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Standardization and quality evaluation of instant tea premix

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Abstract
This study was conducted to standardize the formulation of Instant Tea Premix and determine the anti-oxidant activity. Different tea formulations (F-I, F-II, F-II) with varying levels of tea solids and masala mix (10-15%) were prepared to evaluate their sensory attributes using 9 point hedonic rating scale. Results revealed that among all the samples, formulation F-II sample (tea solids**:** masala mix ratio of 3**:**2) had the overall acceptability score of 8.29±0.32. Further, the optimized formulation also compared with three commercially available tea brands in terms of their phenolic content and antioxidant activity potential. It was found that total phenolic content and % DPPH activity of F-II was highest compared to other samples.

Keywords: Polyphenols, anti-oxidants, instant tea premix, DPPH scavenging activity

Introduction
Tea is native to china having white flowers and dark green shiny leaves. Most commonly two varieties of tea plant *Camelia sinensis*var. *Sinensis* and *Camelia sinensis*var. *Assamica*are used for the different types of tea production. Consumption of tea is significantly exceeding that of beer, wine and soft drinks (Akhlas *et al*., 2003). However, consumption of tea is limited among the people possibly due to their growing concern of health issues (Kim *et al*., 2013 & 2015). But tea has various healthy components such as polyphenols, vitamins, caffeine which can provide various health benefits because of their higher antioxidant potential (Ludwig *et al*., 2013). Anti-oxidant activity is influenced by various growth conditions such as genetic strain, soil profile, climatic conditions, horticultural practices, and growth altitude (Ku *et al*., 2010). Anti-oxidant activity is also influenced by the extraction time, temperature, and surface area. Premix has the largest surface area due to its powder form. Anti-oxidant activity differs in loose leaf, tea bags and instant tea premix. Among these, instant tea premixes has captured the huge amount of market across the globe as being increased modernization for dietary preference and fast-paced culture, busy lifestyle and hectic time schedules. Instant tea premixes comprise of tea extract powder, milk powder with added spices or other flavours and sugar. It's an instant solution for tea consumers who have a preference for this beverage.
As per Mintel's Global product database only 2% of the new products launched has the ingredients promoting anti-oxidant which can address the various health related issues such as cardiovascular disease, skin and anti-ageing, immunity, eye. So instant tea premix along with added spices can contribute to good amount of anti-oxidant activity thereby positioning this beverage has healthy beverage rather than refreshing beverage. Therefore, current investigation aimed to formulate and standardize tea premix based on their sensory attributes and compared with existing products of different brands available in tea market for their antioxidant activity.

Materials and Methods
Tea solids, milk solids, encapsulated spices (ginger, black pepper, cardamom, cinnamon, and cloves), sucrose, maltodextrin and calcium carbonate were the ingredients used for making the instant tea premix. Spice ratio was prepared separately (Table 1) and then later all other ingredients along with prepared spice mix were fed to ribbon blender as per the set formulations. Mixing is done for 10 mins then later it is passed through 19 mesh no. sieve to facilitate uniform mixing.

Table 1: Formulation of masala mix

S. No	Spice	Percentage (%)
1	Cardamom	30
2	Ginger	30
3	Cinnamon	20
4	Black Pepper	10
5	Cloves	10

Formulation of Instant Tea Premix

Formulation of Instant tea premix was done by using tea solids, milk solids, encapsulated spices (ginger, black pepper, cardamom, cinnamon and cloves), sucrose, maltodextrin and calcium carbonate (Table 2). The experiment was divided into three groups on the basis of quantity of tea solids and spice mix percentage at ratio of 10: 15%, 15:10% and 12.5: 12.5%. However total quantity of mix was made to hundred by varying the proportions of other ingredients.

Table 2: Formulation table

S. No.	Ingredients	Ratio of proportion in percentage (%)		
		F-I	F-II	F-III
1	Tea Solids	10	15	12.5
2	Masala mix	15	10	12.5
3	Sucrose	15	15	15
4	Milk solids	30	30	30
5	Maltodextrin	28	28	28
6	Calcium carbonate	2	2	2
Total		100	100	100

Sensory evaluation of instant tea premix

Sensory evaluation of premix in respect of colour, flavour, taste and overall acceptability was done by 25 semi-trained persons including staff and research scholars from NIFTEM (India). Evaluation was done using 9 point hedonic scale. Sensory evaluation was done to select the best formulation among the three and standardise the product. Selected formulation from the three was evaluated for phenolic content and scavenging activity and then later were compared with three commercially available brands (names undisclosed) and labelled as Commercial 1, Commercial 2 and Commercial 3.

Preparation of extract from tea premix

Tea sample of 10 g was extracted with distilled water (100 ml) for 2 h using a hot plate. The re-extraction of initial extracts are processes with 60 ml distilled water at 100-130°C for 30 minutes. The extracted mixture was dried at 40°C and weighed to calculate the yield.

Determination of total phenolic content

Briefly 0.5 ml of powder extracts was dissolved in 35 ml distilled water and then mixed with 10-fold diluted Folin-Ciocalteu reagent (2.5 ml) and 7.5 ml of 20% sodium carbonate solution. Thereafter, volume was adjusted to 50 ml with distilled water and kept for 2 h (23±2°C) at room temperature. Then, 2 h of samples absorbance were taken at 765 nm using UV-spectrophotometer (Sican, 2301, in carp). TPC of each fraction was expressed as mg Gallic acid equivalents/100 g dry weight.

DPPH Radical Scavenging Assay

DPPH radical scavenging activity of water extracts from white, green, black and tea factory wastes against stable DPPH was estimated.by measurement of change in colour (from deep-violet to light-yellow) at 517 nm using a UV-visible light spectrophotometer. Briefly, 0.025 g of dry extract was mixed with 10 ml of hot water. For measurement of DPPH inhibition, a freshly prepared solution of DPPH was prepared in ethanol (6×10^{-5} M). Then, 3 ml of DPPH solution was added to sample extract (77 µl), kept in the dark for 15 min and thereafter decrease in % inhibition was measured using spectrophotometer. Radical scavenging activity was measured using the equation as given below;

% Inhibition = [(AB – AA) /AB)] × 100

Where: AB= blank absorbance (t=0 min); AA- extract absorbance.

Statistical Analysis

The data obtained from testing was carried out in triplicate, subjected to one-way ANOVA test at the 5 % level of significance.

Results

Sensory evaluation of instant tea premix

The result based on the sensory evaluation (Table 3) showed the respondent indicated that formulation has the highest overall acceptability for F-II sample, which has the formulation as tea solids 10%, tea masala 15%, sucrose 15%, milk solids 30%, maltodextrin 25 %, and calcium carbonate 5%. Overall acceptability of F-II was at par with that of F-III sample (7.9±0.21) and significantly superior over that of F-I sample (7.24±0.22). Sensory score for appearance and colour showed that F-II sample has the highest score for colour that is (8.29±0.32). Score of colour and appearance is in order F-II>F-III>F-I.F-II formulation was at par with that of F-III sample (7.9±0.21) and significantly superior over that of F-1 sample (7.24 ±0.22).

Sensory score for flavour showed that F-II got the highest score of 8.48±0.15 followed by F-I sample which got the score of 7.43±0.21. This has the order F-II>F-I>F-III. Sensory score for the taste is as follow for F-I sample has score 7.24±0.19, F-II 8.1±0.12, F-III 7.5±0.08. Score says that F-II sample was at par with that of F-III and significantly higher than that of F-I sample. Hence F-II sample is standardized based on sensory attributes completing our first objective.

Table 3: Sensory score of all three samples.

Sample	Appearance and colour	Flavour	Taste	Overall acceptability
F-I	7 ± 0.08^a	7.1 ± 0.16^a	7.24 ± 0.19^a	7.24 ± 0.22^a
F-II	8.05 ± 0.28^c	8.48 ± 0.15^b	8.1 ± 0.12^b	8.29 ± 0.32^b
F-III	7.6 ± 0.17^b	7.43 ± 0.21^a	7.5 ± 0.08^a	7.9 ± 0.21^b

Means in the columns with different symbols are significantly differ ($P < 0.05$).

Determination of phenolic content

Total phenol content (TPC) was obtained for samples F-II, C1, C2 and C3 in aqueous and methanolic extracts. Figure 1 showed that F-II sample had the maximum phenolic content of 168.96 ± 0.79 mg GAE/100 g followed by C2W which has the value of 168.79±1.06 mg GAE/100 g, C1W has 1540.46±0.00 mg GAE/100 g, C3W has the phenolic content 137.25±0.17 in aqueous extracts. F-II sample was at par with sample C1W and significant higher with the sample C3W in aqueous extracts. Result has the following order F-II>C1W>C2W>C3W.

In the methanolic extracts F-II sample has the value of 201.66±1.12 mg GAE/100 g, C2M has 172.18±1.78 mg GAE/100 g, C1M 125.41±0.95 mg GAE/100g, C3M

154.14±0.18 mg GAE/100 g., sample F-II was at par with the C2M and significant higher than C1M. Result has the following order F-II>C2M>C3M>C1M

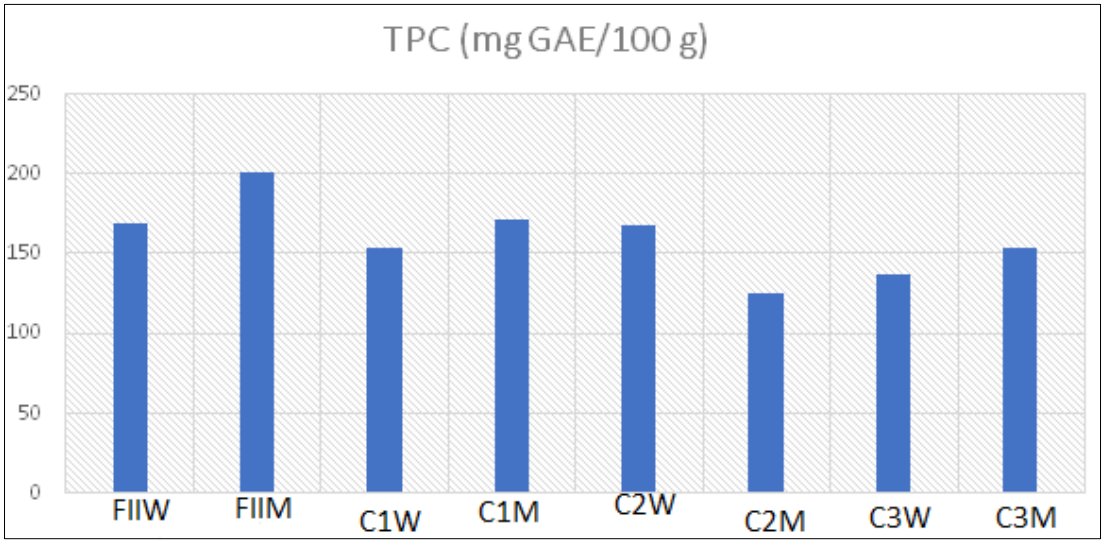

Fig 1: Total Phenol Content in aqueous and methanolic extracts of different tea premix samples.

DPPH Radical Scavenging Assay

Antioxidant activity is generally evaluated with the determination of radical scavenger's activity by rapid and reliable methods DPPH. It's become easy for the scavenging potential of antioxidants to be determined with the measuring absorbance at 517nm. The obtained result (Figure 2) recorded the methanolic extracts of tea premixes and showed highest anti-oxidant activity of F-II i.e. 92.65±1.15 %, followed by 90.74±0.80%, 89.82±0.71%, 58.20±1.17% for C3M, C2M and C1M. F-II sample was at par with the sample C3M and significant higher with the sample C1M.

The recorded result for aqueous extracts showed the highest anti-oxidant activity for the sample F-II i.e. 67.07±0.10 %, followed by 58.20±1.17%, 54.69±0.35%, 47.83±1.19% for C3W, C2W, and C1W. Result also conclude that F-II sample was at par with C3W and significant higher than that of C1W.

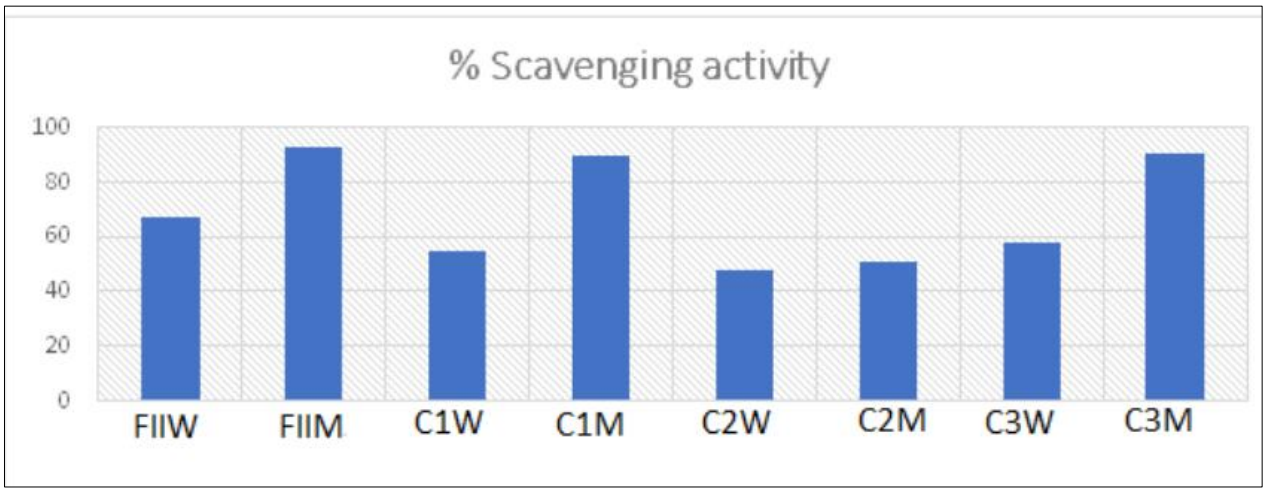

Fig 2: anti-oxidant power of water extracts and methanolic extracts of different tea premix samples.

Discussion

Quality of made black tea is defined by the flavour, colour taste and aroma, which is used by tea taster for sensory evaluation and responsible for fixing prices (Wherkoven, 1974; Liang *et al*., 2008). Among the physical parameter colour value of the brewed liquor has the highest influence on the tea quality (Ladi *et al*., 2012). Results of sensory showed that F-III samples got the highest score which has the tea solids and masala percentage of 15 and 10, having the score of 8.05 for colour, 8.48 for flavour, 8.1 for taste and 8.29 for overall acceptability. The amount of theaflavins (TF) and Thearubigins (Tb) helps out for colour influence in the tea solids (Roberte, 1962; Cloughley, 1980). Thereby manufacturing techniques and natural factors like atmospheric condition, seasonal variation, altitude rainfall, plucking standards, soil fertility etc influence the quality of black tea (Malec & Vigo, 1988; Ashu & Ravindranath, 1996; Tomlins & Mashingaidze, 1997; Fernandez *et al*., 2002). Total phenolic content of the standardized formulation was found to be 168.96±0.79 mg GAE/100 gm in aqueous extracts and 201.66±1.12 mg GAE/100 g in methanolic extracts having the maximum among all the selected brands. Antioxidant activity of the developed premix shows the highest scavenging activity compare to other commercial brands. It has the value of 67.07±0.10% in aqueous extracts and 92.65±1.15% in methanolic extracts. Lowest antioxidant activity is of C1 that is 47.83±1.19% % in aqueous extracts and 51.11±0.90% in methanolic extracts. This is explained as, Theaflavin and

theaburginis which is the major polyphenolic component responsible for anti-oxidant activity. As study conducted by Luczy and Skrzydlewska (2005) they have the effectively and efficiently scavenger's superoxide radicals than epigallocatechin gallate (EGCG) antioxidant activity comparable to the catechin's anti-oxidant activity and they were able to scavenge superoxide radicals more effectively and efficiently than epigallocatechin gallate (EGCG). Van *et al*. (1988) studied theaflavin values of different tea clones according to them explains the highest grade of broken orange pekoe fanning (BOPF) is Theaflavin content. Gupta *et al*. (2014) studied the ginger, black pepper and tulsi combination with tea gives good synergistic anti-oxidant activity. Ginger with tea derived to be the best synergistic combination. His researcher clearly concludes that instead of drinking tea alone spiced tea will be healthier as it has the highest anti-oxidant potential among the mixture studied. Also concluding the antioxidant activity of spices. All the above reference can be taken into consideration for the difference in antioxidant activity and phenolic content of the all the premixes. As tea premixes has tea solids which is made from tea leaves whose quality is governed by several factors ultimately deciding the quality of the end product.

Conclusion

From the study conducted it can be concluded that colour and taste are major attributes for standardizing the formulation based on sensory properties. Tea solids to masala mix ratio of 3:2 can be considered acceptable to optimize the premix based on sensory attributes. Result also shows that there is considerable variation between the developed premix and commercial brand samples taken for the study determined by total phenolic content as well as anti-oxidant properties expressed by DPPH. Difference in antioxidant and total phenolic content of all the premixes is due to the difference in grades of tea used for tea solids, type of processing of tea leaves, spices used in the mixture as they also have the antioxidant capacity, and among all the spices used cinnamon has the highest anti-oxidant capacity. Premixes has the significant amount of anti-oxidant capacity based on the data obtained we can also position these premixes as healthy beverages in the market rather than just refreshing beverage.

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