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Monitoring Tea Pigments Theaflavins and Thearubigins in Dependence on the Method and Duration of Storage

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Abstract: The aim of this research was to monitor the decrease of tea pigments theaflavins and thearubigins in selected types of tea depending on the method and duration of storage. Teas were stored for 12 months in 6 different methods of storage. UV-VIS spectrophotometry was used as the analysis method. In some types of tea in the 1st month of storage a statistically significant decrease in the flavonoid pigments was detected especially in unproperly stored teas.

Key words: Tea, theaflavins, thearubigins, UV-VIS spectrophotometry, storage of teas, Czech Republic

INTRODUCTION

Besides water, tea is the most popular beverage in the world, it is consumed by more than two-thirds of the world's population. It is a drink made of tea leaves infusion. It is prepared in various ways in different parts of the world and among the most widely consumed types are black, green and oolong (Caballero, 2003).

Recently, there has been an ongoing discussion of its significance in terms of pharmacological effects which may significantly affect health of the consumer. The discussed issues include tea pigments such as theaflavins (from here on TFs) and thearubigins (from here on TRs).

TFs and TRs chemically belong to a large group of flavonoids that arise, in contrast to other flavonoid components especially during fermentation or enzymatic oxidation of catechins naturally present in tea leaves with application of enzymes of phenoloxidases and peroxidases. In the above-mentioned process polymerization of up to 75% of catechins occurs where according to the results of Bailey *et al*. (1994)'s collective, the first stage of fermentation is responsible for creating TFs while additional products of catechins are formed as well such as bisflavonols and epitheaflavin acids in subsequent stages the oxidation products occur in form of TRs (Bailey *et al*., 1994; Caballero, 2003). Thus according to Muthumani and Kumar (2007), the maximum increase in TFs is within the first 45 min of fermentation duration and after a longer time, about 110 min, phenoloxidases activity decreases and formation of complexes of which the main polymers are TRs, increases (Gupta *et al*., 2001; Muthumani and Kumar, 2007; Aherne and O'Brien, 2002; Kuhnert, 2010).

TFs and TRs are affected by many factors where among the priority ones belongs the already mentioned fermentation (pH, relative moisture and availability of oxygen). Other factors affecting the total amount of TFs and TRs include geographic location, genetic changes in tea plants and the overall environment including growth factors (Muthumani and Kumar, 2007; Owuor and Obanda, 2001; Owuor, 2003; Owuor *et al*., 2006).

TFs arise as a seven-membered tropolene ring through a reaction of quinone derived catechins with quinone from gallate catechins or their gallates which forms their characteristic construction-the benzotropolene ring (Bailey *et al*., 1994; Belitz *et al*., 2001). TFs which affect the overall pigmentation are often defined as a mixture of theaflavin, theaflavin-3-gallate, theaflavin-30-gallate and theaflavin-3, 30-gallate and constitute about 1-2% of the total dry weight of the final product (Maity *et al*., 2003; Way *et al*., 2004; Owuor *et al*., 2006). TFs are a well-soluble dimeric flavonoids of bright orange to red color and they are very desirable in terms of tea quality; a considerable part of research is focused on increasing their amount during the production of fermented teas (Robertson and Bendall, 1983; Belitz *et al*., 2001).

TRs are the most abundant phenolic fraction of black tea and they are largely oxidized polymer, difficult to define and unstable substance with a wide molecular

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weight. It is a very heterogeneous mixture of red-yellow to orange-brown soluble and insoluble oxidation products (accounting for about 60% of dry matter) with extensive beneficial biological activities and effects on consumer's health but conclusions about their exact chemical structure are highly speculative (Robertson and Bendall, 1983; Way *et al.*, 2004; Kuhnert, 2010).

The above pigments are therefore, significant in tea and tea infusions mainly from the sensory point of view, namely the color. TFs creates a bright orange-red color of the infusion and they have astringent but refreshing taste. On the other hand, TRs create brown-reddish color of the infusion and their flavor is more bitter and more astringent than that of TFs (Schwarz *et al.*, 1994; Hakim *et al.*, 2000; Sharangi, 2009; Bhagwat, 2010).

Often it is therefore, discussed that the quality of tea including tea infusion can be evaluated on the basis of their total content since TRs in reaction with TFs and proteins form a complex of highly polymeric compounds collectively affecting not only the color but also brightness, strength and flavor of the tea infusion (Muthumani and Kumar, 2007; Owuor *et al.*, 2006). From the foregoing it can be assumed that the largest amount of TFs and TRs can be found in fermented teas such as black tea, phu-er or semi-fermented teas (up to a higher level of fermentation) of oolong type. Due to difficult identification of the above mentioned flavonoid pigments, their individual fractions cannot be easily determined. Therefore, most studies are aimed at measuring the overall color of the tea infusion and for example in Owuor *et al.* (2006)'s opinion, the quality of tea and tea infusions cannot be evaluated by individual fractions such as TFs and TRs as each carries different properties including color. In general, most authors agree that a very high amount of compex TRs decreases sensory quality of tea, especially by reducing the overall brightness, bitterness and freshness of tea infusion (Owuor and Obanda, 2001; Owuor, 2003; Owuor *et al.*, 2006).

The aim of the research was to monitor the amount of TFs and TRs in black teas that were then stored in various methods for 12 months and to repeatedly determine stability of TFs and TRs depending on the method and duration of storage. This was decided to discover how significantly can the method of storage of tea keep or impair its sensory value and intensity of the pharmacological effects that are carried by TFs and TRs pigments.

MATERIALS AND METHODS

For the very analysis, 6 loose tea samples were selected from one of the leading processors of tea in the Czech Republic (Oxalis spol. s.r.o., Slusovice). Samples selected for the analysis came from different growing regions (China, India, Kenya, Georgia, Taiwan). Samples for analyses were intentionally selected from black teas that often have undergone a difficult process of fermentation to guarantee the presence of the above mentioned flavonoid pigments and thus their stability over time and storage could be monitored.

Table 1: A summary of all analyzed samples of commercial teas

Samples	Kind	Processor	Area
1	Gruzie OP	Oxalis	Georgia
2	Keemun red	Oxalis	China
3	Kenya, GFOP-I Millima	Oxalis	Kenya
4	Assam mangalam, BPS CL	Oxalis	India
5	Darjeeling namring, Upper FTGFOP1	Oxalis	India
6	Nilgiri	Oxalis	India

Tea samples came from tea harvested in autumn 2009, there were taken from the original distribution packages (designed for small consumer) immediately after their opening. The summary of all analyzed samples of commercial teas is shown in the Table 1.

Removing samples of storage: To compare the changes during storage and differentiation of flavonoid content of the above described pigments, their contents in all samples of tea was determined immediately before the beginning of storage. This analysis was identified as a measurement in the 0th month.

The above samples of teas were stored in various ways for 12 months. As the research was focused mainly on the consumer influence, usual methods of storage that are most commonly used by consumers were applied. Specifically, the tea was stored in closed glass containers (drip flasks with ground glass stopper), in paper and metal closable boxes and a in the original packaging (an inside-protected paper bag with a plastic film on its surface). Teas stored in these ways were put into closable boxes for 12 months which induced dark environment without direct exposure to light and air. In addition, selected samples of commercial teas (Table 1) were again placed in closed glass containers and closable paper boxes and were stored in direct light in the laboratory. All samples were stored at room temperature 20°C.

Sample preparation: Tea sample was weighed in a quantity of 3 g in a glass beaker and it was immediately flooded with water at 85°C. The sample prepared in this way was eluted for 5 or more min (as recommended by the manufacturer). After cooling down to room temperature its absorbance was measured. In all cases, the maximum absorption was checked by measuring the absorption spectrum in a wider range of wavelengths. Each sample (for the entire period of storage and for all storage methods) was measured three times for both analysis of TFs and TRs.

Chemical analysis: In order to determine the TFs and the TRs, the method of UV-VIS spectrophotometry was used applying the analyzer of Cecil CE 7210 in the wavelength range of 190-900 nm when the total pigmentation (absorbance) was measured at a wavelength of 665 nm for TFs and 825 nm for TRs in contrast to water used as blind sample.

The method was taken over (Perkampus, 1992) and adapted to the conditions in the laboratory and it was also earlier published by us.

In the experimental part of the work, at first the amount TFs (mg/100 g of tea) and TRs (mg/100 g of tea) was determined in tea samples immediately after opening the distributional package i.e., in the 0th month and these pigments were analyzed for all used samples of tea in 8 intervals (0-6 and 12 months of storage) within 1 year in weighted tea samples, expressed in mg/100 g of tea.

Statistical analysis: Measured concentration values for TFs and TRs (mg/100 g of tea) were then assessed by statistical analysis using software for computer technology DataLab ver. 2,701, manufacturer Epina GmbH, Austria and the results were checked using theoretical relationships for the statistical analysis (Brown *et al.*, 2009).

At the beginning of the analysis an exploratory analysis of the measured concentrations of TFs and the TRs was performed and it was found that the frequency of the measured values corresponds to the statistical distribution of values according to Gauss. Under extreme value test according to Dixon and Webb the measured concentrations did not occur with extreme values and thus the number of samples we measured (6 samples) was optimal.

In the first stage of statistical analysis, the basic statistical characteristics, among which were the arithmetic mean, standard deviation and coefficient of variation were calculated.

In order to analyze the concentration of pigments depending on the duration and ways of storage, different methods of compliance testing were used according to the survey analysis results such as the Student's t-test F-test, Mann-Whitney and analysis of variance (Brown *et al.*, 2009).

RESULTS AND DISCUSSION

By processing, evaluating and organizing the results acquired from TFs and TRs measuring we obtained a number of values that we present and discuss in this study.

An average amount of TFa and TRs including standard deviations in 0-6 and 12th months is shown in the Table (Table 2 and 3) and for better clarity, graphic interpretation of the average results is presented as shown in the Fig. 1 and 2.

During storage (0-6 and 12 months) of black tea there was a loss of concentration of TFs (mg/100 g of tea) with one exception and that is a statistically significant increase in the second month of storage.

In the study, the present oxidizing enzymes that occur naturally in tea leaves were activated due to manipulation and then the oxidation of catechins which are present in black teas in harmony with Luczaj and Skrzydlewska (2005) in average amount of 10-12%, took place (Luczaj and Skrzydlewska, 2005).

In case of various methods of storage for all the methods the level of TFs decreases by 10-18 mg/100 g of tea. Concentration during the 2nd month of storage

Table 2: TFs concentration (mg/100 of tea) for various types of storage (0-6 and 12th months)

	Storage time (months)							
Method of storage	0	1	2	3	4	5	6	12
Origin packaging	0.39±0.12	0.22±0.08	0.42±0.13	0.30±0.22	0.16±0.04	0.15±0.11	0.17±0.04	0.19±0.09
Metal box	0.39±0.12	0.29±0.09	0.42±0.07	0.23±0.07	0.19±0.28	0.21±0.09	0.18±0.20	0.18±0.07
Paper box-light	0.39±0.12	0.28±0.03	0.42±0.14	0.17±0.09	0.32±0.27	0.22±0.17	0.11±0.07	0.04±0.19
Paper box-dark	0.39±0.12	0.27±0.07	0.43±0.12	0.32±0.18	0.20±0.20	0.28±0.05	0.12±0.08	0.13±0.04
Glass container-light	0.39±0.12	0.24±0.08	0.40±0.22	0.30±0.13	0.34±0.14	0.22±0.16	0.08±0.05	0.06±0.04
Glass container-dark	0.39±0.12	0.25±0.09	0.42±0.11	0.33±0.28	0.31±0.14	0.36±0.21	0.17±0.09	0.18±0.18

Table 3: TRs concentration (mg/100 of tea) for various types of storage (0-6 and 12th months)

	Storage time (months)							
Method of storage	0	1	2	3	4	5	6	12
Origin packaging	7.35±0.18	3.82±0.43	7.10±0.46	3.18±0.33	3.18±0.47	4.35±0.41	4.12±0.41	2.08±0.40
Metal box	7.35±0.18	3.06±0.21	6.79±0.46	3.45±0.39	3.12±0.46	4.88±0.46	2.59±0.42	2.12±0.46
Paper box-light	7.35±0.18	3.80±0.18	5.59±0.20	2.63±0.39	5.47±0.47	6.26±0.48	3.63±0.13	1.11±0.33
Paper box-dark	7.35±0.18	3.67±0.20	5.75±0.19	5.37±0.41	6.40±0.56	4.07±0.50	3.14±0.50	2.90±0.38
Glass container-light	7.35±0.18	4.08±0.31	4.13±0.20	4.15±0.49	5.09±0.16	6.10±0.47	3.49±0.24	1.10±0.42
Glass container-dark	7.35±0.18	3.89±0.38	5.15±0.39	4.08±0.38	5.65±0.37	5.11±0.44	4.22±0.41	2.15±0.47

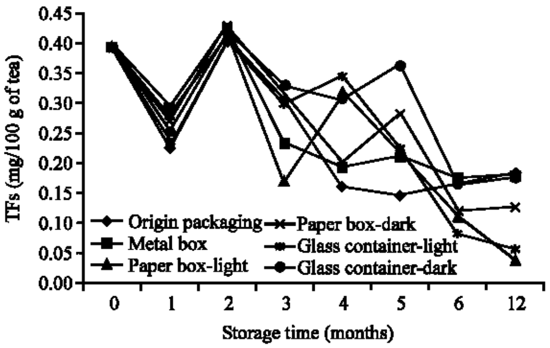

Fig. 1: Average amount of TFs (mg/100 of tea) for various types of storage (0-6 and 12th months)

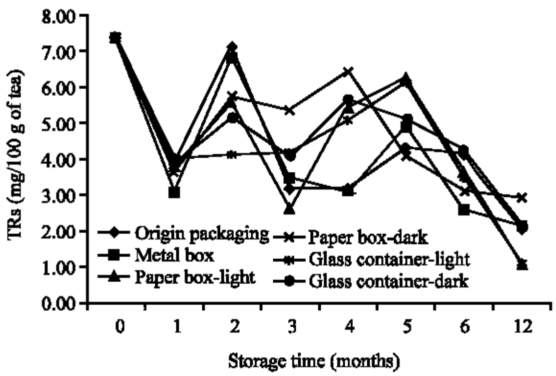

Fig. 2: Average amount of TRs (mg/100 of tea) for various types of storage (0-6 and 12th months)

almost uniformly increases over the original level (at the 0th point of storage) which is also explained, consistently with the research of Gregory and Bendall (1966) by an adaptation of living off of tea-leaf tissue to the storage environment and increased biochemical activity which induces partial restoration of phenoloxidases ant thus intense polymerization of oxidation products and formation of color-significant TFs with almost no impact of the storage environment and material. From the 2nd-12th month of storage the levels of TFs are decreasing with greater influence of the environment and material. Particularly the strongest decline in levels of TFs occurs between the 2nd and the 3rd month of storage, up to 25 mg/100 g in a paper container in the light while the lowest loss (only 36% of the loss in a paper box in the light) was in a glass container in the dark namely only 9 mg/100 g. In the following months there is a statistically demonstrable while various loss in TFs concentrations with the deepening impact of less favorable environment and materials on the stored tea. Storage of tea in a metal container and original packaging (impenetrable to light) stored in the light and glass containers in the dark from the 2nd to the 12th month appeared to be the most favorable, an overall decrease of TFs levels in metal boxes was 51% in the original packaging and in the glass in the dark about 53.84%. For these storage methods there was a statistically significant difference in the amount of TFs in the 3rd month of storage as was also for teas stored in paper containers in the dark. In teas stored in the light (paper package-light, glass container-light) in the 4th month there was an increase of TFs levels to statistically significant level of TFs from the 1st month. Of the original TFs levels the highest loss of TFs throughout the storage period of tea was detected in study containers stored in the light (89.7%), in glass containers in the light (84.6%) and in paper containers in the dark (66.6%).

By evaluating the determined amount of TRs in the monitored samples of tea in 8 intervals within 12 months of storage under different conditions, we found the following TRs levels: at the entrance of tea in the experiment (0th month) 7.35 mg/100 g weight of tea, after 6 months of storage about 3.53 mg/100 g and after 12th months storage of about 1.9 mg/100 g (Table 3).

During the 12th months of storage of black teas in the above intervals, there was a significant decline during the 1st month of storage, a similar increase in levels during the 2nd month as we described above for TFs and subsequent fluctuations in the level of TRs with different environmental and packaging materials influence from the 4th month there was a gradual decline in the concentration of TRs in almost all the stored samples (Fig. 2).

After 12 months of storage, we detected the largest decrease in levels of TRs (85%) in the glass and paper packaging in the light, 71.7% in the original packaging, 71.1% in metal packaging, 70.7% in the glass container in the dark and the smallest loss in paper packaging in the dark (60.5%). During storage and handling with the samples also oxidation of polymeric TRs to its subsequent degradation products occurred which can in harmony Owuor and Obanda (2001), occur naturally due to exposure to long periods of fermentation.

Level fluctuations from the 1st month of storage are strongly influenced by the environment and the material in which the samples were packed.

In the teas stored in metal and original containers there was a statistically demonstrable reduction in concentrations of TRs (mg/100 g tea) in the 2nd month as it was in the tea stored in paper boxes in the dark where the losses were not gradual. In the teas stored in paper containers stored in the light and glass containers stored in the light and in the darkness there was a statistically significant decline of TRs in the 1st month already.

During storage there were variations in levels of TFs and TRs. In the 1st month their losses occurred which was caused mainly by the presence of atmospheric oxygen during sample manipulation and activation of phenoloxidase and peroxidase enzymes which according to Haslam (2003), strongly destabilize structure of the mentioned flavonoid pigments and so fermentation was reactivated. Which is also confirmed in the research by Miyawaki (2006) who state that due to strong bonds of phenoloxidase enzymes in the structure of plant material and thus its higher stability, they probably do not become completely inactivated after heat treatment of the tea material and the effect of oxygen especially in case of handling the tea and improper storage causes activation of enzymes and subsequent instability and aging of tea. Very interesting was the fact that in all analyzed samples the amount of TFs and the TRs during the 2nd month of storage increased when the process of fermentation was likely to continue and oxidation occurred in catechins to TFs and subsequently to TRs. Thus the results correlate to the results of a collective of authors lead by Robertson and Bendall (1983) that are referred to also by other studies who confirm that the storage can lead to activation of phenoloxidases which results in the degradation of analytes such as TFs and formation of substances from the TRs group and can thus lower the overall original quality of tea (Robertson and Bendall, 1983).

When comparing the loss of TFs and TRs, It is discovered that the most significant declines in all methods of storage occurred mainly in TFs. As stated by Owuor (2003), prolonged duration of fermentation leads to degradation of created TFs and the formation of other high-molecular substances which are also classified as TRs (Owuor, 2003). The least significant TFs fluctuations occurred in teas stored in metal and original packagings. Conversely, the greatest changes of TFs occurred in the teas stored in the light in paper and glass containers where from the 4th month on a sharp decline in TFs occurred. The concentration of TRs in black teas has fallen sharply in the 5th month of storage, the largest losses occurred in the teas stored in the light in particular in a paper packaging and glass drip flasks. Conversely, the smallest losses occurred in samples stored in the dark, especially the ones stored in the original and metal containers.

Based on the results, flavonoid pigments can be defined as substances whose amount varies in time and method of storage significantly. The results do not entirely agree with those by Su *et al*. (2003) who refer to these substances in a relatively stable especially during transportation and storage. These researchers however, do not focus on their stability under normal handling processes used by consumers and their storage in less suitable conditions at home (Su *et al*., 2003).

On the contrary, the study conducted by Yao *et al.* (2006) where they compared the results obtained from the colorimetric measurement of teas and sensory analysis, demonstrated that during the storage of tea significant changes in polyphenolic substances in both black teas and green teas occur. They demonstrated that storage of tea causes losing not only the color of tea but also the taste despite the fact that they are still organoleptically acceptable for the consumer (Yao *et al*., 2006).

CONCLUSION

Natural pigments Theaflavins (TFs) and Thearubigins (TRs) affect not only the biological value of tea but also its sensory properties. During the storage further polymerization, increase and decrease in the molecular weight particularly in TRs is probable to occur. The reason for these changes is the reactivation of phenoloxidases, natural enzymes of tea leaves especially due to handling the tea samples and in particular, subsequent influence of atmospheric oxygen.

Quantity of TFs in black tea in the 0th month (beginning of the storage of tea) was 0.39 mg/100 g of tea and of TRs 7.35 mg/100 g of tea. In the 1st month concentrations of both pigments decreased, the TFs value dropped to 0.22-0.29 mg/100 g of tea and TRs values to concentrations of 3.06-4.08 mg/100 g of tea. The lowest values of both pigments were found in the 6th and 12th months. In the 6th month, the amount of TFs ranged from 0.08-0.18 mg/100 g of tea and the amount of TRs from 2.59-4.22 mg/100 g of tea. In the 12th month TFs amount ranged from 0.04-0.19 mg/100 g of tea and TRs from 1.1-2.9 mg/100 g of tea.

In harmony with the above described facts, researchers have demonstrated that the amount of TFs and the TRs is changing already in the first month of storage where statistically demonstrable losses in TFs occurred mainly in samples of tea in paper packages stored in the light and glass containers placed in the light as well as in the dark. On the contrary, for tea samples stored in metal and original packagings we did not discover a statistically demonstrable difference until the 3rd month of storage. In addition, the samples stored in this ways avoided significant fluctuations of recorded levels during storage.

When analyzing the number of TRs, we demonstrated a statistically significant decrease in the 2nd month of storage of tea stored in metal containers and in the original packaging and paper boxes stored in the dark while in other ways there was a statistically demonstrable decline already after 1 month of storage.

From the foregoing it can be assumed that in particular unsuitable handling causes major destability of tea flavonoid pigments that affect the sensory value of the tea infusion. Mainly when stored in air-permeable packaging and containers in direct light, changes arise already in the 1st weeks of storage and thus one can assume that the tea loses its original properties and sensory quality obtained by the production technology carefully respected by the manufacturer.

In a more detailed study of tea, it is necessary to focus on other analytes that contribute to the quality of tea infusions and gain a broader perspective on their quality. Based on the results one can assume that the seller after the removal of the samples from the distribution package or the consumer at purchase and subsequent storage of tea can significantly influence the initial sensory value of teas and tea infusions by the environment and the period of storage.

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REFERENCES

Aherne, S.A. and N.M. O'Brien, 2002. Dietary flavonols: Chemistry, food content and metabolism. Nutrition, 18: 75-81.

Bailey, R.G., H.E. Nursten and I. McDowell, 1994. Isolation and high-performance liquid chromatographic analysis of thearubigin fractions from black tea. J. Chromatography A, 662: 101-112.

Belitz, H.D., W. Grosch and P. Schieberle, 2001. Food Chemistry. 5th Edn., Springer-Verlag, Berlin, pp: 72.

Bhagwat, S., 2010. Flavonoids composition of tea: Comparison of black and green teas. Agricultural Research Service, Report No. 25.

Brown, S.D., R. Tauler and B. Walczak, 2009. Comprehensive Chemometrics: Chemical and Biochemical Data Analysis. Elsevier Science and Technology, Amsterdam, ISBN-13: 9780444527028, pp: 2896.

Caballero, B., 2003. Tea: Chemistry. In: Encyclopedia of Food Sciences and Nutrition, Caballero, B., P. Finglas and L. Trugo (Eds.). Academic Press, Amsterdam.

Gregory, R.P. and D.S. Bendall, 1966. The purification and some properties of the polyphenol oxidase from tea (*Camellia sinensis* L.). Biochem. J., 101: 569-581.

Gupta, S., B. Saha and A.K. Giri, 2001. Comparative antimutagenic and anticlastogenic effects of green tea and black tea: A review. Mutat. Res., 512: 37-65.

Hakim, I.A., U.M. Weisgerber, R.B. Harris, D. Balentine, C.A.J. van-Mierlo and I. Paetau-Robinson, 2000. Preparation, composition and consumption patterns of tea-based beverages in Arizona. Nutr. Res., 20: 1715-1724.

Haslam, H., 2003. Thoughts on thearubigins. Phytochemistry, 64: 61-73.

Kuhnert, N., 2010. Unraveling the structure of the black tea thearubigins. Arch. Biochem Biophys., 501: 37-51.

Luczaj, W. and E. Skrzydlewska, 2005. Antioxidative properties of black tea. Prev. Med., 40: 910-918.

Maity, S., A. Ukil, S. Karmakar, N. Datta and T. Chaudhuri *et al*., 2003. Thearubigin, the major polyphenol of black tea, ameliorates mucosal injury in trinitrobenzene sulfonic acid-induced colitis. Eur. J. Pharmacol., 470: 103-112.

Miyawaki, M., 2006. Control of polyphenol oxidase and pectin methylesterase activities by ultra high pressure. Ph.D. Thesis, Washington State University, Washington DC USA.

Muthumani, T. and R.S.S. Kumar, 2007. Influence of fermentation time on the development of compounds responsible for quality in black tea. Food Chem., 101: 98-102.

Owuor, P.O. and M. Obanda, 2001. Comparative responses in plain black tea quality parameters of different tea clones to fermentation temperature and duration. Food Chem., 72: 319-327.

Owuor, P.O., 2003. TEA Analysis and Tasting. In: Encyclopedia of Food Sciences and Nutrition, Benjamin, C. (Ed.). Academic Press, Oxford, pp: 5757-5762.

Owuor, P.O., M. Obanda, H.E. Nyirenda, N.I.K. Mphangwe, L.P. Wright and Z. Apostolides, 2006. The relationship between some chemical parameters and sensory evaluations for plain black tea (*Camellia sinensis*) produced in Kenya and comparison with similar teas from Malawi and South Africa. Food Chem., 97: 644-653.

Perkampus, H.H., 1992. UV-VIS spectroscopy and its applications. Springer-Verlag, New York, ISBN-13: 9783540554219, pp: 244.

Robertson, A. and D.S. Bendall, 1983. Production and HPLC analysis of black tea theaflavins and thearubigins during *in vitro* oxidation. Phytochemistry, 22: 883-887.

Schwarz, B., H.P. Bischof and M. Kunze, 1994. Coffee, tea and lifestyle. Prev. Med., 23: 377-384.

Sharangi, A.B., 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.): A review. Food Res. Int., 42: 529-535.

Su, Y.L., L.K. Leung, Y. Huang and Z.Y. Chen, 2003. Stability of tea theaflavins and catechins. Food Chem., 83: 189-195.

Way, T.D., H.H. Lee, M.C. Kao and J.K. Lin, 2004. Black tea polyphenol theaflavins inhibit aromatase activity and attenuate tamoxifen resistance in HER2/neu-transfected human breast cancer cells through tyrosine kinase suppression. Eur. J. Cancer, 40: 2165-2174.

Yao, L.H., Y.M. Jiang, N. Caffin, B. D'Arcy and N. Datta *et al*., 2006. Phenolic compounds in tea from Australian supermarkets. Food Chem., 96: 614-620.