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Determination of Inherent Fructose, Glucose and Sucrose Concentrations in Black Tea from Low Country Region in Sri Lanka

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ABSTRACT

Tea is exclusively a natural beverage which is derived from the tender fresh harvestable shoots of the plant *Camellia sinensis* (L.) O. Kuntze; thus, as with all the other chemical constituents, carbohydrates in black tea are also attributed to the components in harvestable shoots. Though numerous studies have been conducted on biochemical constituents in black tea, available information on its sugar components is scarce. Thus, this study attempted to quantitatively determine carbohydrates in the forms of three major soluble sugars, fructose, glucose, and sucrose in black tea using High Performance Liquid Chromatographic (HPLC) technique. This is the first comprehensive study in Sri Lanka, on this regard, and the initial attention was paid to the low-country region, which provides a major contribution to tea production in Sri Lanka. A total number of 737 black tea samples including ten different grades from 77 tea factories was analyzed to represent nearly 21% of the total number of factories in each of the Ratnapura, Galle, Matara, and Kalutara districts. The average levels of fructose in the black tea are 6.77, 7.59, 7.15, and 7.08 mg g^{-1} while the average values of glucose are 6.37, 6.27, 5.86, and 6.96 mg g^{-1} and the average values of sucrose are 5.23, 6.70, 4.91 and 7.01 mg g^{-1} among the selected ten different black tea grades from Ratnapura, Galle, Matara and Kalutara districts, respectively. According to the statistical analysis, for all three sugar components, the concentration values were not significantly different among the selected black tea grades while a significant difference in concentration values for all three sugar components was observed among four different regions in low country elevation.

Keywords: Black tea, carbohydrates, low country, soluble sugars, sugar components

INTRODUCTION

Carbohydrates also called saccharides are the organic compounds with the general formula of $C_m(H_2O)_n$, implicating the hydrates of carbon, consisting of carbon along with Hydrogen and Oxygen, in the atomic ratio of 2:1. This group of organic compounds is further divided into four chemical categories including monosaccharides, disaccharides, oligosaccharides, and polysaccharides, based on the number of saccharides in each category. In general, the monosaccharides and disaccharides, which are smaller and lower in molecular weight, are commonly referred to as sugars. In terms of plant biochemical aspects, carbohydrates are the primary food reserves that play a central role in the metabolism of all green plants. The ultimate source of all carbohydrates in the plant world is "photosynthesis"; the process to harness sunlight, which is carried out exclusively in the green portions of the plant; primarily in the leaves.

Though, photosynthesis is not an exception for the plant *Camellia sinensis (L.)* O. Kuntze, considerable attention has not been paid on carbohydrates in black tea while most other agriculturally important plants have frequently promoted intensive investigations on carbohydrate metabolism and their contents in the ultimate food commodities (Pethiyagoda, 1964). However, Roberts, (1958), states that the dominant role played by the polyphenolic compounds in tea has probably been responsible for this state of diminutive concern on tea carbohydrates. But Sanderson and Perera, (1965) clearly implicated that substances other than polyphenols in tea plants also play an important role in high quality tea production thus emphasizing the significance of investigating carbohydrate compounds in tea. However surprisingly throughout the history of the investigations of biochemical constituents in Ceylon tea, only a few studies were conducted more than five decades back, on carbohydrates in tea, but no studies have been conducted along with the advent of new technologies in quantification. In the study of Sanderson *et al*., (1965) on carbohydrates in tea shoot tip, glucose, fructose, and sucrose have been identified as the soluble carbohydrates using paper chromatography, and the amount of total soluble carbohydrates in shoot tips was quantitatively determined as 1.3-3.1% in dry weight basis. The same study conducted using the clones TRI 740 and TRI 777, on the investigation of the changes of carbohydrates during the manufacture of tea, revealed that soluble carbohydrates are increased during the manufacturing process which may be due to the contribution of insoluble carbohydrates and other cellular constituents. Further, Sanderson and Perera, (1965) explain the changes of reducing and non-reducing sugars in withering and non-withering flush which varied in the range of 1.5 % -2.5% during the period of 18 hours after plucking. Extending the same study, carbohydrates in roots have been studied using the clone TRI 2024, by paper chromatography and the results revealed that fresh root tips contain 0.69% soluble carbohydrates whereas woody roots contain 0.92% including sucrose, glucose, fructose, stachynose, and raffinose. (Sanderson & Perera, 1965). But amazingly throughout the literature, no comprehensive studies were found on the quantitative determination of carbohydrate components in Ceylon tea.

Same as other green plants, tea plant also synthesize glucose, a monosaccharide, as an end product of the photosynthesis process, combining carbon dioxide and water, in chlorophyll in the presence of sunlight (Figure 1). Due to the highly reactive chemical nature of glucose, which may cause some intermediate reactions while transporting, from source to sink, usually shoots to roots, it is then converted to sucrose, a disaccharide comprised of glucose and fructose, within the cytosol of photosynthesizing cells (Figure 2).

So plants transfer glucose and fructose in the form of sucrose, due to high water solubility, energy efficiency, and less reactivity, and it converts back to their monosaccharides to yield energy when it is needed.

$$\text{Carbon dioxide } (CO_2) + \text{Water } (H_2O) \xrightarrow[\text{Sunlight}]{\text{Chlorophyll}} \text{Glucose } (C_6H_{12}O_6) + \text{Oxygen } (O_2)$$

Figure 1. The generalized equation for photosynthesis reaction in plants

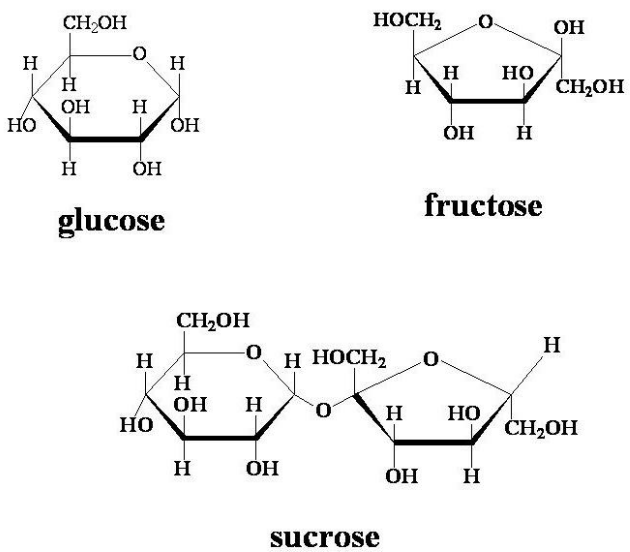

Figure 2. Chemical structures of glucose, fructose and sucrose

According to the ISO 3720:2011, Black tea is defined as "Tea; derived exclusively, by acceptable processes including withering, leaf maceration, aeration, and drying of the tender shoots of varieties of the species *Camellia sinensis* (L.) O. Kuntze". Thus, tea manufacturing is solely a mechanically controlled biological process which, ultimately ends up with a chemical composition derived through the natural transformation of chemical constituents found in fresh leaves. Thus, as to other chemical constituents such as polyphenols, amino acids, catechins, caffeine, etc. it would be beneficial to pay attention towards the carbohydrates, one of the key primary metabolites in the tea plants. According to the research findings of the Tocklai Tea Research Association, free sugars in tea are responsible for the synthesis of catechins in tea shoots; the formation of heterocyclic flavour compounds during the processing of black tea also contributes towards water-soluble solids in tea liquor. Meantime those carbohydrates fuel the enzymatic reactions during the oxidation in tea processing (Keegel., E.L., 1983).

As stated by Nakagawa (1975), the sweetness of green tea comes from sugar whereas the mellowness and sweetness of oolong tea infusion are due to the integrated taste of sugars, non-oxidized catechins, some secondary polyphenolic compounds, caffeine, and free amino acids. (Nonaka et al., 1983; Nagabayashi et al., 1992; Wickremasinghe 1978), states that the combination of carbohydrates with nitrogenous substances in processed tea leads to the augmentation of the natural color of oxidized polyphenolic compounds. According to Liu et al. (1990), different ratios of TFs and TRs of black tea leading to different shades of blackness are produced by the decomposed products of chlorophyll, pectin, sugar, and phenolic compounds. Sharma et al., (2018), states that total soluble carbohydrates which include monosaccharides and disaccharides in tea are one of the important physical and chemical indicators of the quality of tea as they directly affect the taste and quality of tea. It further shows that the presence of slight sweetness in tea may be partly attributed to the presence of sugars.

Accordingly, carbohydrates also play a significant supporting role towards the quality enhancement of black tea. Meantime a comprehensive understanding of the existence of natural sugar components and their levels in tea beverages would provide a positive impact on the tea promotion, as an enhanced tendency for the sugar-free beverages prevails globally. Thus, as the first study in Sri Lanka in this regard, the concentrations of naturally existing soluble sugar components in three hundred and sixty black tea samples were determined during this study, paying the initial attention to the low country elevation in Sri Lanka, which provides the significant contribution towards the Ceylon tea industry.

MATERIALS AND METHODS

Materials and reagents

HPLC instrumentation consisted of Agilent Technologies 1260 HPLC (Agilent Technologies, Germany). Zorbax NH2 column (150 x 4.6 mm (5µm)) were purchased from Agilent Technologies (Germany). Standard D-glucose (Purity - 99.5%), D-fructose (Purity - 99.99%) and D-sucrose (Purity - 99.99%) were purchased from Sigma-Aldrich (Germany). HPLC grade acetonitrile (Purity - 99.99%) was purchased from Sigma-Aldrich (Germany). Ultrapure water was purified using Milli-Q system (Millipore, USA).

Collection of black tea samples

A total number of 77 tea factories (21%) were randomly selected from low country elevation representing Ratnapura, Galle, Matara and Matugama regions. Ten different grades of black tea grades including FBOPF (Flowery Broken Orange Pekoe Fanning's), FBOP (Flowery Broken Orange Pekoe), OPA (Orange Pekoe 'A'), OP (Orange Pekoe), BOPF (Broken Orange Pekoe Fanning's), BOP (Broken Orange Pekoe), BOP1A(Broken Orange Pekoe one 'A'), OP (Orange Pekoe), Pekoe and Dust were collected from each factory. A total number of 737 black tea was analyzed.

Extraction of black tea

Black tea samples were ground through 0.5 mm mesh to homogenize the samples and a weight of 4.00 g of homogenized sample was extracted with 50 mL of distilled water at room temperature. Samples were brewed for 30 mins, in a magnetic shaker and centrifuged. A volume of 1 mL of the extraction was diluted with 4 mL of 100% Acetonitrile. The diluted brew was again filtered through 0.45 µM Nylon filters (Agilent Technologies) prior to the HPLC injection (Jayawardhane et al., 2021). The dry matter content was determined by the loss in mass at 103°C.

Chromatographic Analysis

For chromatographic analysis of sugar components in black tea, AOAC Official Method 977.20, 1977, Separation of Sugars in Honey, Liquid Chromatographic Method was adopted with relevant optimizations and recovery studies relevant to tea matrix (AOAC Official Method 977.20, 1977). HPLC instrumentation consisted of Agilent Technologies 1260 HPLC (Agilent Technologies, Germany). Separation module coupled to Refractive Index Detector (RID) (model G 7162A) system. The system was equipped with a built-in column oven and a vacuum degasser. Zorbax NH2 column (150 x 4.6 mm (5 µm) was used for the analysis. Injection volume and flow rate were 10 µL and 1.5 mL min^{-1} for all the standards and the samples. The method utilized an isocratic system with a mobile phase comprised of 83% Acetonitrile: 17 % water. Column oven temperature is 33 ºC; Detector (RID) Temperature is 33 ºC (Jayawardhane et al., 2021).

Statistical analysis

Analysis of variance (ANOVA) was performed by using SAS version 9.1. Significant differences (P ≤ 0.05) were tested at a 95% confidence level using Duncan's multiple range test as the mean separation technique.

RESULTS AND DISCUSSION

Tea manufacturing is solely a mechanically controlled biological process that initializes with the tender fresh leaves of the plant *Camellia sinensis (L.)* O. Kuntze; thus all the biochemical constituents in black tea are derived from the naturally synthesized biochemically in fresh leaves. This circumstance seems not to be an exception for carbohydrates, thus all three forms of carbohydrates including monosaccharides, disaccharides, and polysaccharides, synthesized in fresh leaves through photosynthesis and other metabolic reactions are found to be in black tea, contributing to different quality attributes. According to Sharma et al., (2018), soluble carbohydrates extracted into tea brew are monosaccharides and disaccharides; and the results of the present study also revealed that the tea brew is comprised of sucrose, a disaccharide along with fructose and glucose; two monosaccharides. This is the first extended study conducted in Sri Lanka with a considerable number of black tea samples, towards a comprehensive understanding of the distribution of inherent sugar components in Ceylon black tea.

Table 1 illustrates the average sugar concentration values in selected ten different grades including FBOPF, FBOP, Pekoe, OPA, OP, Dust, BOPF, BOP, BOP1A, and OP1 of black tea from four different regions of low country elevation in Sri Lanka. Accordingly, average fructose values of 6.77, 7.59, 7.15, and 7.08 g and the average glucose values of 6.37, 6.27, 5.86 and 6.96 while average sucrose values of 5.23, 6.70, 4.91, and 7.01 mg/g among the selected ten different black tea grades from Ratnapura, Galle, Matara and Kalutara regions respectively.

Though few studies have been conducted previously by some colorimetric methods on this regard, they were based on the determination of total soluble sugar contents, but not the contents of individual sugar components. The study conducted by Sanderson et al., (1965) on carbohydrates in tea shoot tips, but not in black tea revealed that glucose and fructose content in shoot tips is 0.3-0.8% whereas the sucrose content is 0.9-2.3% while the total soluble carbohydrate content/sugar was 1.3-3.1% in dry weight basis.

Table 1. The average Fructose, Glucose and Sucrose concentration (mg/g- Dry Weight basis) in ten different black tea grades from Ratnapura (1), Galle (2), Matara (3) and Kalutara (4) regions of low-country elevation in Sri Lanka

		Fructose(F), Glucose (G) and Sucrose (S) concentration (mg/g- Dry Weight basis) in black tea grades from four different regions of low-country elevation									
		FBOPF	FBOP	Pekoe	OPA	OP	Dust	BOPF	BOP	BOP1A	OP1
1	**F**	6.83 ±1.45	6.80±1.60	6.10±1.31	6.66±1.47	6.97±1.43	6.20±0.79	7.04±2.13	7.04±2.37	6.76±1.63	7.07±1.66
	G	6.39±1.88	5.68±2.27	6.05±2.14	6.46±1.68	5.78±1.58	6.47±2.29	6.42±2.39	6.75±2.19	6.77±2.08	5.50±2.13
	S	4.74±3.10	5.24±3.40	5.02±3.06	5.72±2.53	6.27±4.10	5.90±3.22	4.97±2.87	4.67±3.20	5.78±3.90	7.60±4.14
2	**F**	7.86±2.34	7.96±2.57	7.98±3.51	8.08±1.76	8.49±2.34	7.79±1.53	8.16±2.03	8.20±2.99	7.86±2.17	8.28±1.85
	G	6.62±3.1	6.36±2.68	6.40±2.34	7.41±2.52	6.88±2.50	6.22±1.91	7.14±2.43	6.45±2.85	6.73±2.19	5.93±2.04
	S	6.36±3.98	7.29±3.48	4.87±1.83	6.97±3.88	8.02±3.47	7.26±3.49	7.56±4.05	6.91±3.07	9.04±4.26	8.34±3.90
3	**F**	6.75±1.84	6.94±2.03	6.61±2.51	6.88±2.51	6.64±2.44	6.59±1.91	6.39±1.82	7.15±1.61	7.06±2.01	7.06±2.35
	G	5.61±2.25	5.64±2.35	5.93±2.32	6.42±2.08	5.76±2.19	5.92±2.71	5.99±2.72	6.31±2.23	6.44±2.63	5.44±2.54
	S	3.59±2.97	3.19±2.20	2.92±2.73	3.93±2.92	4.02±3.14	3.30±2.00	3.35±1.34	3.33±1.37	4.75±2.30	4.68±3.98
4	**F**	6.83±2.04	6.52±1.39	6.93±1.86	6.82±1.51	6.84±1.77	6.52±1.94	6.63±1.25	6.99±0.80	6.63±1.09	6.88±1.76
	G	5.49±1.60	4.80±1.85	6.07±2.55	5.56±1.69	5.14±1.26	6.01±1.45	5.39±1.33	5.68±1.51	6.04±1.76	4.89±2.60
	S	6.94±4.16	6.78±3.58	5.91±3.69	7.10±3.59	6.83±5.15	8.22±4.82	6.99±3.13	6.37±3.02	8.78±3.02	8.39±5.38

Note: For each of the three sugar components in each black tea grade; the average of 11, 12, 8 and 5 number of factories were taken from Ratnapura (1), Galle (2), Matara (3), and Kalutara (4) regions

Further the study conducted by Ding et al., (2002) using HPLC – Amperometric detector, revealed that Longjing and Bi Luo Chun tea, two different green tea types contain 0.8-0.9 mg/g of Fructose, 1.3-0.9 mg/g of Glucose and 7.8-9.9 mg/g of Sucrose contents whereas the study of Alasalvar *et al.*, (2013), revealed that instant black tea contains 6.92-7.62 g/ 100 g , 3.30-4.14 g/100 g and 0.95-2.48 g/100 g of fructose, glucose and sucrose respectively, which also conform the results of the present study.

"Tea grading" is an important process which involves the separation of tea particles into various shapes and sizes confirming to trade requirements. Though there is no strict definition for each grade due to lack of fundamental standards, it should be emphasized that, in addition to the size and shapes of the particles, the distribution of different portions of the fresh leaves is also represented by different black tea grades. For instance, FBOP is a grade which contains reasonable amount of tip while the particles are longer than average BOP particles. In contrast OP, is slightly longer than FBOP particles, and contains no tip while having very tightly rolled leaf and long tender stalk. Pekoe is the largest of the leafy grades, which derives from well withered leaves (Keegel, 1983). Considering all these facts, ten different black tea grades were chosen from each of the factory for the present study, as low country black tea manufacturing process is comprised with a series of tea grades.

The analytical results of the study implied that within each of the four different regions, all three sugar components varied in a very narrow range among the selected ten different black tea grades. Further the statistical analysis results revealed that the concentrations of all three sugar components are not significantly different among the selected ten different black tea grades (Table 3). Accordingly, to the results, it could be concluded that the shape and the size of the particles and the distribution of different portions such as tip, stalk, etc. of the fresh leaves which represent "different black tea grades" do not have a significant influence on the concentrations of sugar components in black tea. The distribution of Fructose**,** Glucose and Sucrose concentration (mg/g- Dry Weight basis) in black tea from different regions of the low country is illustrated in Table 2. According to the statistical analysis, a significant difference was identified among four different regions, for all three sugar components, Fructose, Glucose, and Sucrose, but for none of those sugar components, a significant difference was not observed among the analyzed black tea grades.

Table 2. The average Fructose**,** Glucose and Sucrose concentration (mg/g- Dry Weight basis) in black tea from low-country elevation

Region	Average concentration (mg/g)		
	Fructose	Glucose	Sucrose
Ratnapura	6.77±1.46	6.37±2.13	5.23±3.08
Galle	7.59±1.86	6.27±1.99	6.70±3.28
Matara	7.15±1.64	5.86±1.96	4.91±2.85
Kalutara	7.08±1.19	6.96±3.07	7.01±3.15

Table 3. One Way ANOVA statistical analysis results to determine the effect of black tea grades on sugar concentration in black tea

Region in the low-country elevation	Significant value obtained for each of the sugar component		
	Fructose	Glucose	Sucrose
Ratnapura	0.9683	0.7194	0.8234
Galle	0.9031	0.5684	0.2865
Matara	0.9648	0.6957	0.7527
Kalutara	0.9954	0.9919	0.4286

Note: In all four regions, for all three sugar components, $P \geq 0.05$ at 95% significant level; thus, in all four regions, either in Fructose, Glucose or Sucrose, there is no significant difference among the selected ten different grades.

This difference may have arisen not because of a single factor but due to the combination of a series of factors that have direct or indirect influences on photosynthetic and other metabolic reactions which consequently determine the soluble sugar compositions in fresh leaves of the plants. Even though all four regions belong to the low-country elevation; the differences in climatic factors in each region, usage of different combinations of clones with the dependency of the bought leaves would be the major factors for this observation. Meantime the existence of a dry spell in the environment at the time of plucking also becomes a causal factor on this regard as in terms of plant physiological responses, plants tend to synthesize more soluble sugar components in leaves to gain an osmotic potential under the dry spell periods. As black tea samples were drawn from the factories in respective regions, the proportions of standard leaves and the degree of existence of mature leaves in different factories would have been varied. This also may have been a causal factor for this observation, as Takayangi *et al.,* (1985) clearly implicates during their study on green tea, that the free sugar content of fresh leaves in the early harvest/ young leaves is higher than that contained in the later harvest/ mature leaves. The results reported in this study are highly consistent with those reported by Sanderson *et al.*, (1965). Thus, to conclude, the combination of a series of factors such as temperature-sunlight-rainfall and other climatic factors, clonal differences, soil fertility, fertilizer use, the degree of plucking standards, etc. All these factors altogether ultimately determine the concentrations of inherent sugar components in black tea.

Table 4. One Way ANOVA statistical analysis followed by general linear model statistical results for the determination of the effect of region on sugar concentration in black tea

	Significant value obtained for each of the sugar component		
	Fructose	Glucose	Sucrose
Black tea from Galle, Matara, Kalutara & Ratnapura regions	0.0001	0.0190	0.0001

Note: In all four regions, for all three sugar components, $P \leq 0.05$ at 95% significant level; thus, for all three sugar components, Fructose, Glucose and Sucrose, there is a significant difference among four different regions.

But the most important point to be emphasized is, though the inherent sugar content in black tea could be varied in a considerable range, due to certain factors, the average total soluble sugar content in black tea does not exceed 3% under any of those possible circumstances. It consequently implies that the tea brew prepared from 2 g of dried fresh leaves, contains less than 0.06 g of total soluble sugars inherently. Thus, due to the presence of considerably very low inherent sugar content in "naturally brewed tea", this would be an ideal beverage option for the consumers, with the increasing tendency for sugar-free beverages globally. Therefore, the results of the present study would necessarily provide a supplementary tool for the promotion of tea drinking.

CONCLUSIONS

As tea is solely a natural beverage derived from the fresh tender leaves of the plant *Camellia sinensis (L.)* O. Kuntze, the existence of sugar components in black tea, derived from the fresh leaves is inevitable. In terms of all three sugar components determined, fructose, glucose, and sucrose; there wasn't a significant difference among the selected black tea grades, but a significant difference was observed among four different regions of production in the low country, Sri Lanka. Though inherent sugar content in black tea could be varied, due to certain factors, such as clonal variations, climatic factors, seasonal variations, plucking standards, etc. The average total soluble sugar content in black tea samples studied did not exceed 3% from the dry weight basis in none of those analyzed samples.

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