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Identifying Suitable Agronomic Practices and Processing Techniques of *Assamica* Tea to Improve the Quality of Green Tea as a Raw Material for Green Tea Ice Cream

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ABSTRACT

The study was carried out to find the most suitable tea cultivar, shading effect and best tea processing technique for production of green tea powder with low astringency and amount of green tea addition to produce ice cream with best flavor, nutritional and medicinal values. Green tea powder was manufactured using five different cultivars *i.e.* TRI 2023, TRI 2026, TRI 2027, TRI 4042 and TRI 4047 grown under shaded and un-shaded conditions with three processing techniques namely; steaming, pan frying and freeze drying. Green tea ice cream was prepared using green tea powder. Total antioxidant capacity (TAC) of green tea powder and green tea ice cream was determined by ferric reducing antioxidant power (FRAP) assay. Colorimetric methods were used to identify total phenolic content (TPC) (Folin-ciocalteu method) and total flavonoid content (TFC) of green tea powder and green tea ice cream. The best quality green tea in terms of colour and flavour can be produced from tea leaves of TRI 4047 cultivar which was cultivated under shaded conditions with freeze drying processing technique. In terms of colour and flavour, 15 g of green tea added for ice cream mixture (500 mL of milk, 125 mL of whipping cream and 150 g of sugar) is the most suitable amount to add for green tea ice cream preparation. Significantly, higher TAC, TPC and TFC in green tea ice cream when compared to normal ice cream (without green tea powder) suggesting the importance of consuming value added green tea ice cream as a healthy food.

KEYWORDS: Consumer preference, Green tea ice cream, Processing technique, Shade effect, Tea cultivar

INTRODUCTION

Tea (*Camellia sinensis* L.*)* is the most popular and the cheapest natural beverage consumed throughout the world next to water. Sri Lanka is the 4th biggest tea producing country in the world and produced 0.34 million tons in year 2014 with a global share of around 8%. Sri Lankan tea is well-known in the world for its high quality and the taste (Perera, 2014).

Six different types of tea are produced from tea leaves. The main types are black tea, green tea and oolong tea. Green tea is prepared from unfermented leaves, oolong tea is produced by partial fermentation and black tea is produced by fully fermentation of tea leaves.

Nowadays, green tea is a popular beverage. Drinking habit of green tea was originated in China and has become in associated with many cultures in Asia from Japan to the Middle East. Recently, green tea has become more widespread in all over the world due to its health benefits (Benzie *et al.*, 1999). Most of the health benefits are given due to the availability of polyphenols in green tea. Green tea contains the highest concentration of polyphenols compared to other types of tea. An average cup of green tea contains between 50 and 150 mg polyphenols (Nugala *et al.*, 2012). Polyphenol is a powerful antioxidant that can neutralize free radicals of oxygen in the human body. Free radicals in the body make health problems as cancers and heart diseases (Thasleema, 2013).

Polyphenols contained in tea are classified as catechins. Green tea contains alkaloids including caffeine, theobromine and theophylline. Due to these beneficial constituents of green tea, it can be used as an ingredient for making value added tea based products.

Ice cream is one of the most popular foods item among consumers, not only children but also adults prefer to eat ice cream. Ice cream is prepared by churning and chilling a mixture containing milk, glucose based sweeteners and flavoring agents. Ice cream is a good source of energy. By adding green tea powder as a flavoring agent to ice cream, beneficial constituents of green tea may contribute to enhance the nutritional value of ice cream too. Therefore, green tea ice cream may be a good value added product of tea in terms of the flavor, nutritional and medicinal value.

However, in Sri Lanka, almost all the commercially-grown tea cultivars are originated from var. *Assamica.* Therefore, the quality of green tea produced in Sri Lanka is not much good due to possessing of higher content of phenolic compounds. However, the amount of astringent compounds in tea leaves vary with many factors, namely cultivar, climate, soil, degree of maturation of shoot and processing

techniques. The suitable cultivars, shade levels and processing techniques have to be identified to produce green powder with low astringency.

Therefore, this study was carried out to find the most suitable tea cultivar, shading effect and the best tea processing technique for production of green tea powder with low astringency and amount of green tea addition to produce ice cream with best flavor, nutritional and medicinal values.

MATERIALS AND METHODS

Location

The experiment was carried out in the laboratory of Department of Plantation Management and Food Processing laboratory of Department of Food Science and Technology, Wayamba University of Sri Lanka from December 2015 to May in 2016.

Plant Material and Experimental Plots

Fresh tender shoots (Bud+Two leaves) of five different tea cultivars *viz.* TRI 2023, TRI 2026, TRI 2027, TRI 4042 and TRI 4047 which were grown in university tea garden were used to produce green tea. Whole tea field was separated into two plots including five cultivars in each plot. One plot was covered with a sixty per cent (60%) shade net whereas the other plot was maintained without shade as a control plot. All management practices were carried out as the recommendation of the Tea Research Institute of Sri Lanka (TRISL).

Processing of Green Tea Powder

Tea flushes (Bud+Two leaves) from TRI 2023, TRI 2026, TRI 2027, TRI 4042 and TRI 4047 cultivars in both plots (Shaded and Un-shaded) were used to produce green tea powder by using three processing techniques of steaming, pan firing and freeze drying.

Steaming

Harvested leaves were withered for 6 h and steamed for 1 min in a steamer to avoid fermentation and dried using a thermostat oven (Memmet, Germany) at 95 °C for 21 min. Dried leaves were ground into fine powder using a coffee blender.

Pan Firing

Instead of streaming, 6 h withered leaves were pan fried for 2 min in a pan at 60-80 °C to avoid fermentation. Other processing steps were similar to steaming.

Freeze Drying

Harvested flushes were freeze dried for 24 h in a freeze dryer (Martin Christ Alpha 1-2 LD plus, Germany) and processed without 6 h withering process.

Preparation of Green Tea Ice Cream

Green tea ice cream was prepared according to "Matcha green tea ice cream recipe" (Jennifer, 2016). Briefly 500 mL of fresh milk was mixed with 125 mL of whipping cream and mixed well. Sugar (150 g) and three egg yolks were added and stirred well using a beater for 10 min. Ten gram of gelatin was boiled and added to the mixture. Four types of green tea ice cream were prepared by adding different quantities of green tea to the mixture as 5, 10, 15 and 20 g. Ice cream was prepared by adding all ingredients except green tea powder as a control. Ice cream samples were kept in a refrigerator and mixture was beaten four times at 40 min intervals.

Preparation of Green Tea Powder and Green Tea Ice-Cream Extracts for Analysis

Green tea powder (0.1 g) was weighed into a 15 mL centrifuge tubes and added 5 mL of 80% methanol. Samples were homogenized for 1 min and vortexed for 40 seconds. Samples were placed in a water bath at 60 °C for 40 min and vortex procedure was repeated in 10 min intervals. Then samples were centrifuged at 4,000 rpm for 5 min and supernatants were decanted into a 15 mL centrifuge tubes. Extraction procedure was repeated once again and both supernatants were pooled. The extracts were stored at -20 °C until analysis.

Ten gram of ice cream was mixed with 50 mL of absolute ethanol and left to stand at room temperature (approximately 28 °C) for 24 h and then centrifuged at 4,000 rpm for 10 min. The clear supernatant was separated and stored at -20 °C prior to analysis (Limsuwan *et al.*, 1994).

Determination of Phenolic Content

The total phenolic content (TPC) was quantified using a modified Folin-Ciocaltue method (Abeysinghe *et al.*, 2007). Gallic acid was used as the standard solution and TPC in one gram of green tea powder or ice cream was calculated and expressed as milligram of gallic acid equivalent (GAE).

Determination of Flavonoid Content

Total flavonoid content (TFC) was determined by a colorimetric method as described by Liu *et al.*, (2002) with slight modifications. Rutine was used as the standard solution and TFC in one gram of sample was calculated and expressed as mg of rutine equivalent (RE).

Determination of Total Antioxidant Capacity

Total antioxidant capacity (TAC) was determined using ferric reducing antioxidant power (FRAP) assay as described by Benzie and Stain (1996). Trolox was used as the standard solution and TAC in one gram of sample was calculated and expressed as mg of trolox equivalent (TE).

Determination of Chlorophyll Content

Chlorophyll content of tea flushes from each cultivar in non-shaded and shaded plots were determined.

Sensory Evaluation

Tasters for tea tasting were initially identified by conducting a triangular test (Rogers and Brimelow, 2001). Tea tasting was conducted to determine the shading effect through the bitterness of green tea liquor. Two grams of green tea from each cultivar from the shaded and the control was weighed into ten tea cups separately and 100 mL of simmering water (avoided boiling water) was added to each cup, covered and allowed to infuse for 3 min. The infusions were stirred and removed from the tea cups to avoid excessive diffusion of tannins. Then the random numbers were given to each cultivar and 10 tea cups were labeled with random numbers. Untrained panelists (30) were used for testing bitterness of prepared green tea liquor. After serving prepared green tea liquor, consumers were asked to rate the bitterness of the liquor following Likert Scale and then the section of the tea field that results the least bitterness in green tea was identified. The section of the field that results least bitterness in green tea was used for further analysis. Same tea tasting panels were used to identify the best cultivar and processing technique.

Likert Scale had five ratings *viz.* 1- Very low bitter, 2- Low bitter, 3- Moderately bitter, 4- High bitter, 5- Very high bitter.

Consumer preference of green tea ice cream was determined by Hedonic Test (Potter and Hotchkiss, 1996). Colour, taste and texture were used as quality parameters of green tea ice cream and 30 untrained panelists were used for this test. After serving prepared green tea ice cream, consumers were asked to rate the given quality parameters according to following Scale; 1- Like extremely, 2- Like moderately, 3- Like, 4- No comment, 5- Dislike, 6- Dislike moderately, 7- Dislike extremely.

Statistical Analysis

Statistical comparisons of mean values for parametric data was performed by general liner model (GLM) of ANOVA followed by Turkey multiple ranges test using SAS (SAS Institute, 1999) and presented as mean±SD with 95% confident level. Mean comparisons of consumer preferences (non-parametric data) were analyzed with Ordinal Logistic Regression using SPSS (version 16) statistical software.

RESULTS AND DISCUSSION

In the study, attempts were made to investigate the variations of content of the most important secondary metabolites (phenolics and flavonoids) and bioactivity such as total antioxidant capacity of green tea powder manufactured from tender flushes (Bud+Two leaves) of *Camellia sinensis var. Assamica* under shaded and un-shaded conditions. Green tea powder produced under un-shaded condition had significantly ($p<0.05$) higher TAC, TPC and TFC when compared to that produced under shaded condition (Table1).

Table 1. TAC, TPC and TFC of green tea powder manufactured from tea flushes under shaded and un-shaded conditions

Treatment	TAC (mg TE/g DW)	TPC (mg GAE/g DW)	TFC (mg RE/g DW)
Shaded			
TRI 2023	445.7±1.9^{d}	256.4±1.1^{ab}	111.4±5.2^{bc}
TRI 2026	415.6±2.4^{g}	220.1±1.6^{d}	107.1±5.2^{c}
TRI 2027	424.3±1.7^{f}	183.8±1.4^{f}	117.62±4.4^{abc}
TRI 4042	433.7±2.9^{e}	237.8±1.2^{c}	114.8±4.4^{abc}
TRI 4047	382.5±3.8^{h}	161.8±2.3^{g}	94.8±3.3^{d}
Un-shaded			
TRI 2023	500.5±2.3^{a}	261.8±4.7^{a}	122.4±4.4^{ab}
TRI 2026	451.9±0.9^{b}	254.1±1.2^{b}	119.5±4.4^{ab}
TRI 2027	479.5±2.9^{c}	242.4±1.1^{c}	125.2±3.6^{a}
TRI 4042	489.1±2.5^{a}	260.6±1.4^{a}	123.8±2.9^{a}
TRI 4047	423.5±2.2^{f}	206.3±0.6^{e}	110.5±4.4^{bc}

Means in a column followed by the same letters are not significantly different at 0.05 level; TE- Trolox equivalent, GAE- Gallic acid equivalent, RE- Rutine equivalent, DW- Dry weight, TAC- Total antioxidant capacity, TPC- Total phenolic content, TFC- Total flavonoid content

Theanine is one of the major amino acids found in green tea and responsible for the refined flavor and sweetness, and is a major flavor compound in green tea. The conversion of theanine to catechins, is catalyzed by sunlight. Cultivation in the shade can restrict this chemical reaction resulting in leaves rich in theanine and caused for lower production of catechins (Nakamura, 2015). Catechins are a type of polyphenol and are the main astringent compound in green tea and have antioxidant properties. Therefore, lower levels of catechine compounds in shaded green tea may lead to produce lesser TAC, TPC and TFC when compared to un-shaded green tea and ultimately affect for low level of astringent compounds, resulting the less bitterness in sensory evaluation (Table 2).

Table 2. Shading effect on bitterness of green tea powder

Shade condition	Number of observations	Mean value
Shaded	150	2.6±0.8
Un-shaded	150	3.2±0.9

Low mean values in the mean value column refers to low bitterness

Total chlorophyll content in tea leaves of the five selected cultivars under shaded condition was significantly ($p<0.05$) higher than total chlorophyll content in un-shaded tea leaves (Figure 1). Higher amounts of chlorophyll may contribute to maximize photosynthesis reaction under limited light condition and makes tea less astringent and with better color and appearance (Mahanta and Baruah, 2006).

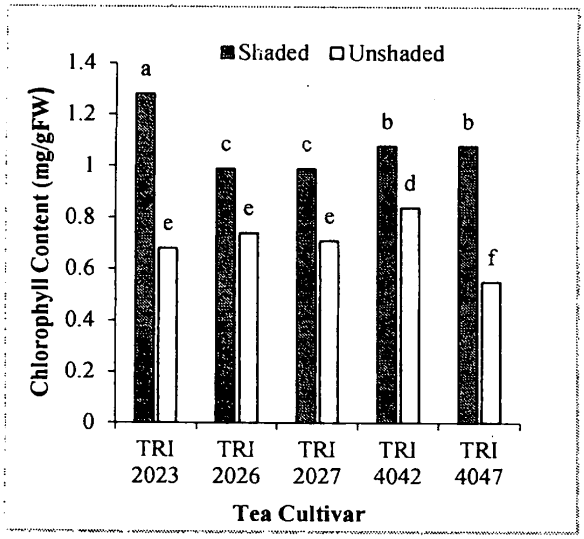

Figure1. Total chlorophyll content of tea leaves under shaded and un-shaded conditions. *Means followed by same letters are not significantly different at $p<0.05$. FW Fresh weight*

Bitterness of green tea extract processed from shaded tea flushes was tested against three processing techniques, *i.e.* steaming, pan firing and freeze drying through consumer preference. Freeze drying technique resulted the least bitterness in green tea compared to steaming and pan firing (Table 3). The least bitterness through freeze drying technique may be due to minimum leaf damage during processing.

Table 3. Identification of the best processing technique based on bitterness

Processing technique	Number of observations	Mean value
Steaming	150	3.0±1.5
Pan firing	150	4.1±0.8
Freeze drying	150	1.9±0.8

Low means in the mean value column refers to low bitterness.

Bitterness of green tea extract which was processed from tea flushes under shaded condition with freeze drying technique was tested against five cultivars *i.e.* TRI 2023, TRI 2026, TRI 2027, TRI 4042 and TRI 4047, through consumer preference. A significant ($p<0.05$) least bitterness of green tea liquor was observed in TRI 4047 cultivar when compared to other four selected cultivars (Table 4). Shaded TRI 4047 has low amount of TAC (423.5 mg TE/g DW), TPC (206.3 mg GAE/ g DW) and TFC (110.5 mg RE/g DW) with compared to other shaded tea cultivars (Table 1). Therefore, lower level of astringent compound may leads to reduce bitterness and enhance the flavor.

Table 4. Identification of the best tea cultivar based on bitterness

Tea cultivar	Number of observations	Mean value
TRI 2023	30	4.6±0.6
TRI 2026	30	3.8±1.1
TRI 2027	30	2.4±0.9
TRI 4042	30	3.0±0.9
TRI 4047	30	1.2±0.1

Low mean values in the mean value column refers to low bitterness

Consumer preference for green tea ice creams which were prepared by varying amounts (5, 10, 15 and 20 g) of powdered green tea (TRI 4047) was tested using a tasting panel. The results revealed that 15 g of green tea powder in ice cream formula gives the best colour, taste and texture with compared to other green tea concentrations (Table 5).

Table 5. Consumer preference for green tea ice cream with varying concentration of green tea

Green tea powder (g)	Mean value for colour	Mean value for taste	Mean value for texture
5	3.23±0.6	3.17±0.2	3.1±0.1
10	2.27±0.8	2.23±0.3	2.37±0.5
15	1.17±0.3	1.23±0.7	1.3±0.2
20	3.27±0.4	4.17±0.5	3.7±0.6

Low mean values in a column refers to higher preference

There were significant ($p<0.05$) variations in TAC, TPC and total TFC in green tea ice cream prepared with varying concentration of green tea powder (Table 6). Green tea ice cream had significantly higher TAC, TPC and TFC when compared to ice cream without green tea powder (control). It was observed that TAC, TPC and TFC values were increasing with increasing concentrations of green tea powder in the ice cream (Table 6).

Table 6. TAC, TPC and TFC of green tea ice cream at different concentration of green tea powder

Green tea powder (g)	TAC (mg TE/100g FW)	TPC (mg GAE/100g FW)	TFC(mg RE/100g FW)
0	6.1±0.1^{e}	2.6±0.2^{e}	0.00±0.0^{e}
5	443.3±13.9^{d}	105.3±12.0^{d}	1.2±0.02^{d}
10	539.2±14.3^{c}	254.3±12.5^{c}	2.3±0.03^{c}
15	757.9±11.0^{b}	324.3±13.8^{b}	3.1±0.02^{b}
20	1018.1±11.2^{a}	447.3±9.0^{a}	3.4±0.02^{a}

Means in a column followed by the same letters are not significant at $p<0.05$. TE- Trolox equivalent, GAE- Gallic acid equivalent, RE- Rutine equivalent, FW- Fresh weight, 0-Control, TAC- Total antioxidant capacity, TPC- Total Phenolic Content, TFC- Total flavonoid content

CONCLUSIONS

This study revealed that the best quality green tea in terms of colour and flavour can be produced from tea leaves of TRI 4047 cultivar which was cultivated under shaded conditions with freeze drying processing technique. In terms of colour and flavour, 15 g of green tea added for ice cream mixture (500 mL of milk, 125 mL of whipping cream and 150 g sugar) is the most suitable amount to add for green tea ice cream preparation. Significantly, higher TAC, TPC and TFC in green tea ice cream when compared to normal ice cream (without green tea powder) suggesting the importance of consuming value added green tea ice cream as a healthy food.

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