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Comparison of proximate composition and antioxidant activity of black and green tea available in Bangladesh

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Abstract

The present study was carried out to compare the black and green tea available in Bangladesh in respect to proximate composition and antioxidant activity. Eight brands of black tea and two brands of green tea were bought from local super market. The variance between every proximate content (moisture, protein, fat, ash and carbohydrate) of black and green tea was found statistically insignificant ($p > 0.05$). The total antioxidant activity was determined by phospho-molybdenum assay method and ferrous chelating activity was monitored by measuring the generation of the ferrous ion ferrozine complex. Green tea performed significantly higher total antioxidant activity (792.87 AAE) than black tea (330.72 AAE).The variance of ferrous chelating activity between black and green tea was found statistically significant ($p < 0.05$) at concentration of 10 ppm, 100 ppm, 200 ppm, 400 ppm, and 600 ppm. Black tea showed maximum inhibition, 50.33% at 600 ppm and lowest 1.93% at 10 ppm. The highest percent inhibition for green tea was found 32.14 at 600 ppm and lowest 1.25 at 10 ppm. IC50 values were found 5.50 ppm and 4.50 ppm for black and green teas respectively.

Keywords: Tea; proximate composition; antioxidant activity; black tea; green tea; Bangladesh.

1. Introduction

Tea is believed to be the second most consumed beverage in the world [1] and in Bangladesh 85.93 million kg tea was consumed in 2017 [2]. It grows better in tropical and subtropical regions with sufficient rainfalls, proper drainage system and sandy loam soil which is slightly acidic in nature [3]. Two basic botanical varieties include: Chinese tea's shrub (Camellia sinensis) as well as Indian tea plant (Camellia assamica) [4, 5]. Tea is manufactured in three basic forms. Green tea is prepared in such a way as to preclude the oxidation of green leaf polyphenols. During black tea production, oxidation is promoted so that most of these substances are oxidized. Oolong tea is a partially oxidized product. While all of these teas originate from the same Camellia sp. plant, their chemical composition varies depending on geographical location, agricultural practices, processing methods and degree of maturation [6]. A cup of tea leaves infusion is "the injection of antioxidants" showing a higher capability for scavenging free radicals than vitamin C and E [7]. The green and black teas have much higher antioxidant activities against peroxyl radicals than 22 common vegetables [8].

Tea has developed in Bangladesh as an agro-based, labour intensive, import-substitution and export- oriented industry over the last 150 years [2]. At present number of tea garden is 166, area under tea is 58,719 hectare; production is 66.26 million kg and yield per hectare increased to 1320 kg. At present Small Holding Tea Sub-sector have newly emerged in northern districts and Chittagong Hill tracts [2]. Mostly black tea is consumed in Bangladesh. Green tea, however, is getting popularity day by day. The proximate composition and the antioxidant activity is the parameters of quality for tea regarding its biological properties. However, little is known about nutrients and antioxidant activity of black and green tea available in Bangladesh which was the key interest of this study to compare the black and green tea in respect to proximate value and antioxidant activity.

2. Methodology

2.1 Sample collection

Eight brands of black tea such as Ispahani, Taaza, Seylon, Finlays, Kazi & Kazi, Fresh, National tea and Tetley and two brands of green tea such as Finlays and

Kazi & Kazi were collected from the local super market at Sylhet city.

2.2 Methods for proximate analysis

Moisture content was determined according to association of official analytical chemists [9]. Ash content was determined according to Ranganna, S. [10]. Estimation of total protein was made by Kjeldahl method [11]. Fat was estimated as crude ether extract of the dry material and the carbohydrate content of tea was determined by difference method [11].

2.3 Determination of antioxidant properties

2.3.1. Extraction of tea sample

Tea sample was extracted with organic solvent methanol (80%) in a ratio of 3:1. The mixture of tea and methanol was shaken for 48 hours and then filtered and the procedure was repeated for three times. The filtrate sample was collected. The methanol was removed by rotary evaporator (R-205, Buchi, Switzerland). Finally freeze drier was used to find the solid extract which was used to determine the antioxidant activity. The method was adopted by ISO [12] with slight modification.

2.3.2. Total antioxidant activity determination

The total antioxidant activity of the extract was evaluated by the phospho-molybdenum assay method which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition. The extract (1 mg/ml, 0.3 ml) was allowed to mix up with 3.0 ml of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate in 4 : 2 : 4 ratio) and the reaction mixture was incubated at 950 C for 90 minutes. After cooling at room temperature, the absorbance of the solution was measured at 695 nm by using a double beam Scientific UV-Vis Spectrophotometer (Model, Analytic Jena Specord (205) against an appropriate blank. The antioxidant activity was expressed as the number of mg equivalents to ascorbic acid per gm of dry extract [13].

2.3.3. Ferrous ion (Fe++) chelating ability

The Ferrous chelating ability of the fractions was monitored by measuring the formation of the ferrous ion ferrozine complex. The Ferrous ions chelating ability of methanol extracts of tea was investigated according to method of Dinis, T.C. et al. [14]. Briefly, 5 ml of each extract solution at different concentrations was mixed with 0.1 ml solution of 2 mM $FeCl_2$.$4H_2O$ and 0.2 ml of 5 mM ferrozine solution. Then mixture was shaken vigorously by vortex mixture and left standing at room temperature for 10 minutes for proper reaction. After 10 minutes, absorbance was measured against a blank at 562 nm using a double beam Scientific UV-Vis Spectrophotometer (Model, Analytic Jena Specord 205).

The Fe++ ion chelating ability of different extractions of tea was measured by the inhibition of the ferrous ion-ferrozine complex formation using a standard curve for ascorbic acid. The percentage of inhibition of ferrous ion-ferrozine complex formation was calculated by the following formula:

$$\% \text{ of inhibition} = \frac{A_0 - A}{A_0} \times 100$$

Where, A0 is the absorbance of the control solution;
A is the absorbance of the sample solution containing tea extract.

2.4 Statistical Analysis

The experimental data were statistically analyzed by IBM SPSS Statistics version 20 statistical software. The results were expressed as Mean and data were statistically analyzed by one-way ANOVA, with the level of significance set at $p<0.05$. The mean values adjusted by Duncan's Multiple Range Test (DMRT) [15].

3. Results and discussion

Moisture, Protein, Fat, Carbohydrate and Ash content were studied to evaluate the nutritional properties and the antioxidant activities were determined by two complimentary methods viz. Total Antioxidant Activity and Ferrous Ion (Fe++) Chelating Ability Assay.

3.1 Proximate composition

The nutritional composition of the collected tea samples is summarized in Table 1. The range of moisture content of black tea was found 8.35% to 6.73%. The moisture content of green tea was found 7.55% for Kazi and Kazi and 8.29% for Finlays. The highest moisture content was found in Finlays green tea and the lowest moisture content was found in Seylon black tea. The average moisture content of black and green tea was found 7.64% and 7.92%. Statistically the variance between the moisture content of black and green tea were found insignificant ($p> 0.05$). In previous investigation, it was found that tea from Lackatoorah, Lungla and Malnicherra tea estates in Bangladesh had moisture content 5.26%, 7.52% and 6.38% respectively [16]. They also found 7.52%, 5.5%, 6.38% moisture content in tea from tea estates of India, Malawi and Indonesia respectively.

The average protein content of black and green tea was found 18.57% and 19.31% respectively, statistically which are not significant difference. It was observed that green tea contained 15-20% protein on dry weight basis[17] which is very similar to the present findings. Fat content of black tea ranged from 1.61% to 2.59% whereas green tea, Kazi and Kazi and Finlays contained 1.69% and 2.23% respectively. The mean value of fat of black and green tea was found 2.0327% and 1.9573% respectively. It was comparable to findings of [18] who found that the fat content of tea on dry weight basis lies

Table1. Proximate composition of black and green tea

Category of tea	Brand of tea	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Black Tea	Ispahani	$7.55^{b}\pm0.34$	$20.34^{e}\pm0.01$	$1.60^{a}\pm0.17$	$64.61^{a}\pm0.22$	$5.62^{ab}\pm0.30$
	Taaza	$7.41^{b}\pm0.06$	$18.55^{c}\pm0.09$	$1.64^{a}\pm0.24$	$66.55^{cb}\pm0.11$	$5.63^{ab}\pm0.07$
	Seylon	$6.73^{a}\pm0.15$	$18.52^{c}\pm0.30$	$2.42^{b}\pm0.30$	$65.56^{b}\pm0.11$	$5.72^{b}\pm0.22$
	Finlays	$8.35^{c}\pm0.29$	$17.35^{b}\pm0.11$	$1.63^{a}\pm0.23$	$67.53^{e}\pm0.06$	$5.57^{ab}\pm0.26$
	Kazi & Kazi	$8.32^{c}\pm0.22$	$16.49^{a}\pm0.17$	$1.70^{a}\pm0.26$	$67.56^{e}\pm0.18$	$5.42^{ab}\pm0.07$
	Fresh	$7.62^{b}\pm0.32$	$18.29^{c}\pm0.05$	$2.39^{b}\pm0.35$	$66.30^{c}\pm0.25$	$5.54^{ab}\pm0.14$
	National tea	$7.60^{b}\pm0.25$	$19.60^{d}\pm0.20$	$2.52^{b}\pm0.36$	$64.57^{a}\pm0.16$	$5.58^{ab}\pm0.13$
	Tetley	$7.55^{b}\pm0.28$	$19.38^{d}\pm0.07$	$2.35^{b}\pm0.38$	$65.31^{b}\pm0.45$	$5.55^{ab}\pm0.28$
Green Tea	Kazi & kazi	$7.55^{b}\pm0.30$	$18.41^{c}\pm0.30$	$1.68^{a}\pm0.30$	$66.72^{d}\pm0.29$	$5.74^{b}\pm0.15$
	Finlays	$8.29^{c}\pm0.14$	$20.20^{e}\pm0.16$	$2.23^{b}\pm0.19$	$64.34^{a}\pm0.20$	$5.27^{a}\pm0.69$

Values are expressed as mean ± SD (n=3), different letters denote significant difference at P< 0.05

between 1.5% to 3%. They studied on tea of Sri Lanka and Indonesia.

The average carbohydrate content of black and green tea was obtained 66.01% and 65.55% respectively. It was reported to have 63 to 67% carbohydrate in Indonesia tea [19]. These show the similarity of the present study. Black and green tea possessed on an average 5.58% and 5.53% ash respectively, statistically which does not differ significantly. The reason is that the black and green tea is made from same source, the difference comes from the manufacturing process which does not affect ash contents

3.2 Antioxidant activity

There are several methods of determination of antioxidant activity. Of those two methods were applied in the present study which is mentioned below.

3.2.1. Total antioxidant activity:

The total antioxidant activity is expressed as the number of equivalents in ascorbic acid. Total antioxidant capacity of the tea is summarized in the figure 1. The range of

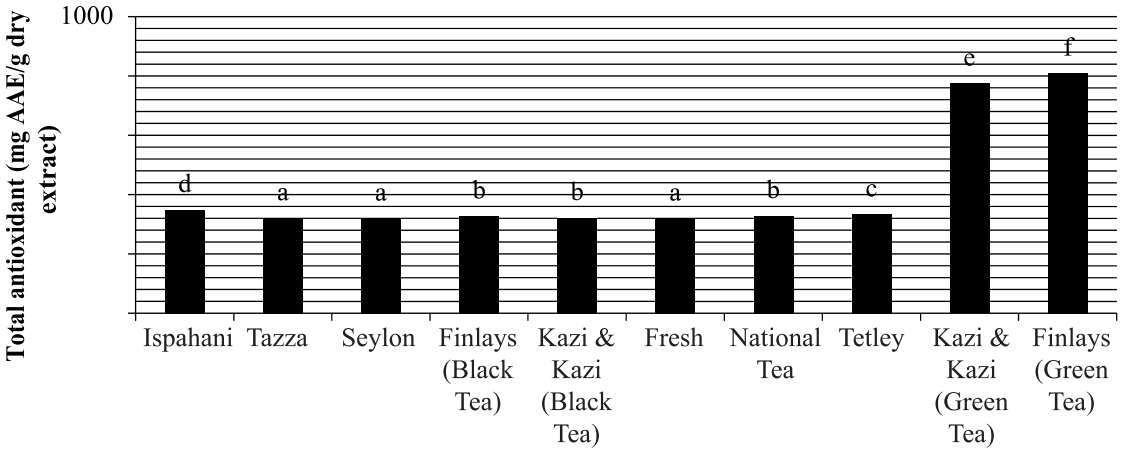

Fig.1. Total antioxidant activity of black and green tea (mg AAE/g dry extract)

Values are expressed as mean±SD (n=3), different letters denote significantly different at P< 0.05

total antioxidant capacity of black tea was found 320.27AAE to 348.93AAE. The total antioxidant activity of green tea was found 775.07 for Kazi and Kazi and 809.07AAE for Finlays. The highest total antioxidant capacity was found in Finlays green tea and the lowest total antioxidant capacity was found in Taaza black tea.

The average total antioxidant activity of black and green tea was found 330.72 AAE and 792.87 AAE respectively. Statistically the variance between the total antioxidant capacity of black and green tea were found significant. Total antioxidant levels were significantly higher in green tea in respect to black teas as reported in previous studies [20, 21]. In another study [22], it was found that total antioxidants of tea estimated by FRAP had widely different in vitro antioxidant power. Here also, total antioxidant capacity in green tea was significantly higher than in green tea ($p<0.05$). Various other studies have reported higher antioxidant activity in green tea than in black tea [23, 24]. From literature survey it is learnt that catechins in green tea impart antioxidant properties due to having three side by side hydroxyl (-OH) groups on β-rings as in the case of EGCG, EGC, GCG and GC that are more potential to scavenge free radicals than two side by side -OH much amount of EGCG and EGC compared to black tea [25]. Antioxidant properties are mainly attributed to black tea due to having thearubigins catechins, phenolic acids and theaflavins [26].

3.2.2. Ferrous chelating activity

The figure 2 presents that both black and green tea showed lesser chelating activity than the standard and green tea has lesser activity than that of black tea. The variance of percent of inhibition between black and green is statistically significant at concentration of 10 ppm, 100 ppm, 200 ppm, 400 ppm, and 600 ppm whereas insignificant at concentration of 20 ppm, 40 ppm and 80 ppm. The highest average percent inhibition for black tea was found 50.33 at 600 ppm & lowest 1.93 at 10 ppm and for green tea the highest inhibition was 32.14 at 600 ppm & lowest was 1.25 at 10 ppm. IC50 values for black and green tea were found to be 599.50 and 866.67 respectively.

Though green tea showed significantly higher total antioxidant activity, black tea had more ferrous ion chelating capability. Fe2+ chelating ability is dependent on degree of leaves fermentation. Green teas showed least metal chelating activity and this does not correlate with antioxidant activity, of course it correlates with the aflavins content which is mostly contained by black tea [27]. In all but the ferrous ion- chelating assays, ethanol extract of green tea exhibited the best antioxidant activity [28]. Ranking in ferrous ion-chelating ability was found like: Boh Bukit Cheeding black tea >Boh Cameron black tea > Sea Dyke green tea [29]. Higher amount of chelating ability might be attributed to black tea due to

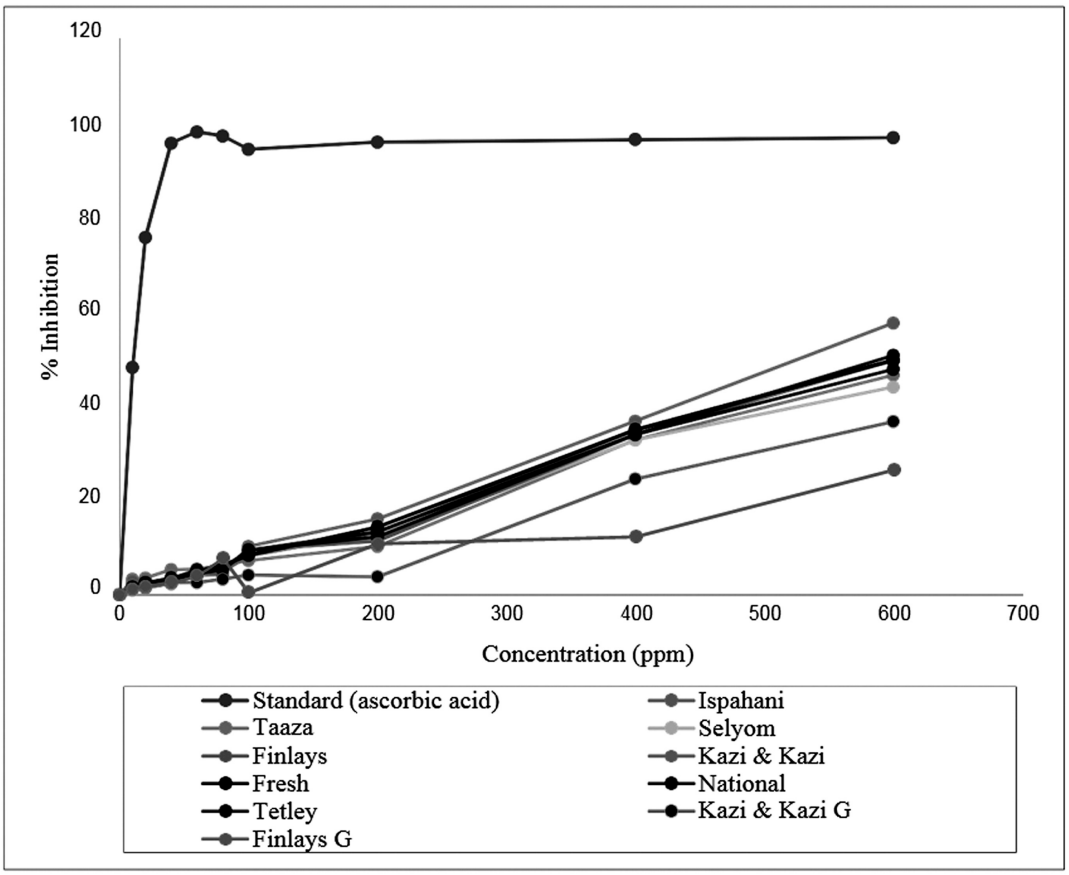

Fig.2. Comparison of ferrous ion chelating ability (% Inhibition) of black and green tea with standard

having large amount of oxidized polyphenols-theaflavins and thearubigenes and gallic acid that are produced during the oxidation process [30].

5. Conclusion

Ten brands of Bangladeshi black and green tea where compared in respect to nutritive value and antioxidant activity. The experiments were conducted in bioactive compounds laboratory, BCSIR, Dhaka and FET laboratory, SUST, Sylhet. The variances between proximate values of black and green tea were found insignificant. Green teas were found to have more antioxidant activity than black teas whereas black tea showed higher ferrous ion chelating ability than green teas. As the green teas exhibited antioxidant activity more than double than that of black tea, it is believed that this study will be a breakthrough to popularize the green tea consumption in Bangladesh.

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References:

1. Mukhtar, H. and N. Ahmad, Tea polyphenols: prevention of cancer and optimizing health. The American journal of clinical nutrition, 2000. 71(6): p. 1698s-1702s.
2. BTB. 2018, Bangladesh Tea Board (BTB), www.teaboard.gov.bd Bangladesh.
3. Graham, H.N., Tea, in Wiley Encyclopedia of Food Science and Technology. 1999, Frederick, J. F., Ed.; John Wiley & Sons: New York. p. 2292- 2305.
4. Balentine, D.A., S.A. Wiseman, and L.C. Bouwens, The chemistry of tea flavonoids. Critical Reviews in Food Science & Nutrition, 1997. 37(8): p. 693-704.
5. Chu, D. and L. Juneja, General chemical composition of green tea and its infusion. Chemistry and applications of green tea, 1997: p. 13-22.
6. Dias, T., et al., White tea (Camellia sinensis (L.)): antioxidant properties and beneficial health effects. Int J Food Sci Nutr Diet, 2013. 2(2): p. 19-26.
7. Vastag, B., Tea therapy? Out of the cup, into the lab. 1998, Oxford University Press.
8. Cao, G., E. Sofic, and R.L. Prior, Antioxidant capacity of tea and common vegetables. Journal of agricultural and food chemistry, 1996. 44(11): p. 3426-3431.
9. AOAC, Official Methods of Analysis. 15 ed. 1990, Artington Virginia, U.S.A: Association of Official Analytical Chemists. 1094.
10. Ranganna, S., Handbook of analysis and quality control for fruit and vegetable products. 1986: Tata McGraw-Hill Education.
11. AOAC, Official Methods of Analysis. 18 ed. Vol. 1. 2005, Gaithersburg, Maryland, U.S.A: Association of Official Analytical Chemists.
12. ISO, I., 14502-2 Determination of substances characteristic of green and black tea-Part2: Content of catechins in green tea-Method using high-performance liquid chromatography. 2005, IHS.
13. Prieto, P., M. Pineda, and M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry, 1999. 269(2): p. 337- 341.
14. Dinis, T.C., V.M. Madeira, and L.M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of biochemistry and biophysics, 1994. 315(1): p. 161-169.
15. Duncan, D.B., Multiple range and multiple F tests. Biometrics, 1955. 11(1): p. 1-42.
16. Iftekhar Ahmad, A.Z.M.S.M., Farjana Begum Sumi, Mohammad Afzal Hossain and Md. Mozammel Hoque., Physico-Chemical Characteristics and Assessment of Cup Quality of Tea Collected from Various Tea Estates of Different Countries. Journal of applied science and technology, 2013. 09(01): p. 107-115.
17. Cabrera, C., R. Artacho, and R. Giménez, Beneficial effects of green tea-a review. Journal of the American College of Nutrition, 2006. 25(2): p. 79-99.
18. Mohammed, M. and M. Sulaiman, Proximate, caffeine and tannin analyses in some brands of tea consumed in Kano Metropolis, Nigeria. Bayero Journal of Pure and Applied Sciences, 2009. 2(2): p. 19-21.
19. de Mejia, E.G., M.V. Ramirez-Mares, and S. Puangpraphant, Bioactive components of tea: cancer, inflammation and behavior. Brain, behavior, and immunity, 2009. 23(6): p. 721-731.
20. Dutta, A.K., et al., Finlay green tea possesses the highest in vitro antioxidant activity among the 20 commercially available tea brands of Bangladesh. Malaysian Journal of Pharmaceutical Sciences, 2013. 11(2): p. 11.
21. Su, X., et al., Polyphenolic profile and antioxidant activities of oolong tea infusion under various steeping conditions. International journal of molecular sciences, 2007. 8(12): p. 1196-1205.

22. Benzie, I.F. and Y. Szeto, Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of agricultural and food chemistry, 1999. 47(2): p. 633-636.
23. Chan, E., et al., Antioxidant properties of tropical and temperate herbal teas. Journal of Food Composition and Analysis, 2010. 23(2): p. 185- 189.
24. Yen, G.-C. and H.-Y. Chen, Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry, 1995. 43(1): p. 27-32.
25. Almajano, M.P., et al., Antioxidant and antimicrobial activities of tea infusions. Food chemistry, 2008. 108(1): p. 55-63.
26. Miller, N.J., et al., The antioxidant properties of theaflavins and their gallate esters-radical scavengers or metal chelators? FEBS letters, 1996. 392(1): p. 40-44.
27. Carloni, P., et al., Antioxidant activity of white, green and black tea obtained from the same tea cultivar. Food research international, 2013. 53(2): p. 900-908.
28. Oh, J., et al., Antioxidant and antimicrobial activities of various leafy herbal teas. Food control, 2013. 31(2): p. 403-409.
29. Chan, E.W.C., Y.Y. Lim, and Y. Chew, Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Food chemistry, 2007. 102(4): p. 1214-1222.
30. Gramza, A., et al., Tea extracts influence on catalytical properties of Fe2plus in lipids. Polish Journal of Environmental Studies, 2004(13).