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Study on Biochemical Compounds, Antioxidant Activity and Organoleptic Taste of Some Spice Tea

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Abstract

Many kinds of food additives are used in the food industry, among them spices are renowned. Spice tea is good for health but little research has been done about this. So a study was conducted to determine the Biochemical compounds, antioxidant activity and consumer acceptability of spice tea. Three spices (cinnamon, cardamom and ginger) were used to develop individual spice tea. The results showed that protein content of cinnamon, cardamom and ginger tea are 14.41%, 18.59% and 17.5% respectively. Caffeine, tannin, carbohydrate, lipid, moisture and ash content were found higher in different spice tea sample. Antioxidant activity of cinnamon tea, cardamom tea, ginger tea and general black tea were found 94.82%, 90.33%, 86.33% and 87.80% respectively, which are higher than black tea (cntrl). During the tasting of cup quality, comparatively better result was found for cinnamon tea and ginger tea than black tea based on infusion, liquor color, briskness, strength and creaming down.

Keywords: Spice tea, Antioxidant activity, Caffeine, Tea, Biochemical compounds.

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1. Introduction

Tea *(Camellia sinensis (L.) O. Kuntze)* was first used in China as a medicinal drink, and later became a popular beverage. Nowadays, Tea is one of the most popular beverages in the world and it is the most consumed beverage next to water. Tea is an important crop in Bangladesh. The total tea growing areas of Bangladesh is divided into three fairly divergent ecological zones- namely Surma Valley in greater Sylhet, Halda Valley in Chittagong and Karatoa Valley in Panchagarh districts [1].At present, Bangladesh producing 63 million kg of made tea per year and from there 93% of tea estates are situated in the Greater Sylhet zone. Herbal teas are often consumed for their medicinal effects and especially for their sedative, relaxative, and stimulative properties. Herbal teas are mostly popular because of their fragrance, antioxidant properties and therapeutic applications [2]. Spices are mainly used for flavoring and they also have certain medicinal properties. A spice is a dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for flavor, color, or as a preservative that kills harmful bacteria or prevents their growth. In Bangladesh; cinnamon, cardamom and ginger are used as spice and they have many medicinal effects. So, addition of these spices with tea can play an important role in public health and can be used for medicinal purposes.

Cinnamon contains unique healthy and healing property due to the presence of active components.Cardamom tea helps treat indigestion, prevents stomach pain, and relieves flatulence. Drinking a cup of cardamom tea is helpful for women who experience mood swings during their menstrual period [3]. Ginger is an energizer and a stimulator. Drinking ginger tea both stimulates and soothes the digestive system. Arthritic people have found ginger tea helpful since it has anti-inflammatory properties. It is good to fight against colds and flu [4]. Spicing of the tea is one way of value addition but limited research has been done on the biochemical effect, antioxidant activity and organoleptic taste of spice tea for consumer acceptability. For this reason, it is very important to conduct this research and develop a spice tea to meet the following objectives.

- To evaluate the Biochemical compounds (polyphenol, caffeine etc.) of some spice tea
- To determine the antioxidant activity of some spice tea.
- And organoleptic taste of some spice tea.

2. Materials and Methods

This experiment was undertaken at the laboratory of Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology (SUST), Sylhet. Before starting the experiment, samples (cinnamon, cardamom, ginger and black tea) were collected from different places of Sylhet.

Preparation of spice tea: Three types of spice (cinnamon, cardamom and ginger) are added with black tea as powder form and black tea without spice as control sample were used for this study. Collected spices were sorted and heated in an oven at 105 ºC for 60 minutes to remove the moisture. Then an electrical grinder was used to powder form the spices. In each sample, 50g of spice was added to 200g of black tea to make the spice tea [5].

2.1. Estimation of Total Carbohydrate

About 1g of each tea sample was ground well (in mortar and pestle) with 100% ethanol. Two ml of the alcoholic extract was diluted to 10 ml with distilled water and one ml of dilutent taken in a test tube and incubated under ice cold conditions; 4 ml of 0.2% ice cold acidified Anthrone reagent was added to it. Contents were then incubated in a boiling water bath for 8 minutes and cooled down to room temperature under running water. Absorbance of the green color developed was read at 630 nm against the reagent blank in a UV-Visible Spectrophotometer and percent total carbohydrate (as dextrose equivalents) was expressed from standard calibration curve of dextrose [6].

2.2. Determination of Protein Content

Protein was determined using Micro-Kjeldahl. 2gm of sample was weighed and inserted in a 250ml Kjeldahl digestion flask and taking care to see that no portion of the sample clings to the neck of the flask. Then 2gm of digestion mixture and 25 ml of conc. Sulphuric acid was also added to the digestion flask. The flask was kept in digestion chamber at 300°C to digest the sample. Heating is continued for 2hrs until the color of the digest pale blue. The digest was cooled and transferred to a 250 ml volumetric flask. The digestion flask was rinsed 3 times with distilled water and transferred to the volumetric flask. The volume was made 250 ml by adding distilled water.

5ml of the digested sample was pipetted to a distillation flask and 60 ml of 40% NaOH was also added to the flask. Then it was transferred to the distillation chamber. On the other hand, 10ml of 2% boric acid, 10ml of distilled water were pipetted into a conical flask with 4 drops of distillation indicator that was worked as receiver. The conical flask was also set in receiving chamber. After that, the distillation flask was heated at 300°C for 1hr. The color of the solution in receiver turned pink to bluish green. The distillation process was turned off when the volume of receiver was made 60-70 ml.After distillation, Titration was carried out with 0.1 N standardized HCl until the blue color was disappeared. For accuracy to determine the end point, the titration was further continued until a faint pink tinge appeared and subtracted from the burette reading 0.02 ml. Blank was also carried out that contained no sample from digestion to titration. The calculation for protein is given below:

$$\text{Nitrogen\%}=\frac{\{Sample\ titre-Blank\ titre\}\times Normality\ of\ HCl\times 14\times volume\ made\ up\ of\ the\ digest\times 100}{Aliquot\ of\ the\ digest\ taken\times Weight\ of\ the\ sample\ taken\times 1000}$$

Protein % = (Nitrogen % × 6.25)

2.3. Quantification of Lipids

1g of tea sample was taken in a separating funnel and 25 ml of chloroform and methanol mixture (2:1) was added into it. Five ml of 0.9% sodium chloride was added and the mixture was shaken well. The mixture was allowed to stand still for 30 minutes and there was a separation of layers. The chloroform layer was carefully transferred to a pre-weighed china dish. Entire extraction procedure was repeated twice and extractants were pooled together. Pooled chloroform extract was evaporated to dryness on a boiling water bath. China dish with lipids was

dried in an oven at 105 ºC and then weighed. From the difference in weights the percentage of lipids present in the leaf material was calculated gravimetrically and expressed as percentage according to Ravichandran and Parthiban [7].

Lipid (%) = (weight of dried dish – weight of empty dish) × 100

2.4. Moisture Content Determination

The moisture content was measured according to the official method 44-o1 of AACC [8]. The moisture content of the sample is calculated using the following formula.

Dry matter (%) = $\{(Wt.of\ dried\ sample(g)) \times 100\}/Wt.of\ fresh\ sample$(g)

Moisture (%) = 100 – Dry matter.

2.5. Determination of Total Ash

Total ash was estimated by directly incineration of sample taken in a crucible according to AACC [8] method 08-01. Ash was calculated as

$$Ash(\%) = \frac{\text{Wt. of Ash}}{\text{Wt. of Sample}} \times 100$$

2.6. Estimation of Polyphenols

About 1g of each tea sample was ground well (in mortar and pestle) with 100% ethanol and one ml of the alcoholic extract was diluted to 50 ml with distilled water. Then two ml of diluted extract was added with 4 ml of 1:1 Folin-Ciocalteu's reagent and water mixture, and 2 ml of 35% sodium carbonate. The contents were further made up to 10 ml with distilled water and the mixture was shaken thoroughly and allowed to stand still for 30 minutes. Absorbance of the blue color developed was read at 700 nm against the reagent blank using UV-Visible Spectrophotometer**.** Quantum of polyphenols present in tea was computed using the standard calibration curve derived from known concentrations (10 to 50 ppm) of gallic acid and the results were expressed as percent gallic acid equivalents [9].

2.7. Estimation of Theaflavin (TF), Thearubigin (TR), Highly Polymerized Substances (HPS) and Total Liquor Colour (TLC)

Two grams of each tea sample were weighed and transferred in a 250 ml conical flask. 100 ml of boiled water was added to the sample and the contents were infused over the boiling water bath for 10 minutes with intermittent shaking. It was then filtered through cotton wool and the analysis was carried out. Solvent extraction of tea extract was carried out in separating funnels with adequate shaking at every stage. Contents of TF, TR, HPS and TLC were calculated from the absorbance values where,

TF (%) = (4.313 x C x 2 x 100) / (Sample weight x DMC);

TR (%) = (13.643 x (B+D-C) x 2 x 100) / (Sample weight x DMC);

HPS (%) = (13.643 x E x 2 x 100) / (Sample weight x DMC);

TLC (%) = (10 x A x 2 x 100) / (Sample weight x DMC).

Multiplication factors of TF and TR were derived from molar extinction coefficients of pure compounds and dilution factor [10]. In the case of TLC, value 10 is the dilution factor [11].

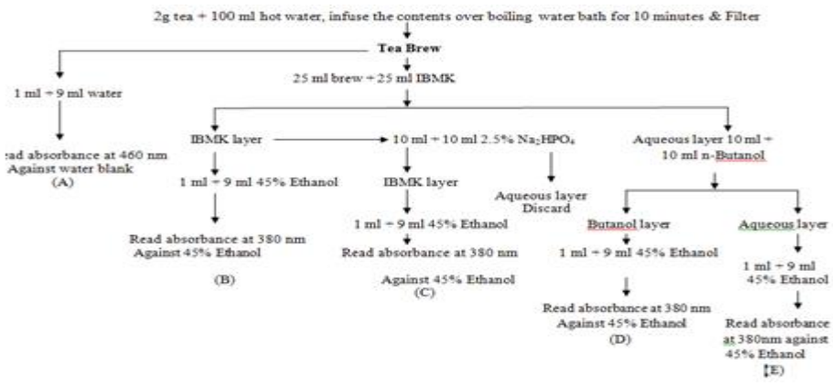

Fig-1. Estimation of TF, TR, HPS, and TLC in black Tea by Spectrophotometer [10, 11]

2.8. Estimation of Caffeine

At first 50 ml distilled water was heated at 40 ºC and then 100mg tea sample was added to the hot water and stirrer for 30 min with magnetic stirrer. Then it was filtered and cooled to room temperature. 50 mi of Chloroform was poured into the infusion and stirrer for 10 min with magnetic stirrer without additional heat. Then the mixture was transferred to a Separating funnel and allows standing still for 30 minute for the separation of caffeine. Organic phase (Chloroform) was separated from the water phase. Organic solution was poured into quartz of UV cell and absorbance was taken in 260nm. Quantum of caffeine present in tea leaves was computed using the standard calibration curve of caffeine and the results were expressed as percent caffeine equivalents [12].

2.9. Determination of Total Tannins Content

Folin-Ciocalteu Phenol reagent was used to determine the total tannins content as reported by Amorim, et al. [13]. At first, 0.2 ml of the sample extract was added with 8.3 ml of distilled water and 0.5 ml of Folin-Ciocalteu Phenol reagent was added and kept at room temperature for 5 minutes. Then 1 ml of 35% sodium carbonate was

added. The mixture was shaken well, kept at room temperature for 20 minutes and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solution of tannic acid was read against a blank. Total tannin content was determined as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve.

2.10. Determination of Antioxidant Activity

The scavenging effects of spice tea samples for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were measured according to the method by Chan, et al. [14]. Briefly, 2.0 ml aliquot of test sample (in methanol) was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark because DPPH is very much light sensitive, and then absorbance was measured at 517 nm in UV Spectrophotometer. The ability to scavenge the DPPH radical was calculated using the following equation:

Scavenging effect (%) = [1 - (A sample – A sample blank)/ A control] × 100

Where, 'A control' is the absorbance of the control (DPPH solution without sample); 'A sample' is the absorbance of the test sample (DPPH solution plus test sample) and 'A sample blank' is the absorbance of the sample only (sample without DPPH solution).

2.11. Organoleptic Taste

The liquor was prepared by pouring boiling water in a mug of a capacity of 142ml (about 0.25 pint) in which 2.5 gm (equal in weight to a 25 paisa coin) was contained. After 5 minutes of brewing the liquor was poured into a bowl and infused leaf was shaken from the mug into the inverted lid, which was placed on top of the mug. Then the dry leaf, the infused leaf and the liquor was evaluated.In order to taste the liquor some of the liquor was taken into the mouth with a sucking noise. The liquor was swilled around the tongue and brought into contact with the plate and gums. In this way the thickness of the liquor was assessed by judging its viscosity, its bitterness by the taste on the back of the tongue, and its astringency and pungency by the sensation apprehended on parts of the cheek and the gums. All these factors together make up the briskness; strength and body of the liquor. Tea aroma and flavor were assessed by drawing liquor to the back of the mouth up to the olfactory nerve in the nose. A mouthful of liquor is thus felt, tasted and smelled and after tasting spit out into a spittoon [15-17].

2.12. Statistical Analysis

The experimental data were statistically analyzed by IBM SPSS Statistics version 20 statistical software. The results are expressed as Mean and data were statistically analyzed by one-way ANOVA, with the level of significance set at $p<0.05$. The mean values adjusted by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Total Carbohydrate, Protein, Lipid, Moisture and Total Ash Content

Tests were conducted to determine these values in different tea sample including a control sample. The results obtained from the tests are given below-

Tea Sample	Carbohydrate %	Protein %	Lipid %	Moisture %	Ash %
Cinnamon	66.25^{d}±0.88	16.41^{a}±1.09	4.80^{c}±0.16	7.45^{a}±0.24	4.71^{a}±0.23
Cardamom	58.85^{a}±0.71	18.59^{b}±1.09	3.89^{a}±0.08	7.51^{a}±0.18	6.38^{c}±0.19
Ginger	62.73^{c}±0.81	17.5^{ab}±1.09	4.28^{b}±0.19	8.38^{c}±0.17	5.69^{b}±0.20
General(Cntrl)	60.75^{b}±0.89	20.78^{c}±1.09	4.41^{b}±0.20	7.92^{b}±0.22	5.75^{b}±0.12

Note: {Values are expressed as Mean±SD of three observations, different letter(s) are significantly different by DMRT (p>0.05)}

From the table it is clear that, the amount of carbohydrate and lipid is higher in Cinnamon tea, protein is higher in general tea. Moisture is higher in Ginger and Ash in Cardamom tea. Graham [18] and Ahmad, et al. [17] found same kind of result in case of black tea [17, 18].

3.2. Estimation of Polyphenol, Caffeine, Total Tannins Content

Polyphenol, Caffeine and Total Tannins value are given below-

Tea Sample	Polyphenol (ppm)	Caffeine (ppm)	Total Tannins (ppm)
Cinnamon	74.62^{c}±1.54	52.72^{c}±1.02	45.00^{c}±1.02
Cardamom	58.98^{a}±1.18	37.72^{a}±0.98	33.87^{a}±1.25
Ginger	68.46^{b}±0.77	43.04^{b}±1.26	32.37^{a}±1.06
General (Cntrl)	77.69^{d}±0.77	38.64^{a}±0.98	37.25^{b}±0.95

Note: {Values are expressed as Mean±SD of three observations, different letter(s) are significantly different by DMRT (p>0.05)}

Analyzing this table we can say that caffeine and tannin are found good in spice teas but in case of polyphenol it was found high in normal black tea.This kind of result also found by Mohammad Shameem, et al. [19] in case of black tea [19].

3.3. Estimation of TF, TR, HPS and TLC

Theaflavin (TF), Thearubigin (TR), Highly polymerized substances (HPS), Total Liquor color (TLC) was analyzed to know the difference between Spice Tea and General Tea.

Tea Sample	TF %	TR %	HPS %	TLC %
Cinnamon	0.5179	5.2777	9.4941	2.6258
Cardamom	0.4878	4.8533	8.9838	2.3679
Ginger	0.5359	5.8675	9.3077	2.4997
General (Cntrl)	0.5621	5.9414	9.7196	2.9095

From the Table, it is clear that, lowest values of TF, TR, HPS and TLC contents are in Cardamom Tea and highest values of TF, TR, HPS and TLC contents are in General Tea (cntrl).

3.4. Determination of Antioxidant Activity

The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of non-radical form DPPH-H by the reaction.

Tea Sample	Scavenging effect (%)		
	500 (mg/ml)	50 (mg/ml)	5 (mg/ml)
Cinnamon	94.82^{d}±0.57	60.81^{c}±0.31	53.74^{c}±0.40
Cardamom	90.33^{c}±0.59	58.16^{b}±0.54	52.95^{bc}±0.28
Ginger	86.33^{a}±0.69	57.79^{b}±0.55	52.43^{b}±0.55
General(Cntrl)	87.80^{b}±0.41	53.42^{a}±0.55	43.54^{a}±0.55

Note: {Values are expressed as Mean±SD of three observations, different letter(s) are significantly different by DMRT (p>0.05)}

From the table, it is clear that the Antioxidant activity of Cinnamon Tea, Cardamom Tea and Ginger Tea is higher than the General Tea. So, Spice Tea shows more scavenging activity than general tea.

3.5. Organoleptic Taste

Different types of Spice Tea were evaluated in regarding to the cup quality of made tea. The Tea was remarked as ‘Excellent’, ‘Above Average’, ‘Average’ and ‘Below Average’ based on the score obtained from organoleptic taste. The Tea obtained above 34 marks was remarked as “Excellent”; above 32 to below 34 was remarked as “Above Average”; above 30 to below 32 was remarked as “Average”; and below 30 was remarked as “Below Average” [15-17].

Cup quality of Cinnamon Tea, Cardamom Tea and Ginger Tea was found as “Average” grade but General Tea was found as “Below Average”. Spice tea was found having comparatively showed the better characteristics. Ginger Tea got the highest total mark. Cinnamon Tea and Ginger Tea was good in liquor color and strength.

Tea Sample	Quality attributes					Total Score 50	Remarks
	Infusion 10	Liquor Color10	Briskness 10	Strength 10	Creaming down10		
Cinnamon Tea	6.19	6.69	6.59	6.48	5.5	31.45	A
Cardamom Tea	6.22	6.13	6.31	6.31	5.81	30.78	A
Ginger Tea	6.69	6.25	6	6.38	6.44	31.76	A
General Tea (Cntrl)	6.28	6.38	5.72	6	5.59	29.97	BA

4. Conclusion

Tea is one of the most popular beverages and plays a vital role as a pharmaceutical agent. There are different brands of black and green tea which are commercially available in the market, having variation in their composition and quality. But addition of Spices with black tea gives a better quality in its composition and also gives a better taste than general black tea. Spice Tea works as herbal and medicinal drinks. From the present study, we find that the anti-oxidant activity of different Spice Tea is higher than General Black Tea. As we know, some spices are used as herbs and works as medicine, so mixing spice with tea gives natural health benefits. From the result of the study show that these health benefits are found increased in tea because of addition of spices with black tea.Spices have a great positive impact at consumer level in different ways and when it added with tea then popularity of spice tea increases. Addition of spices with black tea has a good impact on the panelist rating as shown by the three best rated spice tea than general tea. Ginger spiced tea and cinnamon spiced tea had the highest overall mean liking. Therefore, this study recommends the best spice tea as cinnamon tea and ginger tea since they exhibited high antioxidant activity and the highest overall mean liking. From the obtaining results might be useful to define the formulation of spice tea, enhancing their health effect.

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