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Development of tea concentrate to incorporate in bubble tea using refused black tea and assessment of sensory properties and phytochemical composition

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Abstract
Tea concentrate is a one of major ingredient in trending food product bubble tea, which has a high demand in around the globe, and even in Sri Lankan high end market, yet do not produce its raw ingredients in Sri Lanka. The refused black tea is a waste product made in the black tea production, which has the ability to impart almost same sensory qualities as accepted products, in value addition, but only used in composting and as an animal feed in the industry. Therefore this study was conducted in the objective of developing tea concentrate to be incorporated in bubble tea using the refused black tea. The tea concentrate was therefore made with high grade, low grade and refused black tea, and the sensory qualities were analyzed in order to decide the suitability of using refused black tea over quality tea grades. According to the results refused tea was accepted in regard to sensory qualities and was shown a phytochemical composition of 44.77±0.52 and 246.9 mg/L of tea concentrate respectively for polyphenol content and antioxidant capacity. According to the L*a*b* color values and benchmarking of the final bubble tea product against the commercial product, the refused black tea concentrate was accepted to be used in bubble tea production. The laboratory checked shelf life for the developed tea concentrate was 10 days.

Keywords: refused tea, black tea, bubble tea, tea concentrate

1. Introduction
Bubble tea is a trending food product among the globe, especially in the concentrated populations of Asians, which was considered to originate in the 1980's in Taiwan. This product is originally made with Black tea, Condensed Milk, Honey, and Large Tapioca Pearls, which is served as an instant beverage, by mixing the ingredients upon the order. But yet the product do not have a proper standard method of preparation. Also it is a very expensive product in both local and export market, which demands very high prices due to imported raw materials.

Tea one of the major beverages consumed throughout the world, which is made through the brewing of processed leaves of the evergreen shrub *Camelia sinensis.* This plantation crop is belongs to the botanical family, *Theaceae* which contains about 90 species, wild, and ornamental non-tea species also [1]. With the inherent unique taste, strong reputation, and tradition extending over century for producing the best quality tea, the Ceylon Tea, is popular throughout the world. The Sri Lankan tea industry is accounted for the 15% of total exports, 65% of total Agriculture export, 2% of country's GDP, nearly 2 million direct and indirect employment, 10% of the global market share while being the 4th largest tea producing country and the market leader for orthodox black tea [2].

According to the ISO Standards, the definition of tea is, "Tea derived solely and exclusively, and produced by acceptable processes, notably 'fermentation' and drying, from the leaves, buds and tender stems of varieties of the species *Camellia sinensis* (Linnaeus) O. Kuntze known to be suitable for making tea for consumption as a beverage". Therefore a tea should be in accordance with the above definition and should align to the general and chemical requirements given by the standards to be called as fine "Black tea".

Refused tea is a waste produced during the manufacture of tea. About 4-6% of the total production of tea in Sri Lanka is considered to be refused tea. According to the tea control act No.51 of 1957, refuse tea definition is, "Sweepings, red leafs, fluff, mature stalk, or any other product (not being made tea) obtained in the process of manufacture of tea". The amount of waste tea produced during tea production varies with the manufacturing method, and conditions like, unsuitable leaves by coarse plucking, and over withered, or under withered leaves, etc. And also, Sri Lanka tea factor faced a major issue due to the misusing of Ceylon Tea brand name on illegal trading of refused tea according to the Ministry of Plantation Industries 2015. But yet, there is no proper method is being used in the industry to process waste tea, other than in composting [3], despite the very few researches have done recently. Therefore sustainable value addition of refuse tea other than in non-food industries, is an essential requirement, as even though the refuse tea, does not match with the standards, yet gives very much close brewing properties to low grade teas.

A tea concentrate is a concentrated tea constituent either in powder or liquid form which in the dilution will give a beverage very much closer to the brewed tea. This was mainly produced by concentrating the tea infusion, and in the case of powder followed by freeze drying. In early 1980's a tea concentrate was developed by tea research institute of Sri Lanka with about 4-5% solids. The major drawback in producing tea concentrate which can be incorporated in Ready-To-Drink tea, or any other product is the development of haze and formation of tea cream when the tea get cold. Tea cream is complex substances which are

not sufficiently soluble in cold water, gets precipitated and this leads to discoloration, turbidity, and affect the visual acceptability, flavor, color, as well as the shelf stability [2]. The forming complexes are known to be Caffeine & Tannin complexes [4].

Therefore in the production of tea concentrate, this forming tea cream is separated from the tea before concentrating, by means of chemical adjustments [4], and Centrifugation [5] after brewing at optimal, temperature, time and water to tea leaves ratio.

2. Materials and Methods

The development of the tea concentrate was done with all three high grade, low grade and refused black tea samples according to a self-modified method with the influence of two US patents, after several pre trials.

Initially, 30g of each three tea samples were measured into labeled beakers. Then 300ml of boiling water was added to each sample, where tea leaves: Water ratio was kept at 1:10 [5]. Then the beakers were placed in a water bath and the temperature was maintained at about 800C [5] for 10 – 20 minutes [4]. Then the tea residuals were filtered out and the tea brew was taken and kept aside to cool. The initial, pH, and Brix Values were recorded for each samples. Then in order to accelerate the tea cream formation, minute amount of sodium bicarbonate was added to the tea brew samples, until the pH rises to 5.5. Then 0.1% Sodium carbonate was added as a preservative. Then the samples were kept undisturbed for about 1 hour until the tea cream forms. Than the samples with the cream was centrifuged at 600rpm for 20 minutes, and the clear solution was decanted. The observed tea samples were kept in a shaking water bath for about 3-4 hours, at 800C, until the Brix value rises up to 100. The final samples were again centrifuged at 600rpm, for 10 minutes and hot filled to sterilized glass bottles.

The L*, a*, b* color values for All three tea concentrated tea samples were measured by using Colorimeter (Lovibond® LC100). Color values were taken in 5 replicates.

All three tea concentrates were prepared into Chocolate milk tea, by diluting the concentrate with UHT Chocolate milk at 1:10 ratio. The samples were given to 30 members of a semi-trained sensory evaluation panel of the Department of Food Science and Technology of University of Sri Jayewardenepura. The members asked to evaluate the samples on their aroma, color, taste, flavor and overall acceptability. Panelists were informed that they would be evaluating tea based chocolate milk, and they were presented with three digit code number labels (coded "000"). The order of presentation of the milk tea samples was also random. The panelists were asked to evaluate the samples according to their preference. Samples were evaluated using a 5-point hedonic scale, with 1 for "extremely dislike" and 5 for "extremely like". Water and unsalted crackers were provided to panelists to cleanse their palates between samples. Data acquisition was done using "Minitab 17 Statistical Software". And a benchmarking of the selected product was done against a commercial product using the same panelists and conditions.

For the selected tea concentrate, the total phenolic content of the extracts was measured using ISO 14502-1, Folin-Ciocalteu reagent assay according to the method described by [6] with some modifications.

The total antioxidant capacity was determined using DPPH [1, 1-diphenyl-2-picrylhydrazyl] radical-scavenging activity. For the DPPH assay, the procedure followed the method described by in [7] with some modifications.

Finally a shelf life approximation was done using microbiological stability tests; Total plate count, Yeast and mould count and coliform presence test.

3. Results and Discussion

In the development of this tea concentrate two US patent procedures were used with some modifications. Initially the tea is brewed for all three samples and there the tea leaves to water ratio was taken as 1:10. This was to obtain the maximum amount of tea components to the tea infusion [5, 8].

After tea was brewed with the given conditions the tea was alkaline with sodium bicarbonate (only until pH rises to 5.5). Just after brewing the pH of the high grade, low grade and waste tea was, 4.73, 4.76 and 4.86 respectively. Very minute amount of sodium bicarbonate was added while checking the pH and dissolving thoroughly. Then the samples were kept aside for one hour and then the formed tea cream, causing the turbidity was centrifuged off and the clear decant was taken into beakers. Care is taken to select large beakers, to allow the tea solution to spread in a large area. Initial brix values of the tea samples were, 3.2 for high grade, 3.0 for low grade, and 3.5 for waste tea. Then the samples were kept in a water bath at 80^0C, until the brix values approaches 10. After 4 hours, the final brix values given by the high grade, low grade and waste tea samples are, 10.2, 10 and 9.7 respectively. The low temperature than the boiling temperature was used to retain the volatile tea compounds, and to prevent any burned aromas or flavors being incorporated to the product. If the tea concentrate is to be introduced separately to the industry, then sugar might be added after this concentration step until the brix value increases up to 35-38. But in this study sugar is to be added to the bubble tea as a sugar solution at the point of consumption, adding sugar was not done. After one final centrifuging step to remove any residual particle, if present and then was bottled in sterilized glass bottles while hot. This was done to prevent any microbial contamination. Before store in the refrigerator, the bottled concentrates were kept aside in room temperature to let it cool.

In this study, a higher consideration was given to use refused tea in the development of tea concentrate due to many reasons. Even though refused tea, is a waste product of black tea manufacturing, it contains the mature strokes, leaves parts and dusts of same black tea. This is not suitable to sell as black tea, as it does not obey the terms of the definition. But if processed into tea, as it is hard to recognize by olfactory means many people misuse waste tea in tea production.

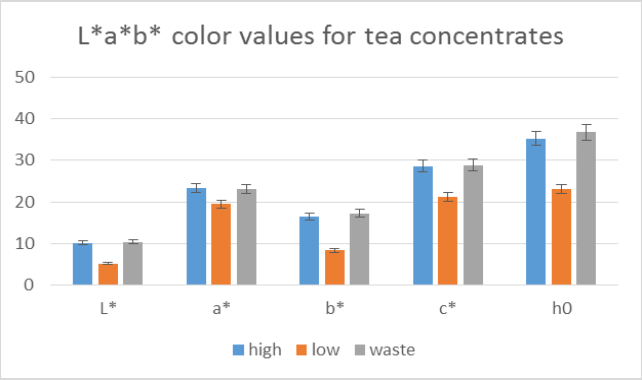
Also refused tea is yet utilized only as animal feed or compost media in the industrial scale. Only a very few researches has being carried out on this phenomena. Therefore using refused tea in a food related industry with some value addition is a much required approach in the current context. If this approach being a success, though refused tea cannot be exported as it is, the value added products may easily capture the export market, without any legal conflict.

Therefore, a sensory evaluation was done to identify whether refused tea concentrate impart any different organoleptic features compared to high grade and low grade teas. Also the colorimeter reading were taken for all three samples to identify any differences, in the appearance context of the teas.

The observed colorimeter readings were as follows.

Table 1: Colorimeter readings of the developed tea concentrates from high grade, low grade and waste tea

	L*	a*	b*	c*	h0
High	10.100±0.123[a]	23.320±0.377[a]	16.480±0.356[a]	28.600±0.464[a]	35.260±0.365[a]
Low	5.160±0.167[b]	19.420±0.832[b]	8.300±0.430[b]	21.120±0.904[b]	23.100±0.283[b]
waste	10.340±0.321[a]	23.080±0.148[a]	17.260±0.270[c]	28.800±o.255[a]	36.740±0.344[c]



* Means that do not share same letter in each column are significantly different

Fig 1: Colorimeter readings of the developed tea concentrates from high grade, low grade and waste tea

This indicates that when consider the L*a*b* color values, waste tea concentrate is much more close to the high grade tea concentrate, but the low grade tea concentrate shows a significantly different value from both waste tea and high grade concentrates in every value.

The sensory analysis was carried out to three chocolate milk tea samples, made with the developed tea concentrates of high grade tea, low grade tea, and refused tea. In that the dilution of the tea concentrate by 10 folds were done by adding UHT chocolate milk. The dilution factor was predetermined by the author, as to the current experience with commercial bubble tea. The results obtained from the sensory evaluation are summarized in the below table.

Table 2: Results of the sensory evaluation of developed tea concentrates

	Aroma	Color	Taste	Flavor	Overall Acceptability
Low Grade	58.5	60	57	58	53.5
High Grade	60	59.5	64	62.5	66
Waste Tea	61.5	60.5	59	59.5	60.5

*For all attributes ($p>0.05$)

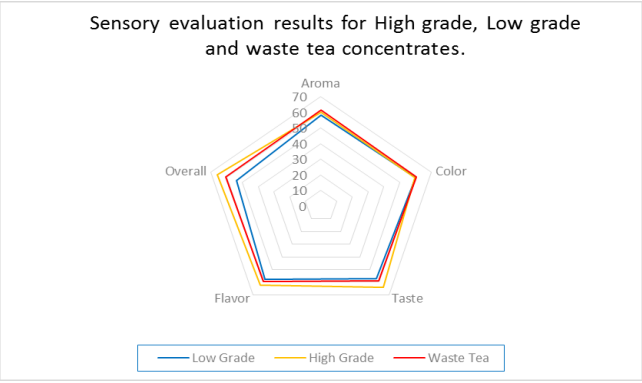


Fig 2: Results of sensory evaluation of developed tea concentrates

As to the results obtain by, it can be clearly seen that there is no significant difference in any attribute for all three tea samples of high grade, low grade and refused tea. Therefore, can come up with the conclusion that when consider the Organoleptic characteristics, refused tea concentrate is compatible with the other accepted tea categories. Also as to the web diagram obtained by the sum of ranks, of the samples, the high grade tea has the highest preference, in all attributes, while the refused tea concentrate has been able to capture the next highest preference, over the low grade tea. But when consider the color attribute, in the sensitivity perception there was almost no difference. Therefore the given difference in colorimeter readings only for b* and h0 can be neglected. The high grade tea shown to have a higher preference in taste and flavor attributes, while the waste tea has dominated the aroma attribute.

Benchmarking can be identified as a process in which our products, or processes are measured against with those who are considered to be the market leaders [9].

In this study, as to the author's experience, and common knowledge, the "Bubble me- Bubble tea" was selected as a competitive and establish bubble tea producer. And a semi trained panel of 30 panelists were appointed to sensory evaluation of the final product, for the benchmarking purpose. Here the panelists were asked to do a preference test on what product they prefer the most. But also they were asked to give a mark to 5 main sensory attributes from a 5 point hedonic scale, which will allow to analyze the sensory attributes separately as well.

The results obtained through the analysis are summarized in the given table.

Table 3: Results of the sensory evaluation of final product benchmarking

	Appearance	Aroma	Texture	Color	Overall Acceptability
Developed Product	50	50	52	46	53
Commercial Product	40	40	38	44	37

* The p values were, Appearance > 0.05, Aroma < 0.05, Texture <0.05, Color > 0.05, Overall acceptability < 0.05

* The p value for the preferred rank < 0.05

As to the p values, the visual sensory attributes; the color and the appearance has shown no significant difference between the two samples. But all other attributes, and the Preferred rank also has shown a significant difference between the two samples, and as to the sum of ranks, the developed product in the research, has gain a much preference over the commercial product.

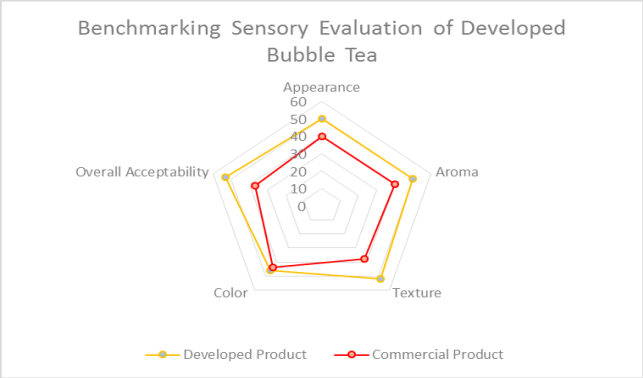


Fig 3: Results of sensory evaluation of final product benchmarking

Even in the attributes, that do not show a significant difference, yet the highest preference was owned by the developed product. This can be clearly visualize through a radar diagram.

The most important and interesting phytochemical in the leaves of *Camelia sinensis* are the polyphenol compounds, as number of biological activities of teas are associated with them [10]. Many of the beneficial health effects of tea are due to these polyphenolic compounds and their antioxidant activity.

But in the case of processing, it is known to reduce the polyphenolic content and the activity too. But also, some responsible compounds are created in the processing steps [11].

Therefore as refused tea concentrate preparation involved many more processing steps, and also it does not have the standard amounts of phytochemicals in the first place, cannot expect a higher value for these polyphenol content and antioxidant activity.

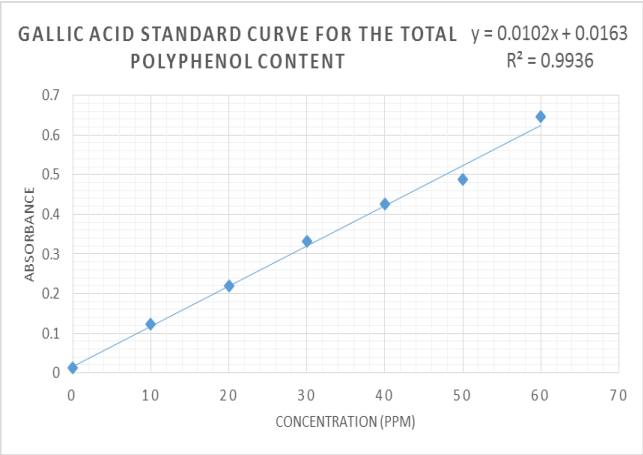


Fig 4: Gallic acid standard curve for total polyphenolic content

As the bubble tea is considered to be a pleasure food, rather than a nutritious food, this is not a limitation for the purpose. But yet the polyphenolic content and the antioxidant activity impart an additional functional property to the tea concentrate. Catechins (catechin, epigallocatechin, epigallocatechin-gallate, epicatechin, and epicatechin gallate), TFs and TRs are the main polyphenols in the tea [10, 12].

Initially, the standard Gallic acid curve was drawn from the obtained data and according to the observations of absorbance and calculations the total polyphenolic content was found.

Table 4: Total polyphenolic content of developed bubble tea beverage

mg/L of Concentrate	mg/L of Beverage	mg/100mL(serving) of Beverage
44.774±0.519X300	1343.23±15.6	134.32±1.56

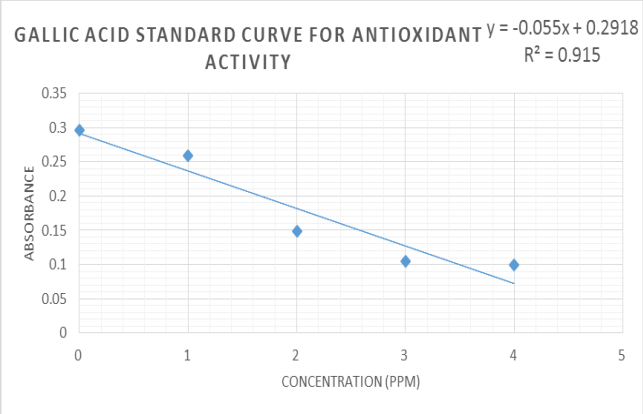


Fig 5: Gallic acid standard curve for antioxidant activity

The antioxidant activity of phenolic compounds is due to their redox properties, allowing them to scavenge reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, nitric oxide, nitrogen dioxide and peroxynitrite, which play important roles in carcinogenesis.

Using the above standard curve gain through the absorbance readings plotted against the concentrations of gallic acid standards. And the inhibition factor was calculated, then the closest inhibition value to 50% was taken, and back calculated to get the Antioxidant activity of the tea beverage.

Table 5: Antioxidant activity of developed bubble tea beverage

Antioxidant concentrate at 47.47% inhibition	Antioxidant concentration of tea concentrate	Antioxidant concentration of beverage	Antioxidant activity per serving
2.469mg/L	2.469X100mg/L	2.469X10mg/L	2.469mg GE per 100mL bubble tea

Finally the product was packaged in sterilized glass bottle, kept in a refrigerator, and microbiological stability was checked for 2 weeks. Any of the tests; Total plate count, yeast and mold count, or coliform test has not shown positive results, within the test period, and therefore the product has a tested shelf life of 10 days in the refrigerated storage conditions.

4. Conclusion

The developed refused black tea concentrate for bubble tea has satisfying sensory qualities with much beneficial economic impact, and considerable shelf life. Therefore can be adopted in the commercial production.

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