inhibitory effect of phenolic compounds could be due to their adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation [44, 45]. It was also observed that none of the concentrations of ethyl alcohol (5–50%) tested exhibited antimicrobial activity against any of the test microorganisms. Similar findings were also observed by Boban *et al.* [46] who reported that among the different components of wine, ethanol had the lowest antimicrobial activity against all tested microbes (*E. coli* [ATCC 25922] and *Salmonella enterica* [ATCC 13076]). An increase in acid concentration (from 0.25% to 1.00%) was accompanied by an increase in antimicrobial activity against all tested microorganisms in the present

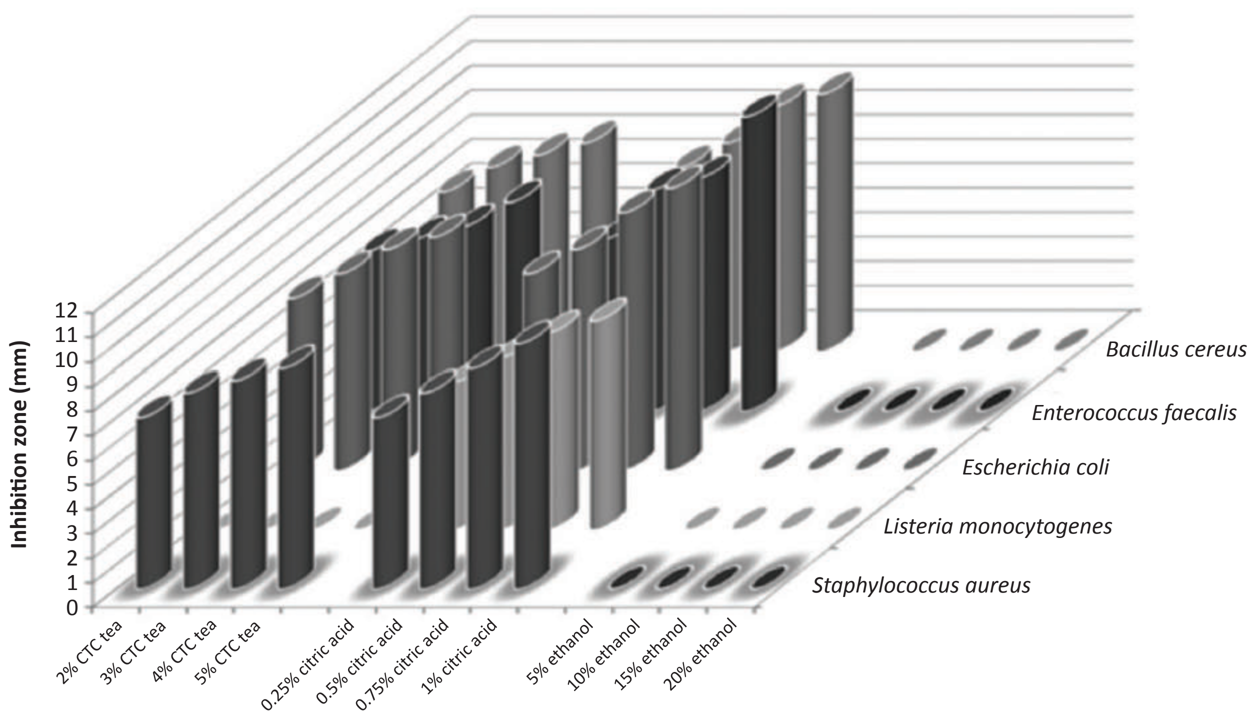

Figure 1 - Effect of the different components (concentration of tea [for polyphenolics], citric acid [acidity] and alcohol) of apple tea wine on antimicrobial activity. *CTC* crush, tear and curl

Test microorganism	CTC tea (per 100 ml apple juice)				Herbal tea (per 100 ml apple juice)				Orthodox tea (per 100 ml apple juice)			
	2 g	3 g	4 g	5 g	2 g	3 g	4 g	5 g	2 g	3 g	4 g	5 g
Apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus*												
Escherichia coli	8.50	9.25	10.75	9.00	9.00	9.25	10.00	11.25	8.00	8.50	10.00	10.50
Staphylococcus aureus	7.00	7.75	8.00	8.00	8.00	8.50	8.75	9.00	6.50	7.50	7.00	7.50
Bacillus subtilis	9.00	9.00	9.50	9.50	9.00	9.00	9.50	9.50	7.00	8.50	9.00	6.00
Bacillus cereus	9.00	9.50	10.00	10.00	7.00	8.50	9.00	9.25	7.00	7.50	8.00	9.00
Enterococcus faecalis	7.00	8.00	8.50	9.00	7.00	7.50	7.50	8.50	6.00	6.50	6.75	7.00
Natural fermentation												
Escherichia coli	8.50	8.50	9.50	10.00	8.25	9.00	9.50	9.50	7.25	7.75	9.25	9.50
Staphylococcus aureus	7.50	8.00	9.50	8.00	7.00	7.50	7.50	8.50	6.50	6.50	7.00	8.00
Bacillus subtilis	7.00	9.50	10.00	9.00	7.00	7.50	8.50	9.00	9.50	8.50	9.50	10.00
Bacillus cereus	8.50	9.00	9.00	9.50	7.00	8.00	9.50	9.50	8.50	8.00	10.50	11.00
Enterococcus faecalis	7.00	7.00	8.00	8.00	6.00	7.00	8.00	8.50	7.50	7.50	8.00	9.50
CTC crush, tear and curl												

Table 5 - Effect of different concentrations and types of tea on antimicrobial activity (inhibition zone in mm) of apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* and by natural fermentation

study. The results of the present study are in line with the findings of Waite and Daeschel [47] who examined the antimicrobial activity of four wine parameters (pH, titrable acidity, sulfur dioxide and ethanol) in various combinations against *E. coli* O157:H7 and *S. aureus*, and reported that pH was the most critical factor in predicting inactivation of tested pathogens.

Antimicrobial activity of apple tea wine fermented naturally and with S. cerevisiae var. ellipsoideus

No clear-cut trend was observed in the antimicrobial activity of the various apple tea wines produced with different types of tea, concentrations and types of fermentation (Table 5). However, all wines exhibited antimicrobial activity (inhibition zone >7 mm) against all pathogenic microbes tested. This might be due to the presence in the wines of organic acid and total phenols which are both important in antimicrobial activity, as shown experimentally (Fig. 1).

Sensory analysis

The sensorial composite scoring of apple tea wine fermented with *S. cerevisiae* var. *ellipsoideus* and by natural fermentation is compared in Fig. 2.

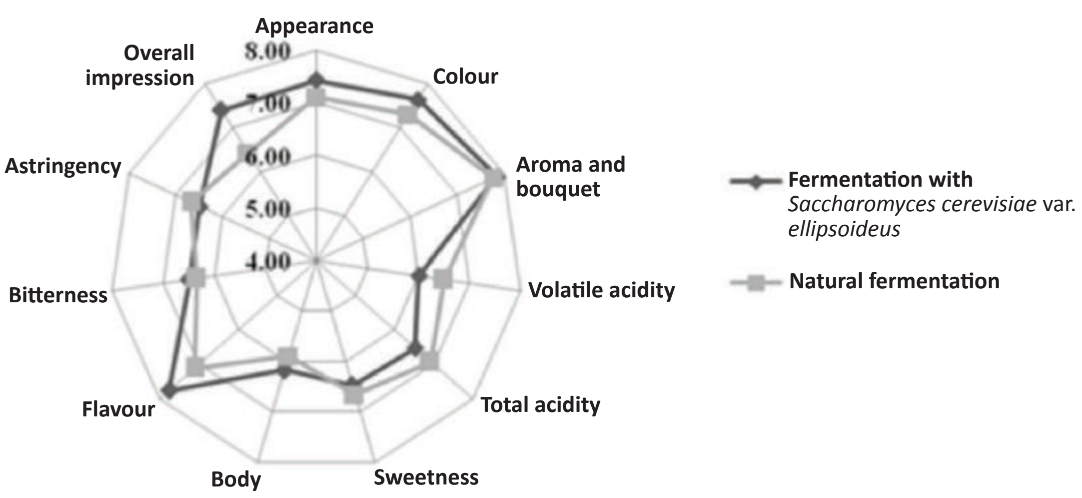

Figure 2 - Sensory evaluation of apple tea wine fermented by different types of fermentation

Fermentation with *S. cerevisiae* var. *ellipsoideus* showed higher scores for most sensory attributes than naturally fermented wine except for volatile acidity, total acidity, sweetness and astringency. Scores showed that wines prepared with *S. cerevisiae* var. *ellipsoideus* and by natural fermentation fell into the 'standard' category [27].

Cluster analysis

A dendrogram of the different treatments of apple tea wine according to the physico-chemical and functional properties of wine fermented with *S. cerevisiae* var. *ellipsoideus* is shown in Fig. 3A.
Cluster analysis grouped wine made with 2 and 3 g tea/100 ml apple juice into one cluster and wine made with 4 and 5 g tea/100 ml apple juice into another cluster, indicating that the physico-chemical characteristics of wines made with 2 or 3 g tea/100 ml apple juice are similar to each other but distinctly different from the characteristics of wines made with 4 or 5 g tea/100 ml apple juice. However, cluster analysis failed to separate type of tea into a cluster, indicating that type of tea did not influence the physico-chemical and functional properties of the wine. However, in case of wine fermented naturally, type of tea or tea concentrations did not affect the physico-chemical or functional properties. Nevertheless, 5 g tea is an outlier as shown in Fig. 3B. The distinctness of this treatment is also shown by the differences among the physico-chemical and functional properties of the treatment. Cluster analysis successfully grouped the two different types of fermentation into separate clusters as shown in Fig. 3C. One cluster comprises wine fermented with *S. cerevisiae* var. *ellipsoideus*, while another comprises naturally fermented wine. Cluster analysis further revealed that the type of fermentation influenced the physico-chemical and functional properties of apple tea wine.

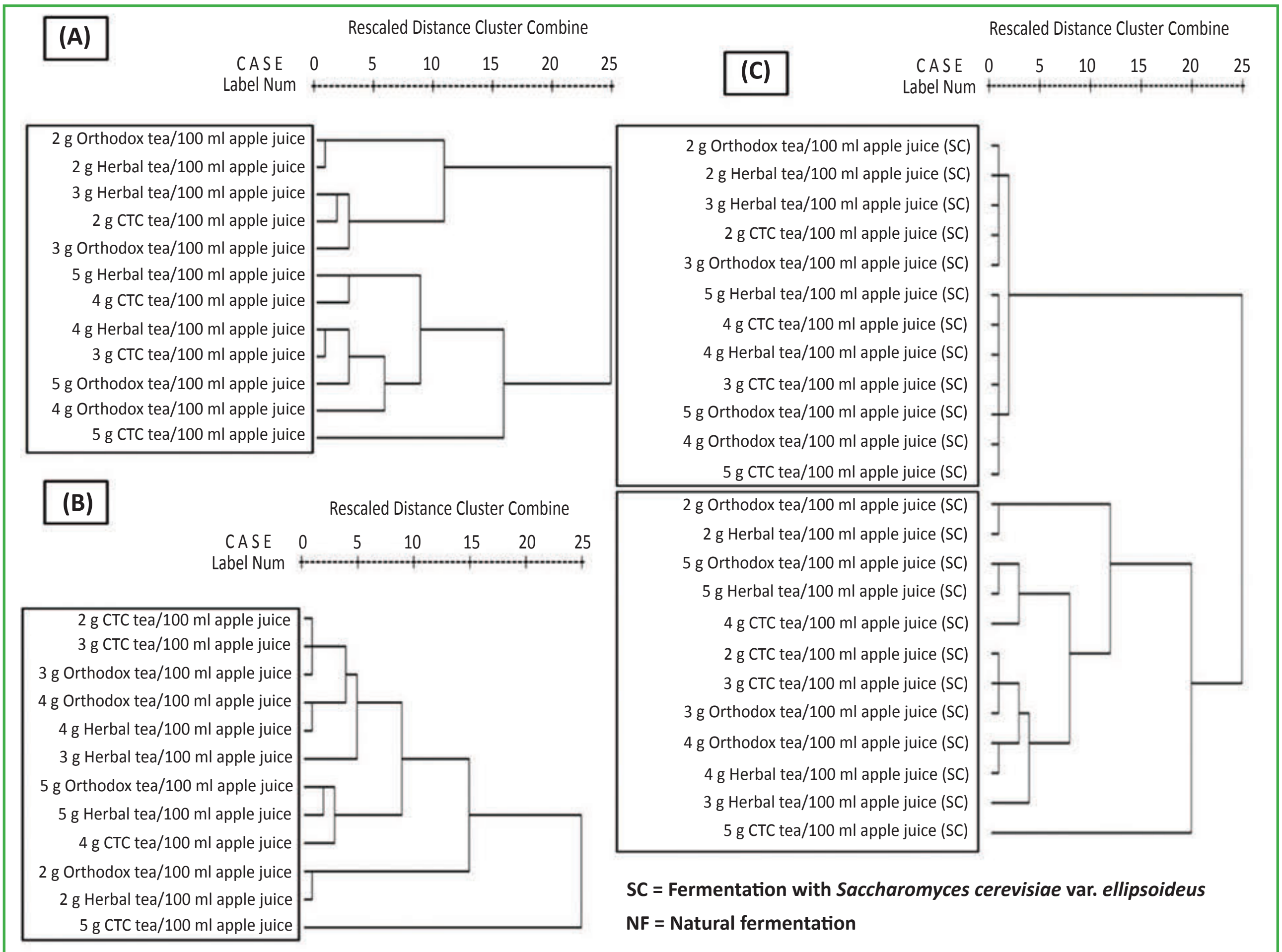

Figure 3 - Dendrograms of different treatments of apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* (A), by natural fermentation (B) and their comparison (C) using physico-chemical and functional properties analysed based on rescaled distance. *CTC* crush, tear and curl

Conclusions

The study shows that the typical microflora involved in the production of apple tea wine differ between fermentation with *S. cerevisiae* var. *ellipsoideus* and with natural microflora, which is reflected in the different characteristics of the respective wines. In both fermentation groups, wine made with CTC tea received significantly ($p \leq 0.05$) higher scores, while wine made with 4 g tea/100 ml apple juice had better results compared to other concentrations of tea. Wine fermented with *S. cerevisiae* var. *ellipsoideus* had higher scores than naturally fermented wines. All wines showed antimicrobial activity (inhibition zone >7 mm) against all pathogenic microbes tested. Clustering of different treatments was observed based on tea concentration as a function of fermentation with *S. cerevisiae* var. *ellipsoideus* unlike natural fermentation. The type of fermentation seems to have an independent effect on the characteristics of the wine as shown by combined cluster analysis of all treatments. The results demonstrated that the best apple tea wine was made with 4 g CTC tea/100 ml apple juice and fermented with *S. cerevisiae* var. *ellipsoideus*. Thus, these findings indicate the desirability of alcoholic fermentation by *S. cerevisiae* for preparing apple tea wine. Although kombucha is naturally fermented, in our study inoculation with *S. cerevisiae* var. *ellipsoideus* produced good results for all wines. Apple tea wine can be considered a wine with the additional medicinal properties of tea. Since natural fermentation is not always predictable, inoculated fermentation is considered to be better than natural fermentation.

Acknowledgements
The authors are thankful to the head of the Department of Food Science and Technology, Dr. Y S Parmar UHF Nauni, Solan (HP) for providing use of the facility and financial support

Conflict of Interest
The authors declare that they have no conflict of interest

Human and Animal Rights
This article does not contain any studies with human or animal subjects performed by any of the authors so human and animal rights are not involved

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