VOL. 9, NO. 9, SEPTEMBER 2014 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences


www.arpnjournals.com

EXTRACTION OF PHENOLIC COMPOUNDS FROM GREEN TEA USING ETHANOL

Puguh Setyopratomo
Chemical Engineering Department, University of Surabaya Jl. Raya Kalirungkut, Surabaya, Indonesia
E-Mail: puguh_sptm@yahoo.com

ABSTRACT

Ethanol was used as a solvent to extract phenolic compounds from dried fresh tea leaves (Camellia sinensis L. Kuntze). The extraction was performed at temperature of 40, 50, and 60 ^{o}C which was maintained using a water bath. Folin-Ciocalteu's reagent was used to determine the total phenolic content spectrophotometrically and gallic acid was used as the calibrant. The highest yield, which was 0.3347 g extract/g dry tea leaves, was obtained at extraction temperature 60 °C and extraction time 240 minutes. While the lowest yield, which was 0.2807 g extract/g dry tea, leaves, was obtained at temperature 40 ^{o}C and 15 minutes extraction time. The value of total phenolic content obtained in this work is between 0.21 - 0.25 mg GAE/mg extract. The study also demonstrated that the extraction of tea leaves with ethanol at relatively low temperature exhibit considerable efficient method to obtain extract with relatively high total phenolic content.

Keywords: ethanol, extraction, green tea, phenolic.

INTRODUCTION

Tea as an important natural source of phenolics

The Camellia sinensis (L.) Kuntze (family Theaceae) is tea plant which can grow in many countries worldwide. It will grow best in areas with considerable rainfall, good trenches, and soil that is slightly acidic, such as in tropical and subtropical area. It found two varieties of tea, i.e. C. sinensis var. sinensis (China tea) is widely planted in China, Japan, and Taiwan, while C. sinensis var. assamica (Assam tea) grows rapidly in south and Southeast Asia, and more recently, Australia [1].

The major component (cover 90% dry weight of phenolic) of total phenolic compounds in tea leaves is composed of flavonoids, in which flavan-3-ols (catechins) become the major constituent, which cover up to 30% of their dry weight. On the basis of the stereochemical configuration of the 3', 4'-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C-ring, catechins can be categorized to two isomers: trans-catechins and cis-epicatechins. Both of them exists as two optical isomers: (+)-catechin and (-)-catechin and (-)-epicatechin and (+)-epicatechin, respectively. While, (-)-catechin can be converted by esterification with gallic acid to produce the esterified or galloyl catechins: (-)-catechin-3-gallate, (-)-epicatechin-3-gallate, (-)-epigallocatechin-3-gallate and (-) - gallocatechin-3-gallate [2], [3].

Tea classification

Besides coffee and cocoa, tea also has been known as a popular beverage since a long time ago. Tea is consumed for daily drinking in about 160 [4]. Base on the processing method where degrees of fermentation become one of the most importance parameter, mostly teas are classified into three main groups, i.e.: green teas (unfermented tea), semi-fermented teas (Oolong teas), and completly-fermented tea (black teas), which represent for 24%, 1%, and 75% of the total tea production in the world, respectively [5], [6], [7]. Meanwhile there is another grouping which classified tea into six groups including black tea, green tea, Oolong tea, yellow tea, white tea, and dark compressed tea [8].

To avoid oxidation, green tea is treated via steaming, roasting, or drying of the tea leaves. Because of its high catechin content which has a very good health impact, green tea is well known as the most popular tea worldwide among those other types of teas. Some of these favourable health effects are its role as antioxidant, anticarcinogenic, antimutagenic, anti-inflammatory and antimicrobial. Therefore, on the nutraceutical and health market, various forms of end products of green tea drinks are become increasingly well-known and popular everywhere [8].

Tea phenolic compounds, which was also known as tea polyphenols (TPs) have been adopted as the main quality parameters or indicators of tea products. Theaflavins are the first stable component produced by oxidation during fermentation. It's produced during the oxidation of cathecin and catechin gallates. During fermentation, it experience further oxidation to form more polymerized thearubigins. Theaflavins cause the brisk and astringent taste and bright golden colour to black tea quality, while thearubigins cause the reddish colour and richness in taste. On the other hand, theabrownins endows tea liquor and leaf with a dark brown colour, which has a negative effect on tea quality [2].

Green tea contains more content of tea polyphenols than oolong and black tea, mainly flavanols. To promote consumption, green tea extracts have been manufactured in various types of cans or bottles. However, in the process of beverage production, production of green tea was in fact more complicated than that of oolong or black tea [9]. The decrease in sensory qualities, including color, flavor and taste, during heat processing has limited the development of green tea beverage products [10]. Moreover, it has been reported that tea polyphenols are sensitive to heat as they are vulnerable to decomposition and isomerization during heat processing, especially for

epigallocatechin and epigallocatechin gallate [11], [12], [13]. If heat is introduced, epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC) and epicatechin gallate (ECG) partially epimerized, then consequently concentration of total catechins decreased [14].

The potential health effect of tea polyphenols

Normal consumption of tea involves brewing the leaves and then consuming the liquor hot or cold. Furthermore, in order to promote the health benefits of tea consumption, besides become popular beverages, tea are also developed in regard with their pharmaceutical and industrial applications [15]. Tea is also often found to be used as an ingredient in various food recipes [16].

It has been known that the health impact associated with tea consumption is in part to the antioxidant and free radical-scavenging activity of the most abundant tea flavonols [17]. Considering the health-benefit effects of the green tea extract, including antioxidative [18], [19], anticancer [20], antimicrobial [21], reducing cardiovascular diseases [22], anti-inflammatory [23], [24], the consumption of green tea is rapidly increasing.

MATERIAL AND METHODE

Fresh tea leaves (Camellia sinensis L. Kuntze) were obtained from tea gardens in the region of PT Perkebunan Nusantara (PTPN) XII Lawang, Malang, East Java, which is located at an altitude of 950-1250 above sea level and at ambient temperatures ranging from 19-25 degrees Celsius. Fresh tea leaves are cleaned of impurities and then drained. Folin Ciocalteu reagent, Na_2CO_3, catechin monohydrate, sodium nitrite, aluminum chloride, sodium hydroxide, Cab-O-Sil and ethanol 90% were purchased from merck.

Fresh tea leaves were sorted and washed, and then were dried in an oven at 100 ^oC. The drying rate was observed by weighing the sample using analytical balance every 20 minutes until a constant weight. Certain amount the dried tea leaves (as much as 358 grams) was then weighed and was inserted in a sealed plastic bag prior to further treatment.

The dried tea leaves were crushed in a blender and then sieved using 70/100 mesh sieve-set. Extraction was performed in 250 ml erlenmeyer flasks by mixing 120 ml of 20% ethanol and 4 grams of dried tea leaves powder. Extraction is done in a water bath at a temperature of 40, 50 and 60 ^oC. The extract was separated by filtration and then evaporates the liquid in an oven at temperature of 50 ^oC to obtain concentrate. The concentrate then was dried by adding Cab-O-Sil powder to form granules. The granules were then put in an oven at a temperature of 50 °C over night to obtain dry granules. The extract content was calculated as follow:

$$\text{weight of extact} = (\text{weight of dry granule}) - (\text{weight of Cab} - \text{O} - \text{sil}) \quad (1)$$

Total phenolic content (TP) was determined spectrophotometrically using Folin-Ciocalteu's reagent according to a modified method of Gordana Rusak, Draz˘enka Komes, Saša Likic´, Dunja Horz˘ic´, and Maja Kovac, 2008 [17]. The method is based on the reduction of phosphotungstic acid ($H_3/P\ [W_3O_{10}]_4$ in alkaline solution to phosphotungstic blue (based on $WO_2.nWO_3$). The value of absorbance which was influenced by the formed phosphotungstic blue is directly proportional to the number of aromatic phenolic groups in the sample and is used for their determination, expressed as gallic acid equivalent [17].

The procedure during the determination of phenolic in the sample is as follow: 0.5 ml of the sample was poured into a 50 ml volumetric flask containing 2.5 ml of Folin-Ciocalteu's reagent, 30 ml of distilled water and 7.5 ml of 20% Na_2CO_3, and the volume was made up with distilled water. During the oxidation of phenolic compounds, phosphomolybdic and phosphotungstic acid, contained in the Folin-Ciocalteu's reagent, were reduced to blue-coloured molybdenum and tungsten oxides. After two hours, the absorbance of blue colouration was measured using UV-VIS spectrofotometer (Hitachi U-2000) at 765 nm against a blank sample. In this work, gallic acid was used as the standard, therefore the results was expressed as mg gallic acid equivalent (GAE)/ mg extract.

RESULTS AND DISCUSSIONS

The drying profile

During manufacturing of green tea extract, the first step is drying of the tea leaves. In this step, the drying process is aimed to reduce the moisture content to certain level where the contamination of green tea extract product by certain substance produced by microbial activities can be avoided. Besides, the drying of fresh tea leaves will destroy the existing wax layer on the outer surface of the leaves and will further break down the cell walls so that it will facilitate the solvent compounds to diffuse easily during the extraction process. Furthermore, tea drying will prevent the tea from oxidizing then the green tea product is a product that is protected from oxidative damage.

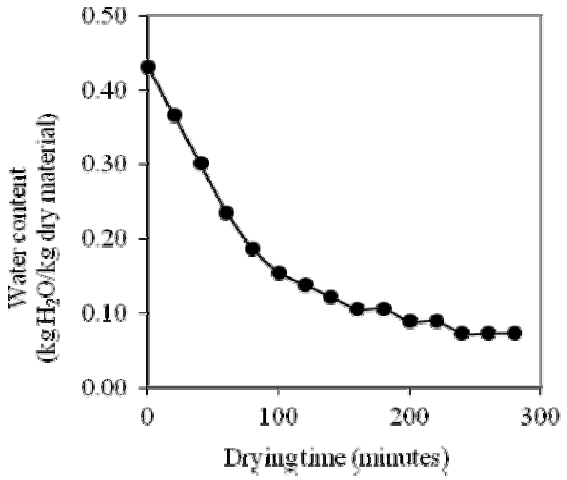

Figure-1. Plot of the water content versus the drying time.

To avoid quality degradation, the drying temperature is maintained at 100 ^{o}C. A maximum tea particle temperature of less than 100 ^{o}C is important for tea quality [25]. The hot drying air removes moisture from the core of the tea leaves by diffusion process [26]. The drying process is also aimed to arrest the fermentation process. The final moisture content of below 7% w.b. is a crucial aspect to get the stable product quality for preservation. There are some drying methods which can be applied for tea leaves drying including thermal drying methods (microwave-, oven-, and sun-drying) and non-thermal drying methods (air- and freeze-drying), thermal drying, however, also resulted in declines in total phenolic content [27].

In this work, the drying profile which was expressed as the reduction of water content (kg H_2O/kg dry tea leaves) along with the drying time was shown on Figure-1. The first 90 minutes drying show a relatively constant rate drying, which was revealed by the relatively straight line of the profile. It indicate that during this first period the drying was controlled by external heat transfer to the material surface. While after the first period, the drying profile form the non linier line which indicate that the falling rate of drying was happen. During the falling rate diffusion of moisture within the material is control the overall drying process.

The extract yield

Different solvents, namely, dichloromethane, ethyl acetate, acetone, acetonitrile, methanol and ethanol have been used by many researchers to extract the phenolic compounds from dried biomaterial. The existing analytical methods which usually use for quantifying the biologically active compounds present in tea leaves consisting of extraction, separation and analysis. Numerous extraction conditions and analysis methods have been applied, resulting in a wide variation in the level of measured concentrations of tea compounds. From many previous studies that have been conducted showed that aqueous ethanol was to be the most effective extraction solvent in prolonged extraction for tea leaves [17], [28]. For this reason, in this research work ethanol was used as solvent for the extracation. Four grams of dried green tea leaves were extracted with 120 ml of 20% ethanol in 250 ml Erlenmeyer flask equipped with a reflux condenser.

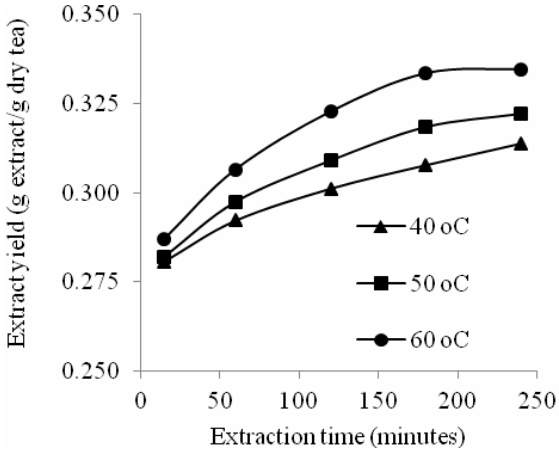

Figure-2. Plot of the extract yield versus the extraction time.

In order to increase the solubility of tea polyphenols and the rate of mass transfer, the conventional extraction technique is commonly carried out by heating, boiling, or refluxing. Most of researcher agrees that many components in tea, such as volatile components, polyphenols, etc., are thermally unstable and could degrade when expose to the heat during thermal extraction. Moreover, processing at high temperature could increase the extraction of protein and pectin; this causes the degradation of the quality of green tea extracts [29]. For this reason, processing in a shorter time and at lower temperature is favorable.

To prevent the degradation of the thermally unstable components when it exposed at high temperature, extraction at temperature of 40, 50 and 60 ^{o}C was applied in this work. The extraction result which was expressed as extraction yield along the experiment was shown in Figure-2. It can be seen from the Figure that higher extraction temperature resulted in a significant increase of the extraction yield. Besides, the result also clarified that the extraction time played an important role in the extraction process. Green tea is rich in phenolic compounds and the extraction efficiency of these compounds strongly depends on the time of extraction and the solvents used [17]. The addition of Cab-O-Sil powder acts as an adsorbent that will absorb the excess of the water in the liquid extract of tea leaves.

The highest yield, which was 0.3347 g extract/g dry tea leaves, was obtained at extraction temperature 60 °C and extraction time 240 minutes. While the lowest yield, which was 0.2807 g extract/g dry tea leaves, was obtained at extraction temperature 40 ^{o}C and extraction time 15 minutes. This result was comparable with green

tea extraction done by Amra Perva-Uzunalic's *et al*. [30] which obtain extraction yield 28 - 34% using ethanol as much as 25 - 100 %vol at temperature of its boiling point. The active ingredients contained in green tea, mainly catechins and caffeine, are usually isolated by extraction with organic solvents, in this case the extraction conditions, such as solvent type, temperature, time, pH, ratio of solvent to material, variously influence the efficiency and quality of the green tea extract [30].

Total phenolic content in extract

The total phenolic content obtained from ethanol extraction at different temperature was shown in Figure-3, where the value is between 0.21 - 0.25 mg GAE/mg extract. The dependency of total phenolic content on extraction temperature is in accordance with the same dependency of the extract yield. It indicate that the main component in the green tea extract is phenolic compound.

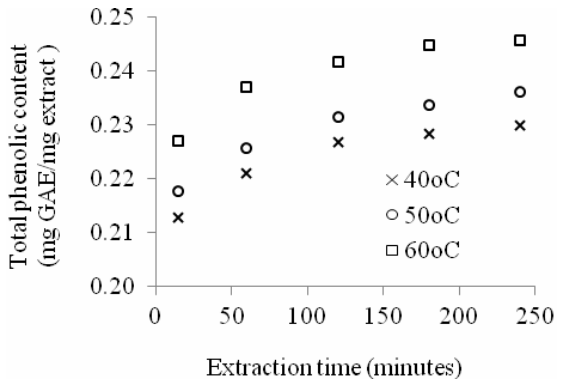

Figure-3. Total phenolic content expressed as galic acid equivalent (GAE) versus extraction time.

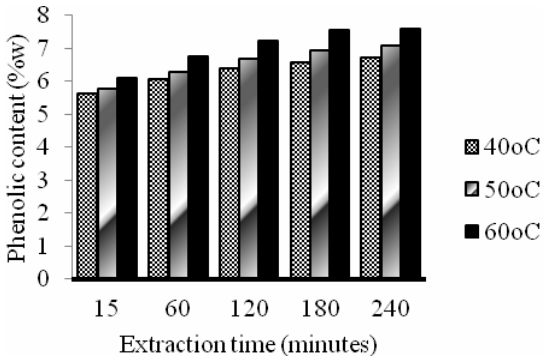

Figure-4. Plot of the phenolic content (%w) versus extraction time.

Catechins and their gallates are the main phenolic compounds in unfermented fresh tea leaves [31], which may constitute up to 30% of dry weight. Principal catechins of young tea leaves are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), epicatechin (EC) and catechin (E.W.C. Chan, 2007). The content of catechins in tea leaf have been reported to be influenced by a number of factors, including season, climate, elevation, agricultural practices, leaf maturity, processing methods, manufacturing practices and storage [31].

The total phenolic content can also be expressed in percent weight of the dry tea leaves such as expressed in Figure-4 where the obtained total phenolic content value is between 5.6 - 7.6 %w. These values agree well with work result by Shiromani Jayasekera, Lovedeep Kaur, Abdul-Lateef Molan, Manohar L. Garg, and Paul J. Moughan (2014) which reported that total catechins in the aqueous extracts from unfermented teas were in the range of 8.4 - 12.1 %w [31]. Extraction of total phenolic content of green tea with various solvent including acetone, methanol, acetonitrile and water (concentration from 25% - 100%) resulted in phenolic content ranged from 5.8 - 569 g/kg dry extract [32].

CONCLUSIONS

Ethanol showed high extraction efficiency for fresh tea leaves. This work has confirmed that green tea is a rich source of phenolics compounds. Higher extraction temperature resulted in a significant increase of the extraction yield. Besides, the result also clarified that the extraction time played an important role in the extraction process. The determination of total phenolic content and the extract yield has confirmed that the main component in the green tea extract is phenolic compound. The study also demonstrated that the extraction of tea leaves with ethanol at relatively low temperature exhibit considerable efficient method to obtain green tea extract with relatively high total phenolic content.

REFERENCES

[1] E.W.C. Chan, Y.Y. Lim, Y.L. Chew. 2007. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. *Food Chemistry.* 102: 1214-1222.

[2] Yao L.H., Jiang Y. M., Caffin N., Arcy B. D., Datta N., Liu X., Singanusong R. and Xu Y. 2006. Phenolic compounds in tea from Australian supermarkets. *Food Chemistry*. 96: 614-620.

[3] Friedman M., Kim S.-Y., Lee S.-J., Han G.-P., Han J.-S., Lee K.-R. and Kozukue N. 2005. Distribution of catechins, theaflavines, caffeine, and theobromine in 77 teas consumed in the United States. *Journal of Food Science.* 70: 550-559.

[4] An B.J., Kwak J.H., Son J.H., Park J.M., Lee J.Y., Jo C. 2004. Biological and antimicrobial activity of irradiated green tea polyphenols. *Food Chemistry.* 88: 549-555.

[5] Krishnan R. and Maru G.B. 2006. Isolation and analyses of polymeric polyphenol fractions from black tea. *Food Chemistry.* 94: 331-340.

[6] Park H.S., Choi H.K., Lee S.J., Park K.W., Choi S.G., Kim K.H. 2007. Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide. *The Journal of Supercritical Fluids.* 42: 205-211.

[7] Hsieh M.H. and Chen S.M. 2007. Determination of amino acids in tea leaves and beverages using capillary lectrophoresis with light-emitting diode-induced fluorescence detection. *Talanta.* 73: 326-331.

[8] Labbé D., *et al*. 2008. Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure. *Food Chemistry.* 111: 139-143.

[9] Zhao W., Yang R., *et al*. 2008. Effect of PEF on microbial inactivation and physical–chemical properties of green tea extracts. *LWT.* 41: 425-431.

[10] Wang L. F., Kim D. M. and Chang L. Y. 2000. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. *Journal of Agriculture and Food Chemistry.* 48: 4227-4232.

[11] Kinugasa H. and Takeo T. 1990. Deterioration mechanism for tea infusion aroma by retort pasteurization. *Agriculture and Biology Chemistry.* 54: 2537-2547.

[12] Zhu Q.-Y. 1997. Stability of green tea catechins. *Journal of Agriculture and Food Chemistry*. 45: 4624-4628.

[13] Chen Z.-Y. 1998. Stabilizing effect of ascorbic acid on green tea catechins. *Journal of Agriculture and Food Chemistry*. 46: 2512-2516.

[14] Kim E.S. 2007. Impact of heating on chemical compositions of green tea liquor. *Food Chemistry*. 103: 1263-1267.

[15] Wang L.F., Lee J.Y., *et al*. 2008. Discrimination of teas with different degrees of fermentation by SPME-GC analysis of the characteristic volatile flavour compounds. *Food Chemistry.* 109: 196-206.

[16] Polat M. and Korkmaz M. 2008. Detection of irradiated black tea (Camellia sinensis) and rooibos tea (Aspalathus linearis) by ESR spectroscopy. *Food Chemistry.* 107: 956-961.

[17] Rusak G., *et al*. 2008. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chemistry*. 110: 852-858.

[18] Liu Z.Q., Ma L.P., *et al*. 2000. Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. *Chemistry and Physics of Lipids.* 106: 53-63.

[19] Tsai T.H., *et al*. 2008. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. *Food Chemistry.* 110: 859-864.

[20] Kuzuhara T., Suganuma M. and Fujiki H. 2008. Green tea catechin as a chemical chaperone in cancer prevention. *Cancer Letters.* 261: 12-20.

[21] Tsai T.H., *et al*. 2008. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. *Food Chemistry*. 110: 859-864.

[22] Lu C.H. and Hwang L.S. 2008. Polyphenol contents of Pu-Erh teas and their abilities to inhibit cholesterol biosynthesis in Hep G2 cell line. *Food Chemistry.* 111: 67-71.

[23] Trompezinski S., Denis A., Schmitt D., Viac J. 2003. Comparative effects of polyphenols from green tea (EGCG) and soybean (genistein) on VEGF and IL-8 release from normal human keratinocytes stimulated with the proinflammatory cytokine TNF alpha. *Arch. Dermatol. Res*. 295: 112-116.

[24] Tedeschi E., Suzuki H., Menegazzi M. 2002. Antiinflammatory action of EGCG, the main component of green tea, through STAT-1 inhibition, Ann. N. Y. Acad. Sci. 973, 435-437.

[25] S. J. Temple, S. T. Tambala, A. J. B. van Boxtel. 2000. Monitoring and control of fluid-bed drying of tea. *Control Engineering Practice*. 8: 165-173.

[26] Partha Pratim Dutta, Debendra Chandra Baruah. 2014. Drying modelling and experimentation of Assam black tea (Camellia sinensis) with producer gas as a fuel. *Applied Thermal Engineering.* 63: 495-502.

[27] E.W.C. Chan, Y.Y. Lim, S.K. Wong, K.K. Lim, S.P. Tan, F.S. Lianto, M.Y. Yong. 2009. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry.* 113: 166-172.

[28] Wan-Joo Kim, Jae-Duck Kim, Jaehoon Kim, Seong-Geun Oh, Youn-Woo Lee. 2008. Selective caffeine removal from green tea using supercritical carbon dioxide extraction. *Journal of Food Engineering.* 89: 303-309.

[29] Jun Xi, Yujing Xue, Yinxiang Xu, Yuhong Shen. 2013. Artificial neural network modeling and optimization of ultrahigh pressure extraction of green tea polyphenols. *Food Chemistry.* 141: 320-326.

[30] Amra Perva-Uzunalic, Mojca S kerget, Z˘ eljko Knez, Bernd Weinreich, Frank Otto, Sabine Gru¨ner. 2006. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. *Food Chemistry*. 96: 597-605.

[31] Shiromani Jayasekera, Lovedeep Kaur, Abdul-Lateef Molan, Manohar L. Garg, Paul J. Moughan. 2014. Effects of season and plantation on phenolic content of unfermented and fermented Sri Lankan tea. *Food Chemistry*. 152: 546-551.

[32] Amra Perva-Uzunalic, Mojca S˘ kerget, Z˘ eljko Knez, Bernd Weinreich, Frank Otto, Sabine Gru¨ner. 2006. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine, *Food Chemistry*. 96: 597-605.