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Comparison of Major Components in Black Teas Manufactured from Korea and Foreign Countries

BY

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DEPARTMENT OF HORTICULTURAL SCIENCE
GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

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(Supervised by Professor **Kwan Jeong Song**, PhD)

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture

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CONTENTS

ABSTRACT

The objective of this study was to compare the quality of black teas manufactured from different countries by analyzing key chemical components in black teas. The major components of black teas manufactured from China, India, Japan, Korea, and Sri Lanka were compared using spectrophotometer and high performance liquid chromatography. Total amino acids and caffeine contents were similar among black tea products of selected countries with the range of 2.2% to 4.4% and 2.6% to 5.6%, respectively. Liquor colors were between 2.2% to 3.9% in Chinese, 1.2% to 3.1% in Indian, 2.3% to 3.1% in Japanese and in Sri Lankan, and 1.6% to 3.1% in Korean black teas. Total polyphenols (TPPs) contents ranged from 12.2% to 16.1% in Chinese, 17.7% to 25.2% in Indian, 7.7% to 10.1% in Japanese, 7.0% to 12.6% in Korean, and 14.8% to 20.7% in Sri Lankan tea products. Total catechins ranged from 21 $mg \cdot g^{-1}$ to 45 $mg \cdot g^{-1}$ in Chinese, 134 $mg \cdot g^{-1}$ to 272 $mg \cdot g^{-1}$ in Indian, 28 $mg \cdot g^{-1}$ to 52 $mg \cdot g^{-1}$ in Japanese, 34 $mg \cdot g^{-1}$ to 74 $mg \cdot g^{-1}$ in Korean, and 60 $mg \cdot g^{-1}$ to 144 $mg \cdot g^{-1}$ in Sri Lankan black teas. Total theaflavins (TFs) contents ranged from 2.3 $mg \cdot g^{-1}$ to 2.8 $mg \cdot g^{-1}$ in Chinese, 1.0 $mg \cdot g^{-1}$ to 5.7 $mg \cdot g^{-1}$ in Indian, 2.0 $mg \cdot g^{-1}$ to 6.3 $mg \cdot g^{-1}$ in Japanese, 2.9 $mg \cdot g^{-1}$ to 4.6 $mg \cdot g^{-1}$ in Korean, and 5.0 $mg \cdot g^{-1}$ to 14.7 $mg \cdot g^{-1}$ except one, 3.9 $mg \cdot g^{-1}$ in Sri Lankan. Total thearubigins (TRs) contents ranged from 9.2% to 12.5% in Chinese, 5.8% to 14.9%, 10.4% to 11.0% in Japanese, 9.7% to 13.3% in Korean, and 9.5% to 18.7% in Sri Lankan. Isobutylmethylketone (IBMK)–souluble TRs fractions ranged from 1.2% to 3.1% in Chinese and Korean, 0.8% to 5.2% in Indian, 1.0% to 1.8% in Japanese, and 2.5% to 7.4% in Sri Lankan. IBMK-insoluble TRs fraction ranged from 5.5% to 8.1% in Chinese, 2.7% to 6.6% in Indian, 4.2% to 5.4% in Japanese, 3.0% to 5.9% in Korean, and 4.0% to 7.8% in Sri Lankan. The result in this study shown that Sri Lankan black teas contained the highest level of TFs and TRs. Indian black teas contained the highest content of TPPs and total catechins which are the dominant chemical components, followed by Sri Lankan tea. This indicates that Sri Lankan and Indian black teas are better than Chinese, Japanese, and Korean in terms of quality analyzing of tea components.

List of Tables

List of Figures

Introduction

Tea is the most widely consumed beverage in the world, aside from water (Kuo et al., 2005; Reto M et al., 2007). It has attracted attention for its refreshing, attractive aroma, taste, potential health benefits such as; prevention of cancer, cardiovascular diseases, and low-density lipoprotein oxidation (Horie et al., 1998; Juneja et al., 1999; Khan et al., 2007; Mckay et al., 2002; Nakagawa et al., 1970; 1975; Yang et al., 2002; Zhang et al., 2009). There are six types of tea, namely green tea, white tea, yellow tea, oolong tea, black tea and dark tea, depending on their processing procedure, mainly the degree of fermentation or oxidation (Yao and Chen et, 2012). Green tea is a type of non-fermented or non-oxidation tea while white, yellow, and oolong teas belong to semi-fermented teas. The green tea is consuming throughout the Asia and Middle East. Oolong tea is popular in China and Taiwan (Khan and Mukhtar, 2007; Wu et al., 2002; Yang et al., 2002). Oolong tea has a distinct flower-like aroma due to a special processing method. Black tea belongs to fully fermented tea and dark tea is post-fermented tea (Hara et al., 1995; Yao and Chen et al., 2012). Black tea is the most widely consuming tea type in the world and it accounts for 80%. It is most popular in Europe, North America, and North Africa (Khan and Mukhtar, 2007; Wu et al., 2002; Yang et al., 2002).

Black tea quality depends mainly on the components and the colour of the tea infusion (Liang et al., 2003). There are specific chemical compositions in tea leaves such as amino acids (AAs), caffeine, catechins, and polyphenols (PPs) (Hu et al., 2001; Liang et al., 1996; 2003; 2005; Mamati et al., 2006; Obanda et al., 1997). AAs have characteristic taste notes of sweet umami (Millin et al., 1967) and play an important role as precursors of some volatile flavor compounds in biosynthesis of tea aroma (Sanderson, 1972). Caffeine is regarded as an important constituent of tea and it has bestowing mood and cognitive-enhancing properties (Bokuchava and skobeleva, 1969). The major catechins in tea leaves are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). There are also minor catechins including gallocatechin (GC), gallocatechin gallate (GCG), catechin gallate (CG), and catechin (C) (Yamamoto, et al., 1997). The oxidation of catechins occurs through enzyme catalyzed reactions to form

theaflavins (TFs) and thearubigins (TRs). TFs are bright and orange-red while TRs are more chemically heterogeneous and tend to be brownish-red (Brown et al., 1966; 1969; Deb and Ullah, 1968; Takino et al., 1964). There are four major TFs; simple TF, TF-3-gallate (TF-3-g), TF-3'-gallate (TF-3'-g), and TF-3.3'-digallate (TF-3,3'-dg). TR is a group of compounds formerly recognized as insoluble fractions and of ethyl acetate (Roberts et al., 1958).

Tea has been strategically developed as a major economic crop by the local government in Jeju, Korea since 2005. The cultivation and production of tea accounted for 341ha and 510 tons, respectively, in Jeju for 2010 (Song et al., 2013). Recently, the consumption of black tea by young people has been increased in Korea. Hence, some tea companies and growers in Korea have been trying to produce good black teas. Moreover, the reliable and accurately measurable of chemical parameters that can be used to estimate black tea quality are desirable in trade, research and breeding programmes (Owuor et al., 2006).

Therefore, the study was conducted to compare the black tea quality through analyzing components in black tea products manufactured in different countries.

Materials and Methods

Black tea samples

Twenty nine black tea samples were used for the present study. There were four Chinese tea, six Indian teas, three Japanese teas, nine Korean teas, and seven Sri Lankan teas.

Table 1. Origin of tea samples and manufacturing methods.

Tea sample	Origin		Processing method
	Country	Region	
CN-A to B	China	Wujishan, Fujian	Orthodox
CN-C to D	China	Yiwu, Yunan	Orthodox
ID-A to F	India	Darjeeling	Orthodox
JA-A to C	Japan	Kagoshinma	Orthodox
K-A to H	Korea	Jeju-Do	Orthodox
K-I	Korea	Gurye, Jeollanam-Do	Orthodox
SR-A to C	Sri Lanka	Nuwara Eliya	Orthodox
SR-D	Sri Lanka	Ratnapura	Orthodox
SR-E	Sri Lanka	Badulla	Orthodox
SR-F to G	Sri Lanka	Dimbula	Crush Tear and Curl, CTC

CN= Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

Determination of components

Total AAs (TAAs)

Three grams of tea powder was infused with 450 mL of freshly boiled distilled water in a boiling water bath for 45 minutes with general shaking at every 10 minutes interval. The tea infusion was filtered using double-layer filter papers and the volume was

fixed to 500 mL after fully cooling down as stock solution for further analysis. Each black tea sample was extracted in triplicate and the mean was generated as the result (Chen and Zhou, 2005).

One milliliter of stock tea infusion was taken into a 25 mL volumetric flask. 0.5 mL phosphate buffer (1/15 M Na_2HPO_4, 1/15 M KH_2PO_4, pH 8.0) and 0.5 mL of 2% ninhydrin ($C_9H_4O_3.H_2O$) solution were added to the flask, then it was boiled for 15 minutes. After cooling down, the volume was fixed to 25 mL with distilled water. The absorbance in 570 nm (A_{570}) was measured using a 5 mm color comparison cell with blank reagents as control in a UV-1650PC spectrophotometer. The amino acids content was calculated using the formula (1).

$$\text{TAAs (\%, on a dry weight basis)} = \frac{CL_1 / 1{,}000 L_2}{Mm} \times 100 \quad (1)$$

Where ***C*** volume (amino acids mg/ A_{570} could be had according to the A_{570} from a standard curve made by theanin or glutamic acid as a standard component using the same method as mentioned above. $\boldsymbol{L_1}$ was the total volume of the tea infusion (mL), $\boldsymbol{L_2}$ was the volume of the infusion taken to reaction (mL), ***M*** was the dry weight of tea sample (g), and *m* was the dry ratio of tea sample.

Total PPs (TPPs)

One milliliter of stock tea infusion from TAAs analysis was taken into a 25 mL volumetric flask, 4 mL distilled water and 5 mL reaction solution (0.1% $FeSO_4$ and 0.5% potassium sodium tartrate, $C_{14}H_4O_6KNa.4H_2O$) were added and the volume was fixed to 25 mL by phosphate buffer (1/15M Na_2HPO_4, 1/15M KH_2PO_4, pH 7.5). The A_{540} was measured using a 10 mm color comparison cell with the blank reagents as control in a UV-1650PC spectrophotometer (Shimadzu Corporation, Tokyo, Japan). The tea TPPs content was calculated using the formula (2).

$$\text{TPPs (\%, on the dry weight basis)} = \frac{A_{540} \times 3.914}{1{,}000} \times \frac{L_1 \times 100}{L_2 Mm} \quad (2)$$

Where L_1 was the total volume of the tea infusion (mL), L_2 was the volume of the infusion taken to reaction (mL), M was the dry weight of tea sample (g), m was the dry ratio of tea sample, and 3.914 corresponded that 1 A_{540} using the 10 mm color comparison cell was equal to 3.914 mg TPPs in the tea infusion.

Liquor color (LC)

Nine grams of tea powder was added to 375 mL of boiling water and stirred by a magnetic bar on a heated (~90^{o}C) magnetic stirrer for 10 minutes. After filtration, the tea solution was allowed to cool down to room temperature. Five milliliters of tea infusion was pipette into 45 mL of distilled water in a 100 mL conical flask. It was shaken well to ensure thorough mixing. The absorbance of this solution at 460 nm was read against distilled water as the blank. The LC was calculated as the basis of the dry matter (DM) content of the black tea samples (Hilton, 1973).

$$LC = (A460 \times 10)/ (DM/100) \quad (3)$$

Catechins, caffeine, and TFs

The tea infusion from the stock solution of liquor color analysis was centrifuged at 15,000 rpm and 4ºC for 15 minutes. The liquor was diluted 1:1 ratio with double distilled water prior to use for High Performance Liquid Chromatography (HPLC) analysis. The chromatographic conditions were used as shown below (Liang et al., 2001):

Column	5μm-Diamonsil^{TM} C_{18}, 4.5 mm × 250 mm
Temperature	35^{o}C
Mobile phase	A : Acetonitrile/acetic acid/water [6:1:193 (v/v/v)] B : Acetonitrile/acetic acid/water [60:1:139 (v/v/v)]
Gradient	30% mobile phase A to 100% mobile phase B for over 60 min
Flow rate	1mL.min^{-1}
Detector	Shimadzu SPD ultraviolet detector, 280 nm
Injection volume	10 μL

TRs

TRs and TRs fractions, Isobutylmethyl-ketone (IBMK)-soluble fractions, TRSI and IBMK-insoluble fractions, TRSII, were analyzed using spectrophotometer by following the method described by Robert and Smith (Robert and Smith, 1963). Fifty milliliters of the cool, well-shaken and filtered tea infusion from total LC analysis were mixed with 50 mL of IBMK and gently shaken to avoid formation of an emulsion. The layers were allowed to separate and 4 mL portion of the IBMK layer was taken and made up to 25 mL with methanol in a volumetric flask (Solution A). Two milliliter portions of the aqueous layer were diluted to 10 mL with distilled water and then to 25 mL with methanol (Solution B). Twenty-five milliliters of the remaining initial IBMK layer were taken in a separate flask and mixed with 25 mL of 2.5% aqueous sodium hydrogen carbonate. The mixture was vigorously shaken before the layers were allowed to separate and the aqueous layer discarded. A 4 mL portion of the washed IBMK layer was made to 25 mL with methanol (Solution C). Two milliliters of a saturated oxalic acid aqueous solution and 6 mL of water were added to 2 mL portion of the aqueous layer left from the first extraction with IBMK, and diluted to 25 mL with methanol (Solution D). The absorbance of solution A, B, C and D at 380 nm and 460 nm were obtained using a CE 393 Cecil Digital grating spectrophotometer with distilled water as the blank.

Calculation of the levels of TRs in black tea liquor by following the above procedures for solvent partitioning of black tea liquor components and, based on the fact that mean absorbance of the TR fractions at 380 nm was 0.733 (Roberts and Smith, 1961;1963), the following equation for estimating TTRs was derived: At 380 nm:

$$\%TR\ (Total) = (375 \times 0.02 \times 6.25[2A_D + A_A\text{-}A_C]) / (0.733 \times 9 \times DM/100) \quad (4)$$

The value $A_A - A_C$ represents the absorbance due to TRSI type for which $A^{0.2\%}_{460\ nm}=0.138$. Thus, at 460 nm and following the above solvent partitioning procedures:

$$\%TRSI = (375 \times 0.02 \times 6.25\ [A_A\text{-}A_C])/ (0.138 \times 9 \times DM/100) \quad (5)$$

Similarly, the value A_B represents absorbance of the IBMK-insoluble thearubigins of SII type and after acidification with oxalic acid, this change to A_D. These acidified SII type TRs have $A^{0.2\%}_{460}$ nm of 0.233, and are more deeply colored than the SI type (Roberts and Smith, 1961, 1963). Hence, at 460 nm:

$$\%TRSII = (375 \times 0.02 \times 12.5\ A_D)/\ (0.233 \times 9 \times DM\ /100) \quad (6)$$

Statistical analysis

Statistical Analysis System (SAS program version 9.1) was used to analyze statistical significance.

Results and Discussions

TAAs, TPPs, caffeine, and LC

AAs are increasing the sweetness of tea. AAs content is high in young leaves. The AAs also play an important role in the development of tea aroma during the processing of black tea (Sanderson, 1972). TAAs contents in black tea produces from five countries were similar. It was ranged from 2.2% to 4.4% (Table 2). TAAs content of black teas varied from 0.7% to 2.7% in the wet season and 0.9% to 2.0% in the dry season (Kottawa et al., 2011). In one research found that, there was a progressive decrease in TAAs concentration as the leaf matured, but the tender stems contain high level of TAAs (Wickremasinghe and Perera, 1973).

The average of TPPs contents in each tea products were recorded as 14.1% in Chinese, 20.8% in Indian, 8.9% in Japanese, 9.6% in Korean, and 17.2% in Sri Lankan tea (Table 2). According to the present study, the highest TPPs contents were recorded from the Indian and Sri Lankan black tea products. This result is similar to the result of previous studies: Indian or Sri Lankan tea varieties (*C. sinensis* subsp. *assamica*) have higher polyphenol contents when compare to the Chinese variety (*C. sinensis* subsp. *sinensis*) (Hara et al., 1995; Harbowy et al., 1997). The geographical variation, environmental factors, climatic factors, agronomic practices and tea processing methods are mainly affecting for above difference (Caffin et al., 2004; Stepen-Thanaraj and Ramaswamy, 1981). The growing environment largely influences the phenolic compound distribution in black tea (McDowell et al., 1991). Not at all, the prolong storage of black or green tea products and the over fermentation during black tea processing may be affect on the lower content of phenolic compound in tea (Caffin et al., 2004). The growing environment and/or cultivar might be one of the main factor influent on the TPPs quality content in black teas. Moreover, the concentrations of chemical compounds in the younger leaves are ideal for the production of a good quality of black tea (Roberts, 2008). Typically, Darjeeling tea is manufactured from the tender shoots and two adjacent leaves (Das et al., 2012). This might be the reason of containing high content of TPPs in Indian black teas.

The average of LC in each tea products were 3.3% in Chinese, 1.9% in Indian, 2.6% in Japanese, 2.2% in Korean tea, and 3.0% in Sri Lankan (Table 2). Change of LC of

black tea depends on fermentation temperature and fermentation duration. The fermentation at the 20^{o}C produced lower color level than at 30^{o}C fermentation temperature in the same fermentation period (Obanda et al., 2001; Owuor et al., 2008). The present study, the LC of Indian black teas were lower than the LC of black teas from other countries. This might be resulted from lower fermentation time and temperature.

The caffeine content in black tea samples were between 2.6% to 5.6%. The mean value of caffeine content in Korean tea was a little lower, 3.5%, while the mean value of caffeine of other countries were similar, 4.3%, 4.5%, 4.1%, 4.1%, in Chinese, Indian, Japanese, and Sri Lankan tea products, respectively. Tea has been valued historically for its caffeine content, which is between 2% to 5% depending on the variety. Caffeine levels vary from 5.30% for 1 bud and 1 leaf, to 4.20% for 1 bud and 2 leaves, to 3.80% for 1 bud and 3 leaves and to 3.20% for 1 bud and 4 leaves (Dev Choudhury et al., 1991).

Table 2. TAAs, TPPs, and caffeine concentration (%) and LC in different black tea products.

Tea sample	TAAs	TPPs	Caffeine	LC
CN-A	2.98±0.0 [z]	15.49±0.6	4.84±0.1	3.73±0.0
CN-B	2.65±0.0	12.17±0.4	4.12±0.2	3.91±0.0
CN-C	3.61±0.0	12.85±0.6	4.33±0.6	3.20±0.0
CN-D	2.46±0.0	16.06±0.3	3.88±0.1	2.17±0.0
Mean	**2.93**	**14.14**	**4.29**	**3.25**
ID-A	2.74±0.0	19.31±0.5	4.24±0.2	1.76±0.3
ID-B	2.72±0.0	20.21±1.6	4.22±0.2	2.04±0.1
ID-C	3.46±0.0	25.21±2.6	5.18±0.2	1.15±0.0
ID-D	2.95±0.1	22.00±2.9	4.83±0.2	1.22±0.0
ID-E	2.45±0.1	20.45±2.5	4.10±0.2	2.17±0.0
ID-F	2.39±0.1	17.69±0.6	4.12±0.3	3.08±0.1
Mean	**2.79**	**20.81**	**4.45**	**1.90**
JA –A	2.36±0.0	8.97±0.4	2.92±0.2	3.06±0.0
JA-B	3.22±0.1	10.10±0.2	5.61±0.5	2.59±0.1
JA-C	2.41±0.0	7.67±0.1	3.82±0.5	2.25±0.0
Mean	**2.66**	**8.91**	**4.12**	**2.63**
K-A	2.87±0.1	6.98±0.4	4.38±0.2	1.94±0.0
K-B	2.18±0.0	7.77±0.1	3.23±0.2	2.79±0.0
K-C	2.99±0.0	8.35±0.2	3.19±0.2	1.68±0.0
K-D	2.25±0.0	12.22±0.5	3.34±0.4	1.60±0.0
K-E	2.59±0.1	8.71±0.1	2.63±0.0	2.31±0.0
K-F	2.65±0.0	8.02±0.2	2.70±0.1	2.05±0.0
K-G	4.40±0.0	12.63±0.3	4.34±0.1	1.97±0.1
K-H	2.93±0.1	11.51±0.3	3.63±0.1	3.14±0.0
K-I	4.01±0.1	10.04±0.2	3.94±0.4	2.53±0.0
Mean	**2.99**	**9.58**	**3.49**	**2.22**
SR-A	2.80±0.0	16.45±0.4	5.29±0.8	3.21±0.0
SR-B	2.56±0.0	18.14±1.3	4.59±0.3	2.21±0.0
SR-C	2.65±0.0	20.72±0.8	4.77±0.1	3.89±0.0
SR-D	3.01±0.0	16.59±1.5	3.51±0.1	2.39±0.1
SR-E	2.79±0.0	18.48±0.9	3.72±0.1	2.36±0.1
SR-F	2.66±0.0	15.48±1.2	3.33±0.5	3.42±0.1
SR-G	2.57±0.1	14.80±1.3	3.47±0.1	3.56±0.4
Mean	**2.72**	**17.24**	**4.1**	**3.01**

[z] values were indicated means (n=3) ± S.E.

CN= Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

Catechins

Eight catechins were analyzed using HPLC. The sum of individual catechin contents was calculated for total catechins contents. The highest totals catechins were recorded in Indian tea products with an average 165 $mg \cdot g^{-1}$ following by the Sri Lankan tea with an average of 98$mg \cdot g^{-1}$. The next highest value products were recorded in Sri Lankan tea with an average 98 $mg \cdot g^{-1}$. The average content of total catechins of other black tea products were relatively similar, 54 $mg \cdot g^{-1}$, 57 $mg \cdot g^{-1}$, and 55 $mg \cdot g^{-1}$, in Chinese, Japanese, and Korean, respectively (Table 3 and Fig. 1). According to other research works, the amount of Total catechins in the black teas depend on the origin of samples, the processing methods and plucking season (Zhang Ding et al., 1992). Further, the changes in the qualitative and quantitative composition of tea leaf catechins and catechins gllates may result from the biochemical activities during the growth of tea plant. Moreover, the catechins contents were found to be lower in the mechanically harvested leaves since there are more mature leaves in these samples than in the hand plucked ones (Caffin et al., 2004). At the 20ºC fermentation temperature, considerable quantities of unoxidized ECG and EGCG still remained in black teas after 120 minutes of fermentation. However, increasing the temperature to 30ºC led to more rapid decline in the EGC and EGCG (Obanda et al., 2004). Darjeeling teas were produced from a bud with two leaves and it contains high concentration of catechins (Table 3). In Table 2; the LC in Indian teas are lower than others. So the highest catechins content in the Indian tea product might also be due to plucking young leaves and shorter fermentation time and/ or lower temperature during the fermentation step.

Table 3. Individual catechin and total catechins concentration ($mg \cdot g^{-1}$) in different black tea products.

Tea sample	GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total catechins
CN-A	2.87±0.2 [z]	21.66±1.0	2.27±0.1	2.74±0.1	3.72±0.0	0.96±0.0	10.91±0.4	0.28±0.1	45.41±1.4
CN-B	4.64±1.2	8.38±1.6	0.50±0.0	1.18±0.1	2.88±0.0	0.32±0.1	2.28±0.0	0.29±0.0	20.48±2.8
CN-C	3.58±0.5	11.65±1.6	1.39±0.6	1.57±0.4	1.33±0.0	1.62±0.2	2.16±0.2	0.19±0.0	23.48±3.3
CN-D	4.69±0.4	42.38±4.1	1.66±0.2	8.03±0.2	55.47±1.3	1.48±0.1	14.04±0.3	0.32±0.0	128.06±6.1
Mean	**3.95**	**10.42**	**1.46**	**3.38**	**15.85**	**1.10**	**7.44**	**0.27**	**54.36**
ID-A	3.83±0.3	42.36±2.2	3.30±0.5	7.78±1.7	45.55±1.9	3.61±0.4	26.89±2.8	0.25±0.0	133.55±7.3
ID-B	4.59±0.14	40.43±1.6	4.11±1.2	5.36±0.2	61.79±7.9	2.85±0.6	28.47±0.5	0.23±0.0	143.23±9.0
ID-C	7.70±0.5	73.50±3.6	6.31±0.5	9.69±0.7	129.51±3.3	9.18±1.2	35.88±1.4	0.17±0.0	271.94±7.2
ID-D	7.61±0.1	56.74±1.5	7.56±0.2	15.40±0.4	95.02±5.5	5.44±0.2	36.75±1.3	0.30±0.1	217.22±8.7
ID-E	4.30±0.2	40.41±2.6	4.02±0.3	7.21±0.2	56.22±5.5	4.30±1.0	45.42±7.9	0.32±0.0	157.89±10.9
ID-F	2.14±0.7	21.78±3.2	2.14±0.2	3.32±0.6	17.16±1.9	3.74±0.1	19.12±0.5	0.24±0.0	67.51±4.6
Mean	**5.03**	**45.87**	**4.57**	**5.85**	**67.54**	**4.85**	**32.09**	**0.25**	**165.22**
JA-A	1.70±0.1	16.88±1.4	1.24±0.2	1.93±0.4	3.97±0.2	0.53±0.1	1.97±0.0	0.20±0.0	28.42±2.2
JA-B	3.12±0.6	27.86±5.4	2.23±0.4	4.37±0.3	7.62±0.6	1.11±0.2	4.79±0.4	0.51±0.1	51.61±3.4
JA-C	4.97±1.6	21.33±2.4	0.95±0.1	1.75±0.3	2.08±0.3	0.18±0.0	2.02±0.3	0.15±0.0	33.43±4.3
Mean	**3.26**	**22.02**	**1.47**	**2.68**	**4.56**	**0.61**	**2.93**	**0.29**	**57.95**
K-A	3.06±0.1	26.75±0.8	1.90±0.1	3.03±0.6	4.32±0.8	0.34±0.1	3.68±0.3	0.40±0.1	43.47±2.3
K-B	1.94±0.4	16.79±1.3	1.83±0.6	3.55±0.3	6.43±0.8	0.72±0.0	2.55±0.3	0.30±0.0	34.11±1.5
K-C	2.86±1.2	30.84±1.9	1.76±0.2	5.87±0.3	12.95±0.6	0.79±0.1	4.66±0.2	0.33±0.0	60.06±3.6
K-D	4.82±0.5	58.84±4.3	1.95±0.2	10.38±1.4	35.45±3.6	0.69±0.1	9.22±1.0	0.67±0.1	122.02±10
K-E	2.72±0.3	27.19±2.8	1.71±0.4	3.32±1.1	3.23±0.3	0.88±0.3	2.28±0.2	0.41±0.1	41.75±5.2
K-F	2.30±0.3	29.01±2.1	1.98±0.2	2.94±0.5	3.03±0.3	1.18±0.0	2.72±0.5	0.42±0.1	43.59±2.2
K-G	3.02±0.2	28.90±1.4	1.94±0.2	5.99±0.4	18.22±0.2	1.80±0.3	13.00±0.3	0.75±0.0	73.61±2.4
K-H	2.15±0.1	18.30±1.4	1.48±0.3	3.55±0.7	6.05±0.7	0.75±0.3	4.98±0.3	0.40±0.1	37.65±3.8
K-I	2.72±0.6	15.65±2.5	2.15±0.3	3.99±0.6	5.66±0.7	2.00±0.3	4.86±0.6	0.74±0.1	37.76±1.9
Mean	**2.84**	**28.03**	**1.86**	**4.74**	**10.59**	**1.02**	**5.33**	**0.49**	**54.88**
SR-A	3.54±0.7	40.14±3.9	3.54±0.4	4.60±0.7	4.81±0.1	2.07±0.2	8.01±0.4	0.71±0.0	67.42±4.4
SR-B	5.51±0.3	59.83±3.1	4.64±0.2	10.96±0.6	36.24±2.1	3.54±0.3	22.96±1.1	0.24±0.0	143.92±7.6
SR-C	4.62±0.4	55.02±3.0	5.54±0.5	10.68±0.5	12.61±1.9	4.88±0.7	16.38±1.0	1.12±0.2	110.85±7.7
SR-D	4.12±0.2	37.28±0.3	4.29±0.0	4.42±0.2	23.94±1.7	3.38±0.0	12.71±1.3	0.38±0.2	86.40±3.2
SR-E	5.71±0.1	49.58±0.8	5.66±0.1	11.20±0.5	36.02±1.2	4.22±0.3	19.71±0.5	0.16±0.0	126.55±2.5
SR-F	3.93±0.1	54.41±1.7	8.27±0.3	9.45±1.4	7.02±0.1	3.25±0.1	10.67±0.3	1.33±0.1	94.40±3.8
SR-G	8.08±2.3	38.12±1.8	3.71±0.7	4.62±0.6	3.22±0.2	1.38±0.1	7.25±0.5	1.17±0.2	59.47±3.3
Mean	**5.07**	**47.77**	**5.09**	**7.99**	**17.69**	**3.25**	**13.96**	**0.73**	**98.43**

[z] values were indicated means (n=3) ± S.E.

CN= Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

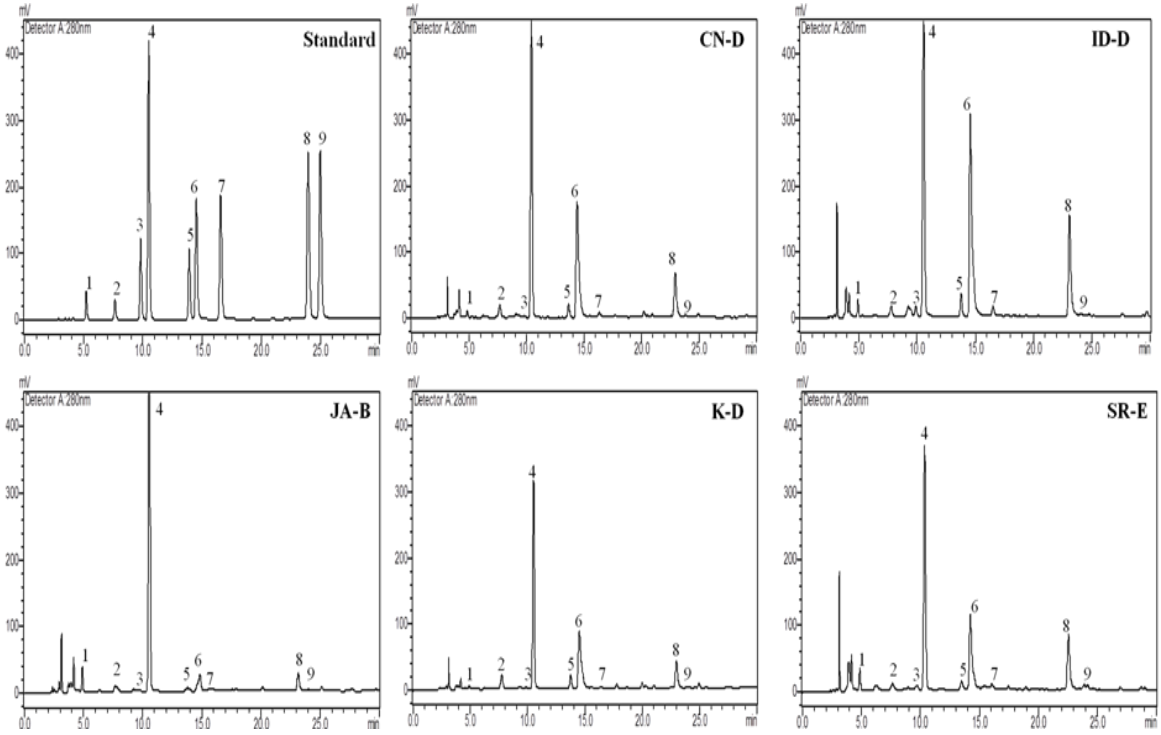

Fig. 1. HPLC chromatograms of individual catechin and caffeine standard and extracts of black tea produced from different countries. 1=GC, 2=EGC, 3= C, 4=Caffeine, 5=EC, 6=EGCG, 7=GCG, 8=ECG, and 9= CG. **CN=** Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

TFs and TRs

Total TFs are known as fermentation products and provide a bright, yellowish appearance to the beverage and have long been positively correlated with the quality and market value of black tea (Roberts, 1958). Four individual TF was analyzed using HPLC. The sum of individual TF was calculated as total theaflavins (TFs). The average of TFs content in different country products were 2.6 $mg \cdot g^{-1}$ in Chinese, 3.5 $mg \cdot g^{-1}$ in Indian, 3.5 $mg \cdot g^{-1}$ in Japanese, 3.8 $mg \cdot g^{-1}$ in Korean, and 8.1 $mg \cdot g^{-1}$ in Sri Lankan tea (Table 4 and Fig. 2). It is shown that Sri Lanka black tea contain high level of TFs. It is believed that, during the formation process, TFs are continuously being formed and/or degraded (Robertson, 1983). The change in TFs level is depending on both temperature and time of black tea processing (Obanda et al., 2001). The over-fermented and/or pro-long storage may result in lower values of the TFs (Harbowy et al., 1997). In this experiment, almost all samples were manufactured by orthodox method except two samples (SR-F and SR-G) were produced by CTC method. Even though the same manufactured methods were used, the TFs contents still varied among different countries as well as among the region in the same country (Table 4). The concentrations of chemical compounds in the younger leaves are ideal for the production of a good quality black tea (Roberts et al., 2008). In addition, previous researchers have found that TFs formation is highest in tender stem and lowest in the fifth leaf since the coarser leaves and stems have a low concentration of polyphenol oxidase, a most important enzyme in black tea processing and as well as other compounds compared to the tender leaves (Takeo and Baker, 1973; Wickremasinghe and Perera). The optimum temperature range for reaction of polyhenol oxidase is 25-30ºC (Robert, 2008). The TFs level in Indian black teas were low (Table 4), although the Indian tea leaves were plucked from a bud and two leaves. Thus, there might be some differences of the controlling time and temperature during fermentation step among those black tea products.

The average of total TRs contents were between 11.0% in Chinese, 9.4% in Indian, 10.7% in Japanese, 11.2% in Korean, and 13.8% in Sri Lankan tea (Table 5). The average of TRSI contents were 2.5% in Chinese, 2.9% in Indian, 1.5% in Japanese, 1.9% in Korean, and 4.6% in Sri Lankan (Table 5). Also, the average of TRSII contents were 7.0% in Chinese, 4.4% in Indian, 4.8% in Japanese, 4.2% in Korean, and 5.5% in Sri Lankan tea (Table 5). The quantity of TFs and TRs will be related to the period of fermentation as well as to the temperature of the fermenting leaf. An optimum condition for maximizing

the formation of TFs and TRs in fermentation time is ranged from 90 to 120 minutes (Robert, 2008). For black tea produced in Kenya, although the contents of TFs and TRs varied with localities and the patterns of variation change from clone to clone, there was no significant effect of location or altitude on the contents of theaflavins and thearubigins (Owuor et al., 1987). Hence, highest content of TFs and TRs in Sri Lankan black teas might be due to the appropriate plucking leaves and the good control of fermentation time and temperature.

Table 4. Individual TF and TFs concentration ($mg \cdot g^{-1}$) in different black tea products.

Tea sample	Simple TF	TF-3-g	TF-3'-g	TF-3,3'-g	TFs
CN-A	0.32±0.0 [z]	0.62±0.0	0.98±0.1	0.69±0.1	2.62±0.2
CN-B	0.22±0.0	0.54±0.1	0.86±0.1	0.64±0.1	2.27±0.2
CN-C	0.53±0.3	0.62±0.0	0.57±0.0	1.05±0.1	2.77±0.4
CN-D	1.17±0.4	0.74±0.3	0.70±0.1	0.18±0.0	2.80±0.8
Mean	**0.56**	**0.63**	**0.78**	**0.64**	**2.62**
ID-A	1.30±0.0	0.98±0.0	0.89±0.0	0.69±0.0	3.86±0.0
ID-B	1.20±0.0	2.03±0.7	1.71±0.6	0.79±0.1	5.72±1.3
ID-C	0.55±0.0	0.21±0.0	0.17±0.0	0.04±0.0	0.97±0.0
ID-D	0.65±0.0	0.25±0.1	0.32±0.1	0.02±0.1	1.25±0.0
ID-E	1.21±0.1	0.98±0.1	1.00±0.1	0.64±0.2	3.82±0.4
ID-F	0.99±0.2	0.87±0.0	1.65±0.6	1.56±0.4	5.07±1.2
Mean	**0.98**	**0.89**	**0.96**	**0.62**	**3.45**
JA-A	0.43±0.0	0.89±0.1	0.56±0.2	0.43±0.2	2.31±0.5
JA-B	1.19±0.1	1.96±0.1	1.48±0.1	1.69±0.2	6.32±0.5
JA-C	0.50±0.1	0.85±0.1	0.49±0.1	0.15±0.0	2.00±0.2
Mean	**0.71**	**1.23**	**0.84**	**0.76**	**3.54**
K-A	1.03±0.1	1.69±0.1	1.02±0.1	0.86±0.1	4.60±0.4
K-B	0.65±0.1	0.99±0.1	0.82±0.1	0.80±0.1	3.26±0.2
K-C	1.07±0.1	0.93±0.0	0.86±0.0	0.50±0.1	3.37±0.1
K-D	1.53±0.4	1.07±0.3	0.81±0.2	0.19±0.1	3.59±1.0
K-E	1.46±02	1.47±0.2	0.71±0.1	0.20±0.0	3.83±0.4
K-F	1.54±0.0	1.57±0.1	0.91±0.1	0.55±0.0	4.57±0.1
K-G	0.77±0.0	1.02±0.0	0.67±0.1	0.46±0.1	2.92±0.4
K-H	0.63±0.0	1.39±0.0	1.25±0.1	1.33±0.2	4.61±0.4
K-I	0.55±0.1	1.00±0.1	0.81±0.0	0.97±0.1	3.34±0.4
Mean	**1.03**	**1.24**	**0.87**	**0.65**	**3.79**
SR-A	1.57±0.0	2.30±0.1	1.98±0.2	3.05±0.9	8.91±0.8
SR-B	2.37±0.2	1.69±0.2	1.81±0.2	1.25±0.2	7.12±0.6
SR-C	1.57±0.2	2.33±0.2	2.00±0.2	2.48±0.3	8.39±1.0
SR-D	1.04±0.1	1.07±0.0	0.99±0.1	0.84±0.2	3.94±0.2
SR-E	1.40±0.2	1.22±0.1	1.43±0.1	0.99±0.2	5.04±0.6
SR-F	3.65±0.2	4.95±0.4	3.72±0.2	2.38±0.3	14.70±0.9
SR-G	1.43±0.1	2.38±0.1	1.99±0.1	1.79±0.3	7.60±0.2
Mean	**1.86**	**2.28**	**1.99**	**1.82**	**8.1**

[z] values were indicated means (n=3) ± S.E.

CN= Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

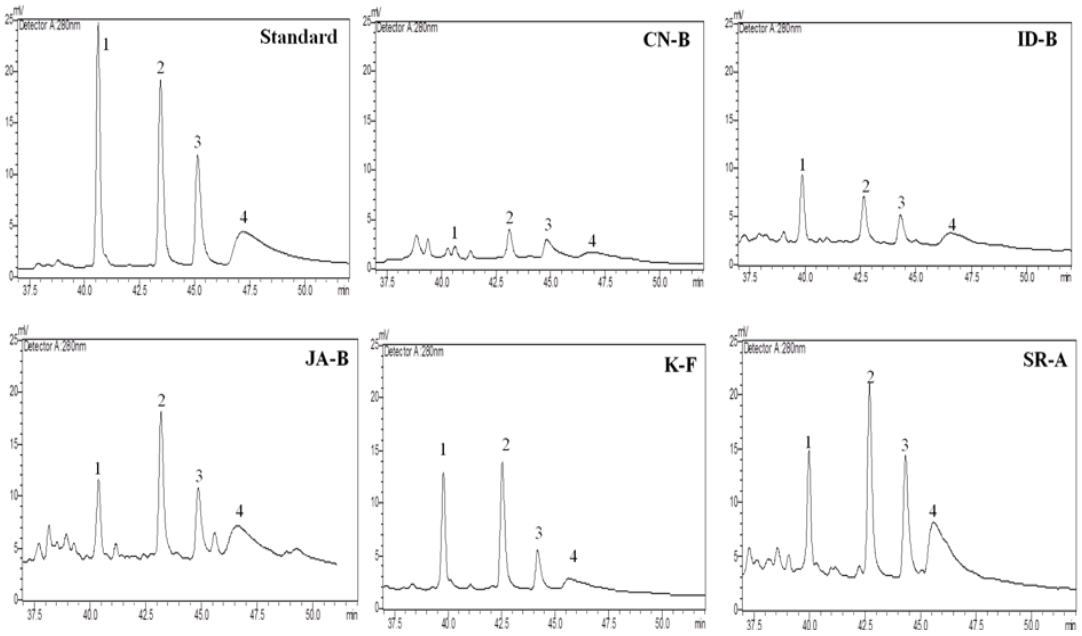

Fig. 2. HPLC chromatograms of individual TF standard and extracts of black tea produced from different countries. 1=TF, 2=TF-3-g, 3= TF-3'-g, and 4= TF-3,3'-g. **CN=** Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

Table 5. Total TRs, TRSI, and TRSII concentration (%) in different black tea products.

Tea sample	Total TRs	TRSI	TRSII
CN-A	12.50±0.3 [z]	3.08±0.3	8.00±0.3
CN-B	11.60±0.1	2.50±0.2	8.08±0.1
CN-C	10.60±0.3	3.11±0.2	6.39±0.3
CN-D	9.20±0.5	1.17±0.2	5.50±0.2
Mean	**10.98**	**2.47**	**6.99**
ID-A	14.30±0.9	5.24±0.1	6.28±0.4
ID-B	9.10±0.2	2.91±0.0	4.28±0.1
ID-C	4.30±0.1	0.18±0.0	2.66±0.1
ID-D	5.80±0.2	0.80±0.1	2.75±0.2
ID-E	14.90±0.3	3.41±0.1	4.01±0.1
ID-F	8.10±0.2	4.60±0.2	6.64±0.0
Mean	**9.42**	**2.86**	**4.44**
JA-A	11.00±0.5	1.79±0.2	4.70±0.3
JA-B	10.70±0.2	0.97±0.2	5.38±0.1
JA-C	10.40±0.7	1.74±0.4	4.16±0.3
Mean	**10.70**	**1.50**	**4.75**
K-A	10.60±0.2	2.43±0.1	3.68±0.1
K-B	10.00±0.3	1.57±0.1	4.75±0.2
K-C	9.70±0.5	1.15±0.1	3.01±0.2
K-D	10.40±0.3	1.17±0.1	3.55±0.1
K-E	11.80±0.4	1.65±0.1	3.48±0.6
K-F	11.80±0.4	2.29±0.3	4.58±0.1
K-G	11.80±1.5	2.45±0.7	4.21±0.2
K-H	13.30±0.5	3.05±0.4	5.88±0.2
K-I	11.40±0.8	1.53±0.4	5.01±0.5
Mean	**11.2**	**1.92**	**4.24**
SR-A	13.00±0.3	5.58±0.5	7.75±0.6
SR-B	12.50±0.5	2.78±0.4	4.64±0.2
SR-C	10.20±0.3	2.45±0.5	3.97±0.0
SR-D	15.70±0.6	3.96±0.1	4.93±0.4
SR-E	9.50±1.1	4.03±0.3	4.00±0.6
SR-F	18.70±0.1	7.41±0.5	5.52±0.3
SR-G	12.00±0.4	5.72±0.5	7.51±0.4
Mean	**13.08**	**4.56**	**5.47**

[z] values were indicated means (n=3) ± S.E.

CN= Chinese tea product, **ID=** Indian tea product, **JA=** Japanese tea product, **K=** Korean tea product, and **SR=** Sri Lankan tea product.

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초록

홍차의 주요 화학 성분을 분석함으로써 서로 다른 국가에서 제조된 홍차의 품질을 비교하고자 본 실험을 수행하였다. 중국, 인도, 일본, 한국 및 스리랑카에서 제조된 홍차의 주요 성분들은 분광광도계 및 HPLC 를 사용하여 분석하였다. 총 아미노산과 카페인 함량은 각각 2.2~4.4%와 2.6~5.6% 내외로 유사하였다. 우려낸 찻물의 색은 중국산 2.2~3.9%, 인도산 1.2~3.1%, 일본산과 스리랑카산 2.3~3.1% 그리고 한국산에서 1.6~3.1% 내외로 나타났다. 총 폴리페놀 (TPPs) 함유량은 중국산 12.2~16.1%, 인도산 17.7~25.2%, 일본산 7.7~10.1%, 한국산 7.0~12.6% 그리고 스리랑카산에서 14.8~20.7% 내외로 조사되었다. 총 카테킨 함량은 중국산 21~45 $mg \cdot g^{-1}$, 인도산 134~272 $mg \cdot g^{-1}$, 일본산 28~52 $mg \cdot g^{-1}$, 한국산 34~74 $mg \cdot g^{-1}$, 그리고 스리랑카산에서 60~144 $mg \cdot g^{-1}$ 내외였다. 총 데아플라빈 (TFs) 함량은 중국산 2.3~2.8 $mg \cdot g^{-1}$, 인도산 1.0~5.7 $mg \cdot g^{-1}$, 일본산 2.0~6.3 $mg \cdot g^{-1}$, 한국산 2.9~4.6 $mg \cdot g^{-1}$, 그리고 스리랑카산에서 5.0~24.7 $mg \cdot g^{-1}$ 내외로 나타났다. 총 데아루비긴 (TRs) 함량은 중국산 9.2~12.5%, 인도산 5.8~14.9%, 일본산 10.4~11.0%, 한국산 9.7~13.3% 및 스리랑카산 9.5~18.7% 내외로 조사되었다. Isobutylmethylketone (IBMK)-soluble TRs fraction 은 중국산과 한국산에서 1.2~3.1%, 인도산 0.8~5.2%, 일본산 1.0~1.8%, 그리고 스리랑카산에서 2.5~7.4% 내외로 나타났다. IBMK-insoluble TRs fraction 은 중국산 5.5~8.1%, 인도산 2.7~6.6%, 일본산 4.2~5.4%, 한국산 3.0~5.9%, 그리고 스리랑카산에서 4.0~7.8% 로 조사되었다. 본 연구결과, 스리랑카산 홍차에서 가장 높은 TFs 와 TRs 함량을 보였으며, TPPs 와 총 카테킨 함량은 인도산에 가장 많이 함유되어 있었으며 그 다음이 스리랑카산으로 조사되었다. 그러므로 스리랑카산과 인도산 홍차가 성분면에서 중국산, 일본산 및 한국산 홍차보다 더 좋은 품질을 나타내었다.

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