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Effect of Different Types of Fermentation (Inoculated and Natural Fermentation) On the Functional Properties of Apple Tea Wine.

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ABSTRACT

Study was conducted to determine the effect of different fermentations on the functional properties of apple tea wine. With increase in concentrations of tea from 2 to 5 g, a increase in protein content, pH, colour, total phenols, epicatechin and caffeine was recorded among the tea leaves extracts with apple juice and apple tea wine fermented by both the fermentations, whereas, a decrease in quercetin and antioxidant activity in the must and wine took place. As compared to the composition of tea leaves extract in apple juice, a decrease in pH, colour, total phenols, epicatechin and caffeine of apple tea wines (by both fermentations) was observed, in contrast to the protein content and antioxidant activity an increase was observed. However, apple tea wine prepared from 5 g tea had the highest protein content, pH, total phenols, epicatechin, caffeine and antioxidant activity. Among the apple tea wine based on different types of tea, CTC tea based apple tea wines had highest proteins, total phenols, epicatechin and caffeine, whereas, Herbal tea based apple tea wine (fermented with *Saccharomyces cerevisiae* var. *ellipsoideus*) had the highest quercetin content, whereas, in case of natural fermented apple tea wines, it was highest in orthodox tea based apple tea wine. Among the different types of tea, difference for antioxidant activity was non-significant.

Keywords: Apple, Tea, apple tea wine, *Saccharomyces cerevisiae,* natural fermentation

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INTRODUCTION

Wines are made from complete or partial alcoholic fermentation of grape or any other fruit like apple, plum, peach, pear, berries, cherries, currants, apricot [1, 2]. Presently, apple juice is fermented to manufacture the cider, a sparkling and refreshing fruit flavored beverage, consumed in many countries of the world [3, 4] along with wine and brandy. Tea (*Camellia sinensis* L.) is the most important non-alcoholic beverage consumed as a morning drink, contain more than 700 chemical constituents, among which flavonoids, amino acids, vitamins (C,E,K), caffeine and polysaccharides are important to human health. The stimulative effect of tea is due to caffeine [5]. But polyphenolics are most important constituents acting as antioxidant known, to play a very significant role in human health.

Black tea has a strong body due to tannins, which are a group of astringent polyphenolic compounds such as flavonoids (theaflavin and thearubigin) and others derivatives of polyphenols. The natural tannins are powerful reducing agents and exhibit a marked tendency to absorb oxygen, thereby, making tea infusions a possible health drink due to its antioxidant property. Not only in tea, tannins impart body to various fermentation products especially in wines and fermented fruit juices, besides enhancing their flavour profile [6]. Black tea can be considered as a good fermentation medium because its infusion contains proteins, aminoacids, volatile compounds, lipids, enzymes and more importantly polyphenols [7].

Alcoholic beverages in several parts of the world are prepared by natural fermentation though at commercial scale such beverages are prepared by inoculated fermentation (*Saccharomyces cerevisiae* var *ellipsoideus*). But, there many areas in natural fermentation which are still not well understood [8] especially the role of numerous non-*Saccharomyces* yeasts normally associated with grape must and wine. These yeasts, naturally present in all wine fermentations to a more or less extent, have been found to be metabolically active and their metabolites can impact on wine quality. Rather, non-*Saccharomyces* wine yeasts have been found to influence the fermentation kinetics of fermentations even when inoculated with starter cultures of *Saccharomyces cerevisiae* [9]. It is well established that the industrial wine fermentations are currently conducted by starters of selected wine yeast strains of *Saccharomyces cerevisiae* in contrast to traditional spontaneous fermentations conducted by the flora that originate from the grapes and winery equipment without deliberate inoculation to start the process [10, 11]. Natural fermentation is also practised to make apple wine but pure culture of yeast *Saccharomyces cerevisiae* is preferable as the final quality of wine is predictable. Moreover, the physico-chemical characteristics of the beverages fermented by the pure yeast culture were found to be desirable from the enological and toxicological angles [1, 12]. From the glimpse of the literature, it is clearly visible that different types of tea leaves are the richest sources of different polyphenolics. The polyphenols of tea can be utilized to improve the phenolics composition of wine by mixing apple juice with tea extract. Therefore, the present study was aimed for characterization of tea leaf extracts with apple juice and to study the effect of different fermentation on the composition of wine.

MATERIALS AND METHODS

Materials: Golden variety of apple was procured from the local market of Solan, whereas, Orthodox tea (Dhauladhar, Natural Organic Orthodox Kangra Tea) was procured from HPKV, Palampur; Herbal Tea (Himalayan Brew, Kangra Special Herbal Tea) was procured from local market of Palampur and CTC tea (Tajmahal) was procured from local market of Solan (H.P.). The yeast culture viz. *Saccharomyces cerevisiae* var. *ellipsoideus*, (UCD 595) used in the study was obtained from Indian Institute of Horticulture Research, Bangalore. Sucrose, the common sugar was procured from the local market for the preparation of apple tea wine. The pectinesterase used was manufactured by M/S Triton Chemical, Mysore (India) and was used at a concentration of 0.5%.

Preparation of apple tea wine: To study the effect of different types of fermentation on the functional properties of apple tea wine, different quantities/concentrations (2, 3, 4 and 5 g) of different tea (CTC, orthodox and herbal tea) were used. The infusion/extract of these tea leaves was made with apple juice by boiling for 3 minutes at 100 ^{o}C. After sieving, these infusions were bottled and used for further analysis. The infusions so prepared were also used as a fermentation medium. To these extracts, diammonium hydrogen phosphate (DAHP) at the rate of 0.1% as nitrogen source and 0.5% pectinase enzyme for clarification were added. The total soluble solids (TSS) was raised to 20°B with addition of sugar and sulphur dioxide (100 ppm) was added to kill the natural microflora. After 2 hours, the respective must were inoculated with 5% of

activated culture of *Saccharomyces cerevisiae* var. *ellipsoideus*. Each treatment study was carried out in 2.5 l capacity narrow mouth dark glass bottles, filled up to 75% of their capacity. After the completion of fermentation, the wines were racked, filtered and filled-in 200 ml bottles keeping 2.5 cms head-space, followed by crown corking and mild pasteurization and used for further analysis and characterization. Natural fermentation (uninoculated) for all the treatments was carried out at room temperature. When a stable TSS was reached the fermentation was considered complete. Air locks were fitted in the mouth of glass bottles near the end of fermentation. The apple tea wine so prepared was siphoned/racked, bottled and used for further analysis and characterization.

Analysis of Biochemical parameters

Proteins were estimated by the standard procedure as described by Sadasivam and Manickam [13]. pH was estimated by digital pH meter while the colour was estimated UV-Vis Spectrophotometer at 440 nm in terms of optical density [14]. The total phenols content in apple tea wines were determined by Folin Ciocalteu procedure given by Singleton and Rossi [15]. Phenolic compounds (epicatechin and quercetin) and caffeine content were evaluated by reversed phase - high performance liquid chromatography (RP-HPLC) with direct injection without any particular treatment except filtration [16, 17]. Antioxidant activity (Free radical scavenging activity) was measured as per the method of Brand-Williams *et al*. [18].

Statistical analysis

Statistical analysis of the quantitative data of chemical parameters obtained from the experiments was done by Completely Randomized Design (CRD) Factorial.

RESULTS AND DISCUSSION

Effect of tea concentration

Protein and pH of apple tea leaves extracts increased almost linearly with increase in tea concentration (Table 1), which is apparently the contribution of tea leaves (wt/wt) as it is the richest source of proteins [19, 20, 5]. Protein content of apple tea wine prepared from the highest concentration of tea was found higher than protein content of the tea leaves extract in apple juice. A comparison of the protein contents of the tea leaves extract with apple juice and with that of respective apple tea wine revealed an increase in the protein content of the apple tea wine with 4 or 5 g tea leaves extracts (Table 1). Although tea leaves extracts were also prepared with 2 and 3 g of tea leaves extract but the protein content decreased instead of increase as happened in high concentrations. It is known that phenols/tannins, react with proteins and precipitates resulting in decrease in concentrations of both protein and phenols [1]. But in case of high concentrations of protein, the net result might be an increase in protein content. Testing the sediments of various apple tea wines revealed the presence of total phenols in the sediments obtained by the fermentation. Thus, an increase in protein content of apple tea wines with more tea leaves concentration was obtained. Besides this, the extra-cellular proteins secreted by yeasts during the fermentation time may also have affected the concentrations of proteins in the end products [21]. Proteins content of naturally fermented apple tea wines were higher than the apple tea wines fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* which might be due to the extra cellular proteins secreted by the microflora including yeasts during the fermentation time [21]. A gradual and significant increase in pH of apple tea wine was observed with increase in tea concentration which is apparently the contribution of tea leaves (w/w), but the increase was lesser than the pH of tea leaves extract in apple juice which might have been caused by more CO_2 absorption, accumulation and dissolution of excess of CO_2 which might have resulted in the formation of carbonic ions in the must and that ultimately increased the acidity [22]. Low pH in case of naturally fermented apple tea wine as compared to the apple tea wines fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* was observed which might be due to the results of presence of natural micro-flora including both yeast and bacteria. Colour (measured as OD, 440 nm) increased with increase in the concentration of tea (Table 1), which might be due to the subsequent increase in tea concentration, resulting in an increase in colour of the extracts. A decrease in OD of colour was observed in the apple tea wines prepared by both fermentations as compared to the apple tea leaves extracts which might be the effect of clarification of wine with the action of enzyme, precipitation and sedimentation during fermentation.

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Table 1: Changes in physico-chemical characteristics of apple tea leaves extract as influenced by concentrations of tea and types of fermentation

Concentrations of tea (per 100ml apple juice)	Protein Content (mg/100 ml)			pH			Colour (OD 440 nm)		
	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine
2g tea leaves	613	541	590	4.56	4.52	4.16	3.03	1.78	1.89
3g tea leaves	694	709	692	4.64	4.59	4.30	3.42	2.19	2.39
4g tea leaves	727	758	861	4.72	4.66	4.32	3.86	2.39	3.00
5g tea leaves	778	825	1035	4.76	4.73	4.37	5.55	2.35	5.10
$CD_{(p>0.05)}$	7	6	7	0.07	0.10	0.09	0.07	0.14	0.14

Table 2: Changes in physico-chemical characteristics of apple tea leaves extract as influenced by concentrations of tea and types of fermentation

Concentrations of tea (per 100ml apple juice)	Total phenols (mg/l)			Epicatechin (ppm)			Quercetin (ppm)		
	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine
2g tea leaves	449	252	415	171	140	166	14.73	11.74	13.13
3g tea leaves	510	311	418	215	191	207	12.11	11.72	12.06
4g tea leaves	576	367	516	265	217	256	11.61	10.47	11.63
5g tea leaves	661	398	579	297	243	313	11.25	7.68	11.50
$CD_{(p>0.05)}$	14	6	6	12	12	12	0.96	0.44	0.87

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Table 3: Changes in physico-chemical characteristics of apple tea leaves extract as influenced by concentrations of tea and types of fermentation

Concentrations of tea (per 100ml apple juice)	Caffeine (ppm)			Antioxidant activity (%)		
	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine
2g tea leaves	444	374	397	81.47	81.41	81.60
3g tea leaves	592	475	458	81.13	82.40	81.87
4g tea leaves	656	542	570	80.80	83.33	82.33
5g tea leaves	756	627	671	79.33	83.83	82.87
$CD_{(p>0.05)}$	24	19	22	NS	NS	NS

Total phenols, epicatechin and caffeine content tea leaves extracts in apple juice increased almost linearly with increase in tea concentration (Table 2 and 3), which is apparently the contribution of tea leaves (w/w) as it is the richest source of polyphenols and caffeine [19, 20, 5]. Total phenols and epicatechin content of apple tea wines were however lower than that of tea leaves extracts in apple juice, which might be result of precipitation of some of the tannins by protein and absorption by the yeast cells [1]. Higher values for total phenols and epicatechin contents were recorded in naturally fermented apple tea wine which was higher than that of fermentation with *Saccharomyces cerevisiae* var. *ellipsoideus*. This might be due to the low fermenatbility of the naturally fermented apple tea wine and thus, resulting in low precipitation of the phenolics during fermentation. A decrease in caffeine content of all apple tea wines was observed than the caffeine content of the apple tea extracts. The results of the present study are in line with the findings of Malbasa *et al.* [23] who reported that caffeine was one of the major constituents of black tea and as a source of nitrogen, in addition to other xanthine derivatives, was essential for the microbial growth during *Kombucha* fermentation, it decreases continually and such dynamics was independent of tea concentration. Caffeine content of naturally fermented apple tea wines was found higher as compared to the apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* which might be due to the low fermentability in case of natural fermented apple tea wines which might have resulted in lower degradation of caffeine than *Saccharomyces cerevisiae* var. *ellipsoideus* fermented apple tea wines.

Quercetin content of the tea leaves extracts decreased with the increase in tea concentrations (Table 2) which is apparently the contribution of the apple juice for quercetin. Kahle *et al.* [24] found that the quercetin content in apple juice ranged between 0.4-27 mg/L. Quercetin content of different apple tea wine fermented with *Saccharomyces cerevisiae* var. *ellipsoideus* decreased with increase in tea leaves concentrations. It is in accordance with the results of tea leaves extract in apple juice as apple is a good source of quercetin [24]. With the increase of tea leaves concentration, naturally the concentrations of apple juice decreased resulting in decrease in quercetin. Higher value for quercetin content was recorded in naturally fermented apple tea wine and was more as compared to the fermentation with *Saccharomyces cerevisiae* var. *ellipsoideus*. It can be attributed to low precipitation of phenolics in the inoculated fermentation than the natural fermentation as discussed earlier.

It was observed that with the increase in tea concentration, there was a non-significant decrease in antioxidant activity of tea leaves extract in apple juice, which might be due to the decrease in acidity of the tea leaves extract and is correlated with pH of the extracts (Table 3). The antioxidant activity depends upon pigments like carotenoids and anthocyanins [25], ascorbic acid [26] and total phenolic content [27] in the product. A slight increase in antioxidant activity of apple tea wine in both the fermentations was observed as compared to the apple tea leaves extract and is desirable.

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Table 4: Changes in physico-chemical characteristics of apple tea leaves extract as influenced by concentrations of tea and types of tea

Types of tea	Protein Content (mg/100 ml)			pH			Colour (OD 440 nm)		
	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine
Orthodox tea	652	608	759	4.66	4.63	4.28	4.35	2.85	3.00
Herbal tea	622	665	649	4.70	4.65	4.26	3.62	1.59	3.35
CTC tea	836	852	975	4.66	4.60	4.32	3.93	2.09	2.93
$CD_{(p>0.05)}$	6	5	6	NS	NS	NS	0.06	0.12	0.12

Table 5: Changes in physico-chemical characteristics of apple tea leaves extract as influenced by concentrations of tea and types of tea

Types of tea	Total phenols (mg/l)			Epicatechin (ppm)			Quercetin (ppm)		
	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine
Orthodox tea	492	290	460	187	154	217	14.21	10.32	12.29
Herbal tea	496	312	448	234	204	210	11.72	10.82	12.08
CTC tea	659	394	539	291	236	279	7.38	10.07	11.87
$CD_{(p>0.05)}$	12	5	5	11	10	10	0.83	0.38	0.75

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Table 6: Changes in physico-chemical characteristics of apple tea leaves extract as influenced by concentrations of tea and types of tea

Types of tea	Caffeine (ppm)			Antioxidant activity (%)		
	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine	Apple tea leaves extract	Apple tea wine fermented with *Saccharomyces cerevisiae* var *ellipsoideus*	Naturally fermented apple tea wine
Orthodox tea	527	371	407	81.20	82.74	82.75
Herbal tea	571	495	539	80.05	83.44	81.90
CTC tea	738	647	626	80.80	82.04	81.85
$CD_{(p>0.05)}$	21	16	19	NS	NS	NS

Influence of types of tea

Among the different types of tea, extracts of CTC (Black) tea with apple juice had the highest protein, total phenols, epicatechin and caffeine content (Table 4, 5 and 6), which are in line with the findings of Harbowy and Balentine [19] and Horzic *et al*., [28]. It is attributed to different manufacture steps of black tea i.e. withering, leaf distortion, fermentation and firing. In withering step, freshly plucked tea leaves undergo certain biochemical changes like increase in amino acid, simple carbohydrate and caffeine levels; maximal activity of polyphenol oxidase, loss of pectinase activity and breakdown of chlorophyll. Antioxidant activity of orthodox tea was the highest out of the CTC tea and herbal tea (Table 6). Similar results have also been recorded by Horzic *et al.* [28], who reported that the per cent inhibition determined by DPPH assay, displays slightly different sequence of antioxidant capacities of teas and infusions: Oolong tea > black tea > green tea > white tea > chamomile infusion > linden infusion.

The difference regarding proteins, total phenols, epicatechin and caffeine content among the apple tea wines having different tea is due to the composition of the respective tea. In both the fermentations, CTC tea base apple tea wine had highest amount of total phenols, epicatechin and caffeine which can be correlated with the composition of the respective tea as discussed earlier. In both fermentations, CTC tea based apple tea wine recorded the highest protein content but it was more in case of the natural fermented CTC based apple tea wine which might be due to the extra cellular proteins secreted by yeasts during the fermentation time [21]. Herbal tea based apple tea wine (fermented with *Saccharomyces cerevisiae* var. *ellipsoideus*) had higher quercetin content than natural fermented apple tea wines. It was highest in orthodox tea based apple tea wine. Among the different types of tea base apple tea wine, difference for antioxidant activity was non-significant.

CONCLUSION

It is concluded that with increase in tea leaves concentration irrespective to the types of tea, there was gradual increase in pH, colour, total phenols, epicatechin and caffeine, whereas, among the different types of tea, apple tea wine having CTC tea had highest proteins, total phenols, epicatechin and caffeine content. Results of both the fermentations were comparable. But fermentation by *Saccharomyces cerevisiae* var *ellipsoideus* resultant in good fermentability and have low phenolic and tannin content which is responsible for the increasing the palatability of the apple tea wine. Hence, on the basis of physico-chemical analysis apple tea wine having 4 g CTC tea (fermented with *Saccharomyces cerevisiae* var *ellipsoideus*) was the best.

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