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PURIFICATION PROCESS INFLUENCE ON GREEN TEA EXTRACTS' POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY

Anna Gramza-Michałowska, Józef Korczak, Marzanna Hęś
Agricultural University of Poznań

Abstract. The research examined green tea ethanol extract, subjected to different purification processes with use of active carbon, bleaching earth, and mixture of acetone, acetic acid, water, with or without earlier hexane treatment. Purified extracts were examined according to total polyphenols content, antioxidant activity in linoleic acid emulsion and antiradical activity in DPPH$^{\bullet}$ radical scavenging method. Highest polyphenol content was evaluated in the extract purified with bleaching earth, lowest however in purified with solvents mixture. Antioxidant activity of green tea extracts in linoleic acid emulsion indicated that highest antioxidative effectivity coefficient (Aec) values represented sample purified with active carbon and solvents mixture. Highest DPPH$^{\bullet}$ radical scavenging activity was found in sample purified with solvents mixture, other samples however represented close activity. The present research indicated that plant extracts purification processes resulted in decrease of total polyphenols content, however without its antioxidant activity decrease.

Key words: green tea, *Camellia sinensis*, ethanol extract, antioxidant activity, linoleic acid emulsion, polyphenols, antiradical activity, DPPH$^{\bullet}$, purification

INTRODUCTION

For several years there have been growing interests in antioxidants present in plant products. A lot of investigations turned to examinations of tea leaves *Camellia sinensis* L. It was found that tea leaves consist of strong antioxidative compounds, polyphenols – flavonoids. Main green tea leaves polyphenols are catechins: (+)-catechin C, (-)-epicatechin EC, (+)-gallocatechin GC, (-)-epigallocatechin EGC, (-)-epicatechin gallate ECG, (-)-epigallocatechin gallate EGCG [Balentine et al. 1997, Gramza and Korczak 2005, Gramza-Michałowska and Bajerska-Jarzębowska 2007].

Green teas infusion pharmacological proprieties had been well-known for centuries, confirmed by the last year's investigations [Ramarathnam et al. 1995, Sato and Miyata 2000, Yang and Landau 2000, McKay and Blumberg 2002, Wu and Wei 2002]. Ac-

Corresponding author – Adres do korespondencji: Dr inż. Anna Gramza-Michałowska, Department of Food Technology and Human Nutrition of Agricultural University of Poznań, Wojska Polskiego 31, 60-624 Poznań, Poland, e-mail: angramza@au.poznan.pl

cording to tea polyphenols activity in scavenging superoxide radicals it was found that tea brewing could be tool in oxidative stress related diseases prevention [Halliwell et al. 1995, Unno et al. 2000].

Lipids undergo the oxidation processes, causing a sequence of unfavorable changes, like color and texture changes, rancidity and nutritious value decrease [Frankel 1998]. High antioxidative activity in lipids and lipid emulsions and antiradical activity of tea extracts were presented by many research laboratories [Wanasundara and Shahidi 1996, Koketsu and Satoh 1997, Chen et al. 1996, Gramza et al. 2006]. There are many limitations in using plant origin extracts [Houlihan and Ho 1985, Gramza and Korczak 2005]. Plant extracts usually impart intensive color; carry specific taste and aroma and posses low solubility in fat, temperature and light resistance. That is why the present research aimed to evaluate the purification processes influence on green tea extracts' total polyphenol content and antioxidant activity in selected model systems.

MATERIAL AND METHODS

Reagents

All chemicals used were analytical grade: ethanol, methanol, acetone, acetic acid, hexane [POCH], active carbon [Merck], bleaching earth [Sigma], 2,2-diphenyl-1-picrylhydrazyl DPPH [Sigma-Aldrich], Folin Ciocalteu reagent [Fluka], catechin [Sigma].

Plant material

Yunan green tea leaves were bought at special tea store. Ethanol extract was prepared according to Gramza et al. [2006]. Extracts were prepared by triplicate 24 hours maceration of grinded tea leaves (100 g) with 250 ml of 95% ethanol at ambient conditions. Collected extracts were filtered and centrifuged (4500 rpm, 15 min), ethanol was evaporated on rotary evaporator (RVO 200A, INGOS). The powdered ethanol extract was kept frozen (–18°C) until further use. On the basis of the earlier experiments on green tea extract antioxidative activity, the concentration of 1000 ppm was chosen for the further research. The range of extracts concentration was determined experimentally. Obtained extract was purified with use of four different methods. First two methods based on extracts treatment with active carbon or bleaching earth, filtration and drying [Chen et al. 1992]. Other two methods based on extracts treatment with acetone, water and acetic acid in proportion respectively: 70:29.8:0.2 afterwards the mixture was heated, filtered and evaporated on rotary evaporator [Wollgast et al. 2001]. One part of the samples was initially treated with hexane, and afterwards the purifying process with solubilizers' mixture continued.

Total polyphenol content

The levels of total polyphenols in purified extracts were determined according to method by Horwitz [1970]. The results were expressed as catechin equivalents in $mg \cdot g^{-1}$ of the dry extract. Standard concentrations of (+)-catechin between 0-600 $\mu g \cdot ml^{-1}$ were used to prepare the calibration curve.

Lipid substrates and antioxidants

Purified green tea extracts antioxidant activity was examined in 10 mM emulsion of linoleic acid [Lingnert et al. 1979]. Emulsions were freshly prepared in: phosphate buffer (pH 7.0) with Tween 20 (Sigma) and incubated in darkness in temperature of 37°C for 19 hours. Samples were compared with that of control sample. Oxidation stage of the emulsion was examined by conjugated linoleic acid dienes content measurement (CLA), and expressed as antioxidative effectivity coefficient Aec – the ratio of difference between conjugated dienes content increase in emulsion sample with no antioxidants added and conjugated dienes content increase in the sample with additives, to conjugated dienes content increase in emulsion sample with no antioxidants. Results expressed as Aec > 0 represents antioxidative properties, Aec < 0 represents prooxidative properties of a substrate.

DPPH$^{\bullet}$ radical scavenging method

Antiradical activity of purified extracts was measured according to method by Sanchez-Moreno et al. [1998]. An aliquot of ethanol (0.1 ml), solution containing extracts concentration of 1000 ppm was added to 3.9 ml of DPPH$^{\bullet}$ 0.025 g·litre^{-1} in ethanol prepared daily. The decrease in absorbance at 515 nm was measured after 30 min on a Carl Zeiss Spectrophotometer (Jena Optik). Ethanol was used to zero the spectrophotometer. DPPH$^{\bullet}$ stock solution was stored at 4°C until it was used. The absorbance decrease is connected with the radical scavenging ability by the antioxidants contained in the extracts. The faster the absorption decreases, the stronger antioxidant, possessing higher ability of hydrogen donation. The range of extracts concentrations and measurements frequency were established experimentally. The absorbance measurements also were taken in time intervals (2 min), until reaction reached plateau [Gramza et al. 2005]. The percentage of remaining DPPH$^{\bullet}$ was plotted to obtain the amount of antioxidant needed to decrease the initial radicals concentration by 50%. The time needed to reach the steady state to EC_{50} concentration (T_{EC50}) was calculated graphically. Lower T_{EC50} and EC_{50} value proves the higher antioxidant ability of studied substrate.

Statistical analysis

The results were obtained from a minimum of six independent experiments and averaged. Data were analysed by the analysis of variance ($p \leq 0.05$) to estimate the differences between values of compounds tested. Results were processed by the computer program Statistica 6.0.

RESULTS

There are many limitations in plant origin extracts usage. Plant extracts are usually colored, carrying specific taste and aroma. To purify the Camellia sinensis extracts different solvents have been used: an acetone, acetic acid and water, additionally one sample was treated with hexane before the extraction. Other two samples were purified with use of bleaching earth and active carbon. In purified extracts total polyphenol con-

tent was measured. Table 1 represents results of total polyphenols content in purified extracts. It was found that purification process decreased, for almost 50% the initial green tea ethanol extracts polyphenols content. The highest losses were found in the sample purified with solvents after hexane treatment (357 $mg \cdot g^{-1}$ extracts dry weight) as compared to extract not purified (837 $mg \cdot g^{-1}$ extracts dry weight). Sample of extract purified with bleaching earth was characterized by the highest content of total polyphenols (442 $mg \cdot g^{-1}$ extracts dry weight). Results suggested that during the green tea extract purification process, a large amount of polyphenols could be removed. Purification with use of chemicals like active carbon or bleaching earth could be better than the other examined methods because of lower polyphenols losses.

Table 1. Total polyphenol content in purified green tea ethanol extracts
Tabela 1. Zawartość polifenoli ogółem w oczyszczonych ekstraktach herbaty zielonej

Green tea extract Ekstrakt herbaty zielonej 1000 ppm	Total polyphenol content $mg \cdot g^{-1}$ dry weight Zawartość polifenoli ogółem $mg \cdot g^{-1}$ s.m.
Purified with active carbon Oczyszczony za pomocą węgla aktywnego	422 ± 0.01 a
Purified with bleaching earth Oczyszczony za pomocą ziemi bielącej	442 ± 0.01 a
Purified with solvents mixture after hexane treatment Oczyszczony za pomocą mieszaniny rozpuszczalników oraz heksanu	357 ± 0.01 b
Purified with solvents mixture Oczyszczony za pomocą mieszaniny rozpuszczalników	377 ± 0.01 b
Not purified Nieoczyszczony	837 ± 6.03 c

Results presented as mean values of three replicates. Values followed by different letters significantly differ at $\alpha < 0.05$.

Wyniki przedstawiono jako średnią z trzech niezależnych powtórzeń. Wartości oznaczone innymi literami różnią się istotnie na poziomie $\alpha < 0{,}05$.

Next step of the research aimed at purified extracts antioxidant activity evaluation. Results were presented as the antioxidative efficiency coefficient (Aec) in linoleic acid emulsion. It was found that all samples showed similar activity in linoleic acid emulsion (Table 2). Among purified green tea extracts lowest Aec however was found in the extract purified with solvents mixture of acetone, acetic acid, water and hexane (Aec = 0.40, $p < 0.05$). Results showed that apart from large quantities of removed polyphenols purified tea extracts activity was still high, suggesting that not all strong antioxidants could be removed.

Evaluation of antiradical activity of purified extracts showed that all extracts presented high radicals scavenging activity (Table 3). Highest scavenged radicals' content was found in sample purified with solvents mixture with earlier hexane treatment (95.0%). Other samples were nearly active; accept for sample purified with solvents mixture. Research with $DPPH^{\cdot}$ radical showed that the time needed to scavenge 50% of remaining radicals (T_{EC50}) was significantly shorter in sample of extract purified with

Table 2. Antioxidative effectivity coefficient (Aec) of purified green tea ethanol extracts
Tabela 2. Współczynnik efektywności przeciwutleniającej oczyszczonych etanolowych ekstraktów herbaty zielonej

Green tea extract Ekstrakt herbaty zielonej 1000 ppm	Extracts Aec in linoleic acid emulsion Aec ekstraktów w emulsji kwasu linolowego
Purified with active carbon Oczyszczony za pomocą węgla aktywnego	0.60 ± 0.01 b
Purified with bleaching earth Oczyszczony za pomocą ziemi bielącej	0.59 ± 0.02 b
Purified with solvents mixture after hexane treatment Oczyszczony za pomocą mieszaniny rozpuszczalników oraz heksanu	0.40 ± 0.12 a
Purified with solvents mixture Oczyszczony za pomocą mieszaniny rozpuszczalników	0.62 ± 0.01 b
Not purified Nieoczyszczony	0.60 ± 0.01 b

Results presented as mean values of three replicates. Values followed by different letters significantly differ at $\alpha < 0.05$.

Wyniki przedstawiono jako średnią z trzech niezależnych powtórzeń. Wartości oznaczone innymi literami różnią się istotnie na poziomie $\alpha < 0{,}05$.

Table 3. DPPH scavenging activity of purified green tea ethanol extracts
Tabela 3. Zdolność zmiatania rodnika DPPH przez oczyszczone etanolowe ekstrakty herbaty zielonej

Green tea extract Ekstrakt herbaty zielonej 1000 ppm	DPPH$^{\bullet}$ radical scavenging activity, AA% Zdolność zmiatania rodnika DPPH$^{\bullet}$, AA%	T_{EC50} [min]
Purified with active carbon Oczyszczony za pomocą węgla aktywnego	95.5 ± 0.24 ab	1.3
Purified with bleaching earth Oczyszczony za pomocą ziemi bielącej	95.7 ± 0.24 ab	1.75
Purified with solvents mixture after hexane treatment Oczyszczony za pomocą mieszaniny rozpuszczalników oraz heksanu	95.0 ± 0.52 a	1.8
Purified with solvents mixture Oczyszczony za pomocą mieszaniny rozpuszczalników	96.2 ± 0.52 b	1.4
Not purified Nieoczyszczony	95.4 ± 0.12 ab	1.6

Results presented as mean values of three replicates. Values followed by different letters significantly differ at $\alpha < 0.05$.

Wyniki przedstawiono jako średnią z trzech niezależnych powtórzeń. Wartości oznaczone innymi literami różnią się istotnie na poziomie $\alpha < 0{,}05$.

active carbon (1.3 min) and solvents mixture (1.4 min). Other samples needed longer time to scavenge 50% of radicals in the examined system.

DISCUSSION

There are no many data on fractionation of tea leaves or extracts. Zadernowski et al. [1995] treated plant seeds with water and butanol receiving two fractions. One of them consisted of phenolic acids and its derivatives, in aqueous phase however catechins, procyanidines and flavonols were present. Other research of Xie et al. [1993] examined the tea extracts divided into a chloroform, ethyl acetate and butanol. Similarly to Sava et al. [2001] they found that oxidized polyphenols are insoluble in water and organic solvents (ethanol, hexane, acetone, chloroform). Those substances are soluble in alkali and are similar to melanin.

According to present research it was found that purification process did remove almost half of polyphenols content. Its quality and quantity will be the subject of other publication. Apart from total polyphenol content, activity of purified extracts was not influenced. It could be the effect of different solvents and chemicals used, helping to clean the extracts, but removing probably weakest antioxidants, high molecular weight, leaving the low molecular substances presenting strong antioxidative activity in the examined conditions.

CONCLUSIONS

1. Green tea extracts purification resulted in 50% decrease of total polyphenol content in the examined samples. The greatest amount of total polyphenols was determined in the extracts purified with active carbon and bleaching earth.

2. The research showed that apart from large quantities of removed polyphenols purified tea extracts activity was high, suggesting that not all strong antioxidants could be removed.

3. The best radicals scavenging ability of $DPPH^{\bullet}$ was found in the extract purified with solvents mixture, treated with hexane.

4. In the analysed samples Tec50 was shorter in extracts purified with active carbon and solvents mixture. Other samples needed longer time to scavenge 50% of radicals in the examined system.

5. On the basis of the obtained results it was found that purification processes contributed to total polyphenol content decrease, without its antioxidant activity decrease.

REFERENCES

Balentine D.A., Wiseman S.A., Bouwens L.C.M., 1997. The chemistry of tea flavonoids. Crit. Rev. Food Sci. Nutr. 3, 693-704.

Chen Q., Shi H., Ho C.T., 1992. Effects of Rosemary extracts and major constituents on lipid oxidation and soybean lipoxygenase activity. JAOCS 69, 10, 999-1002.

Chen Z.Y., Chan P.T., Ma H.M., Fung K.P., Wang J., 1996. Antioxidative effect of ethanol tea extracts on oxidation of canola oil. JAOCS 73, 375-380.

Frankel E.N., 1998. Lipid oxidation. The Oily Press, Scotland.

Gramza A., Khokhar S., Yoko S., Gliszczynska-Swiglo A., Hes M., Korczak J., 2006. Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. Eur. J. Lipid Sci. Tech. 108, 4, 351-362.

Gramza A., Korczak J., 2005. Tea constituents (*Camellia sinensis* L.) as antioxidants in lipid systems. Trends Food Sci. Tech. 16, 351-358.

Gramza A., Pawlak-Lemańska K., Korczak J., Wąsowicz E., Rudzińska M., 2005. Tea extracts as free radical scavengers. Pol. J. Env. Stud. 14, 6, 153-157.

Gramza-Michałowska A., Bajerska-Jarzębowska J., 2007. Leaves of *Camellia sinensis*: Ordinary brewing plant or super antioxidant source? Food 1, 56-64.

Halliwell B., Murcia M.A., Chirico S., Auroma O.I., 1995. Free radicals and antioxidants in food and in vivo: what they do and how they work? Crit. Rev. Food Sci. Nutr. 35, 7-20.

Houlihan C.M., Ho C.T., 1985. Natural antioxidants. In: Flavor chemistry of fats and oils. Eds D.B. Min, T.H. Smouse. AOCS, Champaign, IL: 117-143.

Horwitz W., 1970. Official methods of analysis of the association of Official Analytical Chemists (AOAC). 10 Ausgabe Washington, 15.049-15.055.

Koketsu M., Satoh Y.I., 1997. Antioxidative activity of green tea polyphenols in edible oils. J. Food Lipid. 4, 1-9.

Lingnert H., Vallentin K., Eriksson C.E., 1979. Measurement of antioxidative effect in model system. J. Food Proc. Preserv. 3, 87.

McKay D.L., Blumberg J., 2002. The role of tea in human health: an update. J. Am. Coll. Nutr. 21, 1-13.

Ramarathnam N., Osawa T., Ochi H., Kawakishi S., 1995. The contribution of plant antioxidants to human health. Trends Food Sci. Tech. 3, 75-82.

Sanchez-Moreno C., Larrauri J.A., Saura-Calixto F., 1998. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. 76, 270-276.

Sato T., Miyata G., 2000. The nutraceutical benefit. Part I: green tea. Nutr. Pharm. 16, 315-317.

Sava V.M., Yang S.M., Hong M.Y., Yang P.C., Huang G.S., 2001. Isolation and characterization of melanic pigments derived from tea and tea polyphenols. Food Chem. 73, 177-184.

Unno T., Sugimoto A., Kakuda T., 2000. Scavenging effect of tea catechins and their epimers on superoxide anion radicals generated by a hypoxantine ans xantine oxidase system. J. Sci. Food Agric. 80, 601-606.

Wanasundara U.N., Shahidi F., 1996. Stabilization of seal blubber and menhaden oils with green tea. J. Am. Oil Chem. Soc. 73, 1183-1190.

Wollgast J., Pallaroni L., Agazzi M.E., Anklam E., 2001. Analysis of procyanidins in chocolate by reversed-phase high-performance liquid chromatography with electrospray ionisation mass spectrometric and tandem mass spectrometric detection. J. Chromat. A, 926, 211-220.

Wu C.D., Wei G.X., 2002. Teas as a functional food oral health. Nutr. Oral Health 18, 443-444.

Xie B., Shi H., Chen Q., Ho C.T., 1993. Antioxidant properties of fractions and polyphenol constituents from green, oolong and black teas. In: Proc. Natl. Counc. Repub. China B, 17, 77-84.

Yang C.S., Landau J.M., 2000. Effects of tea consumption on nutrition and health. J. Nutr. 130, 2409-2412.

Zadernowski R., Nowak-Polakowska H, Lossow B., 1995. Naturalne przeciwutleniacze tłuszczu w nasionach wybranych gatunków roślin [Natural lipid antioxidants in selected plants seeds]. Acta Acedem. 107-118.

WPŁYW PROCESÓW OCZYSZCZANIA NA AKTYWNOŚĆ PRZECIWUTLENIAJĄCĄ EKSTRAKTU ZIELONEJ HERBATY

Streszczenie. Przedmiotem badań był ekstrakt etanolowy herbaty zielonej, poddany różnym procesom oczyszczania: za pomocą węgla aktywnego, ziemi bielącej, mieszaniny wody, acetonu i kwasu octowego przed i po uprzednim odtłuszczeniu próby heksanem. W otrzymanych ekstraktach oznaczono eksperymentalnie zawartość polifenoli ogółem, aktywność przeciwutleniającą w układzie zemulgowanego kwasu linolowego oraz zdolność zmiatania rodnika DPPH$^{\bullet}$. Największą zawartością polifenoli ogółem charakteryzował się ekstrakt oczyszczony z użyciem ziemi bielącej, natomiast najmniejszą – z wykorzystaniem mieszaniny rozpuszczalników. W dalszym etapie badań określono aktywność przeciwutleniającą ekstraktów w układzie zemulgowanego kwasu linolowego, gdzie najwyższym współczynnikiem ochronnym, a tym samym największą aktywnością przeciwutleniającą, charakteryzował się ekstrakt oczyszczony za pomocą węgla aktywnego oraz mieszaniny rozpuszczalników. W układach z wolnym rodnikiem DPPH$^{\bullet}$ największe zdolności zmiatania tego rodnika stwierdzono dla prób oczyszczonych z użyciem mieszaniny rozpuszczalników, pozostałe próby wykazywały zbliżoną aktywność. Na podstawie badań stwierdzono, że procesy oczyszczania ekstraktów roślinnych przyczyniły się do zmniejszenia zawartości polifenoli ogółem, jednakże bez obniżenia ich aktywności przeciwutleniającej.

Słowa kluczowe: zielona herbata, ekstrakt etanolowy, aktywność przeciwutleniająca, kwas linolowy, polifenole, aktywność przeciwrodnikowa, DPPH$^{\bullet}$, oczyszczanie

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