ACTA SCIENTIARUM POLONORUM Acta Sci. Pol., Technol. Aliment. 6(1) 2007, 27-36

THE INFLUENCE OF TIME AND TYPE OF SOLVENT ON EFFICIENCY OF THE EXTRACTION OF POLYPHENOLS FROM GREEN TEA AND ANTIOXIDANT PROPERTIES OBTAINED EXTRACTS

Beata Drużyńska, Agnieszka Stępniewska, Rafał Wołosiak
Warsaw Agricultural University SGGW

Abstract. The aim of the study was to investigate the influence of the type of solvent and time on efficiency of the extraction of polyphenols and antioxidant properties extracts obtained from green tea. Extraction was conducted at room temperature using four solvents: water and 80% ethanol, 80% methanol and 80% acetone (water solutions, v/v) at 15, 30 and 60 minutes. Extracts were analysed for contents of polyphenols and catechins. The antioxidant properties have been determined by two methods: scavenging activity against $DPPH^{\cdot}$ and the method with $ABTS^{\cdot+}$. The abilities of extracts to chelate iron ions (II) have been investigated too. On the basis of findings it turned out that both type of solvent and time have a significant influence on extraction of polyphenols from green tea. The best solvent for the extraction of total polyphenols was 80% acetone, whereas for catechins was water. The increase of extraction of polyphenols with prolonged extraction time was observed. All extracts had antioxidant properties against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ and abilities to chelate iron ions (II).

Key words: efficiency of extraction, polyphenols, green tea, antioxidant properties

INTRODUCTION

Polyphenol compounds occurring commonly in the world of plants have drawn the attention of researchers in recent years. Polyphenols are a group of compounds synthesized exclusively by plants, especially for the protection against UV-radiation and activity of pathogens. About 8000 plant polyphenol compounds have been identified so far whereas only some hundred occur in edible plants. They are present in fruits, flowers, leafs, roots, and woody parts of plants, whereas external tissues include bigger amounts of these components [Manach et al. 2004, Oszmiański 1995]. Their antioxidant properties are of main interest due to potential application of polyphenol preparates in food technology for the protection of easy oxidizing food components as well as in the ther-

Corresponding author – Adres do korespondencji: Dr inż. Beata Drużyńska, Department of Biotechnology, Microbiology and Food Evaluation of Warsaw Agricultural University SGGW, Nowoursynowska 159 C, 02-787 Warsaw, Poland, e-mail: beata_druzynska@sggw.pl

apy of many diseases at the bottom of which lies disordered balance of oxidizing processes in the body [Scalbert et al. 2005, Robinson 2001]. Properties of polyphenols are subject of many studies but a problem of effective output of these compounds is not discussed too often in spite of the fact that this stage of analytic conduct is most important at any types of quantitative marking and it is a way out to conclude on the features of polyphenol compounds and any possible application of their preparations in various industry branches.

The extraction of polyphenols is difficult for two main reasons: first, these compounds are extremely different in relation to their structure – they may occur in plant tissues combined with sugars, proteins or they may create polymerized derivatives of various dissolubility. Their chemical structure and interactions with other food components are not fully known and it is a very important information at the selection of a solvent and terms and conditions of extraction. Second, polyphenols are susceptible to oxidation, furthermore high temperature and alkaline environment cause their degradation, thus the extraction process and further stages of the preparation of a trial to specify the contents of polyphenols are subject of great caution [Tura and Robards 2002].

In spite of the development of new extraction techniques, classic extraction dominates in many laboratories mainly due to its simplicity and low economic outlay. The efficiency of the process can be widely regulated here by the selection of suitable solvents and application of possibly effective terms and conditions of extraction under special pressure on its duration and applied temperature.

This work focuses on the research of the efficiency of polyphenols' extraction with the application of solvents commonly used in the laboratory practice (ethanol, methanol, acetone, water) paying special attention to the duration of the process. Observations were made on the example of green tea – a product that due to its high contents of polyphenol compounds of strong antioxidative properties has been of high interest of scientists for several years.

MATERIAL AND METHODS

The research material was green tea commonly available on the market "Gun Powder" from Bio-Active. The firm purchases the product from the biggest supplier of tea from China-China Tea Co. Ltd.

A preparation with polyphenols was made as follows: 5 g tea added in turn for 100 ml extraction solutions (water, 80% ethanol, 80% methanol and 80% acetone – water solutions, v/v) [Perva-Uzunalic et al. 2006]. Extractions were performed on a WL-1 shaker at room temperature for 15, 30 and 60 minutes in each of extraction solutions. Liquid from the deposit was poured off to conical butt with a cork and stored in freezing temperatures (–18°C) in conditions of limited air and light access. Extracts were used for marking last two weeks. Water extracts, from the point of view of capability of loss of polyphenols during the storage were prepared on the day of the research.

Chemical characteristic of the received extracts included determination of contents of total polyphenols (result calculated into gallic acid) [Singleton and Rossi 1965] and total catechins (result calculated into (–)epicatechin) [Sun et al. 1998]. Antioxidant properties were examined to stable and synthetic DPPH radicals and cation radicals $ABTS^{\bullet +}$.

The DPPH method consisted in adding antiradical compounds to a methanol solution of DPPH which in a radical form shows the absorbance at 517 nm. Value of this absorbance drops after the addition of an antiradical compound [Song et al. 1999].

In this study antioxidant activity is also researched against synthetic radicals $ABTS^{\bullet +}$. The research was performed according to modified method described by Re et al. [1999]. Cation-radicals ABTS were generated as a result of ABTS oxidation by potassium persulfate. The addition of antioxidant resulted in the reduction of ABTS and drop of the colour intensiveness of ABTS solution. The reduction level of ABTS was determined by a spectrophotometric method. The ABTS solution revealed maximum absorbance at 734 nm, the addition of the antiradical compound resulted in the drop of the absorbance.

The ability to chelate Fe(II) by green tea extracts was also determined. The ability to Fe(II) chelate by polyphenols was tested in extracts of polyphenols with the addition of iron chloride (II) and ferrozine. The absorbance of a colourful complex was measured 10 minutes after the addition of ferrozine at the wave length of 562 nm in spectrophotometer Shimadzu UV-160A [Lai et al. 2001].

All markings were produced in at least three replications. Values of average and standard deviations were calculated with the use of the program Microsoft Office Excel 2003. Statistical analysis of two-agent experience and correlation indexes were calculated with the help of the program STATGRAPHICS Plus 4.1.

RESULTS AND DISCUSSION

Most efficient extractor of polyphenols among the examined solvents seemed to be 80% acetone solution (Fig. 1). A fifteen minute long extraction of tea with this solvent enabled higher output of polyphenol compounds (9.80 g/100 g d.m. tea) than an hour long extraction with other solutions (for water this output amounted to 9.21 g, for 80% ethanol 6.85 g and for 80% methanol 7.09 g/100 g of dry mass tea). Water extracted polyphenols better than ethanol and methanol solutions.

Turkmen et al. [2006], while examining the efficiency of various solvents in the output of polyphenols from black tea also stated high extraction ability of 80% acetone solution, but water for the terms of the process applied by the researchers extracted polyphenols weaker than alcohol solutions. Baraniak et al. [2002] examined simultaneously the influence of a solvent type and extraction time for the output of polyphenols from a cauliflower and found that the greatest amount of them was included in the extract obtained as a result of a 30-minute long shaking of material with 80% ethanol solution. In case of acetone solvent, maximum amount of phenol compounds was isolated already after 15-minute extraction but only in case of the application of 80% methanol the level of these compounds in extracts increased with the prolongation of the duration time of the process.

Figure 2 presents the influence of extraction conditions on the output of catechins from tea. The greatest content of catechins was evidenced by shaking tea with water – a 15 minute long extraction gave better results (7.28 g/100 g dry mass tea) and proved to be less effective only than a 60 minute long extraction with 80% acetone (7.75 g/100 g dry mass of tea).

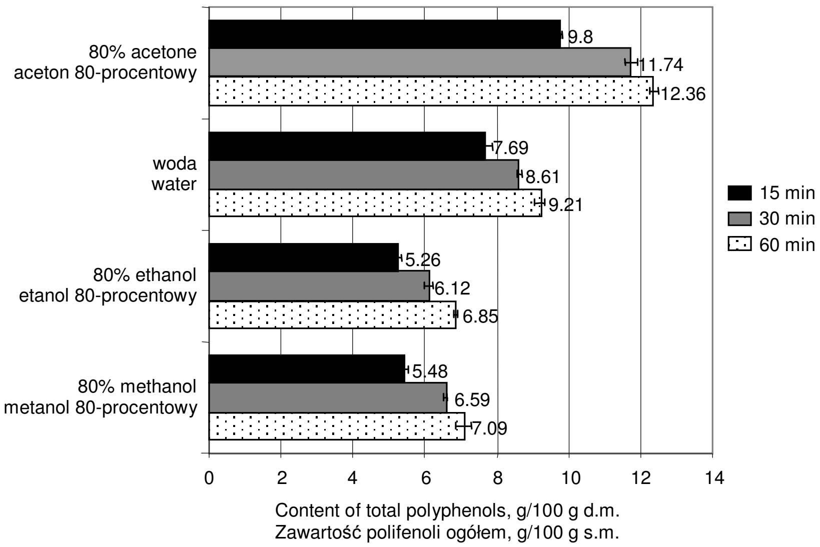

Fig. 1. Content of total polyphenols in green tea extracts depending on solvent and extraction time
Rys. 1. Zawartość polifenoli ogółem w herbacie zielonej zależnie od użytego ekstrahenta i czasu ekstrakcji

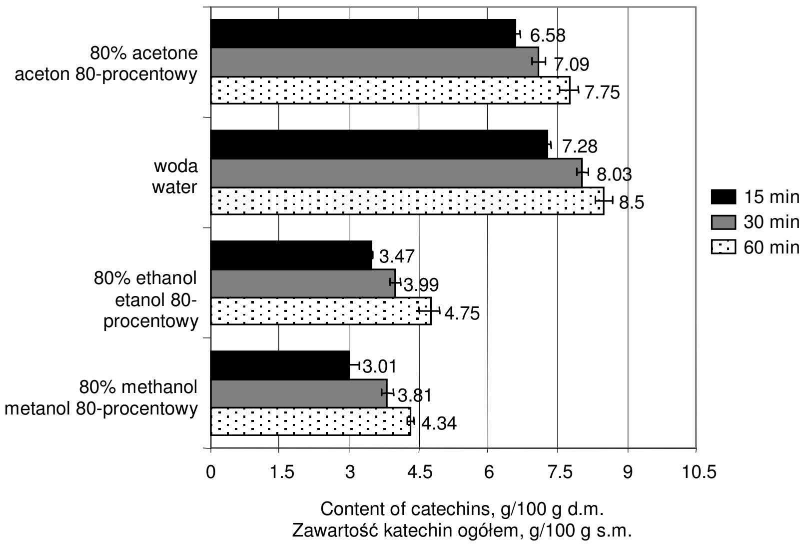

Fig. 2. Content of catechins in green tea extracts depending on solvent and extraction time
Rys. 2. Zawartość katechin w herbacie zielonej zależnie od użytego rozpuszczalnika i czasu ekstrakcji

Perva-Uzunalic et al. [2006] who have dealt with the research of extraction efficiency of catechins from green tea stated that an 80% acetone solution acts more effectively (95.2% of all catechins present in tea came to the solution) than 80% ethanol (recovery of catechins at the level of 89.1%) and 80% (output of catechins at the level of 77.9%). Water, that in this study extracted catechins most effectively, had, according to the authors, the weakest activity but it should be noted that they conducted a two hour long extraction at the boiling point of a solvent, and in these terms and conditions, as mentioned previously, catechins may be decomposed and probably that is why the efficiency of catechins' extraction was the lowest with the use of water.

It has been observed in the study that with the prolongation of the extraction time the output of polyphenols in total and catechins in each examined solvent increases. Many researchers turn their attention to the oxidation possibility of polyphenol compounds during long-term extractions which may lead to lowered results. In order to limit the occurrence of unprofitable changes, extraction can be conducted in the atmosphere of a neutral gas (i.e. nitrogen) at limited access to light or with the addition of reducing substances (i.e. ascorbic acid) [Tura and Robards 2002]. Especially big losses of catechins are caused by extractions conducted simultaneously in increased temperature and for a long period of time with the use of water as a solvent. Apart from oxidation, degradation of catechins and their epimerization can take place – especially catechins of green tea, having an epicatecholic form, they transform in catecholic forms [Perva-Uzunalic et al. 2006].

The study does not prove a decrease of the content of catechin with the prolongation of extraction time with water, probably because the process was conducted at the room temperature.

The conducted analysis of alternation (ANOVA) for a two-agent experiment (where agent A – type of solvent occurred at four variability levels, and agent B – extraction time on three variability levels) showed a very important influence ($p < 0.0001$) of a type of solvent and extraction time on the output of polyphenols in total and catechins from tea on the importance level $\alpha = 0.05$. In addition, in case of polyphenols in total, the interaction between a type of solvent and extraction time has been proved which means that in case of this marking method, the prolongation of the extraction time significantly increased extraction ability of each examined solvent. In case of catechins, the interaction between two agents proved to be not important from statistical point of view, and used test of Student-Newman-Keuls for the division of means into homogeneous groups did not show any important similarities in the output of catechins by single solvents at assumed extraction times.

Antioxidant properties of green tea extracts obtained during the process in various terms and conditions were marked with three methods. Their ability to deactivate stabile $DPPH^{\bullet}$ radicals has been examined, deactivation cation radicals $ABTS^{\bullet+}$ and chelating iron ions (II) activity.

Figure 3 presents the percentage ability of single green tea extracts for scavenging $DPPH^{\bullet}$ radicals. The conducted research showed that all extracts had scavenging ability for synthetic $DPPH^{\bullet}$ radicals. It has been proved that for each solvent, with the prolongation of the extraction time, obtained solutions deactivate radicals to a higher extent. As the increase of the output of polyphenol compounds with the prolongation of the extraction time had been stated previously, a relation between the contents of polyphenol compounds in solutions and antiradical activity of these solutions was searched for.

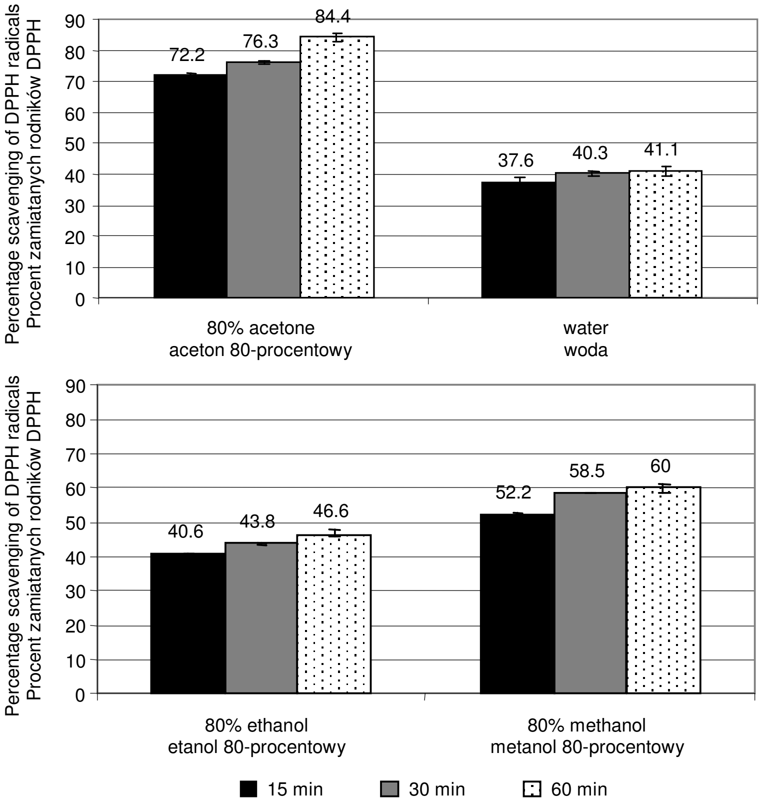

Fig. 3. Antioxidant activity of extracts against DPPH radicals
Rys. 3. Aktywność przeciwrodnikowa ekstraktów zielonej herbaty wyrażona jako procent zmiatanych rodników DPPH•

Strongest properties to DPPH• radicals showed acetone extracts (% scavenging radicals at the level of ca. 75) and methanol extracts (in average 55% scavenged radicals), the weakest activity showed water extracts of green tea (% scavenged radicals was at the level of 40).

Since water extracts had the greatest amount of catechins, it should be supposed that they did not decide on the antiradical activity of extracts to DPPH• radicals. Though the contents of condensed tannins in examined solutions has not been marked, it seems probable, that they could have an influence on scavenging radicals by green tea extracts [Saint-Cricq de Gaulejac et al. 1999].

According to Yokozawa et al. [1998], the gallatte of catechins (and they prevail among green tea extracts), have got the strongest inhibition ability of stabile DPPH• radicals. This study has not proved, however, any significant statistical relation between the contents of catechins in the extracts and their ability to reduce DPPH• radicals. The statistical analysis was conducted at the significance level $\alpha = 0.1$.

Based upon the conducted research, it has been found that all solutions have the ability of scavenging synthetic cation-radicals $ABTS^{\bullet+}$ (Fig. 4). These properties were stronger

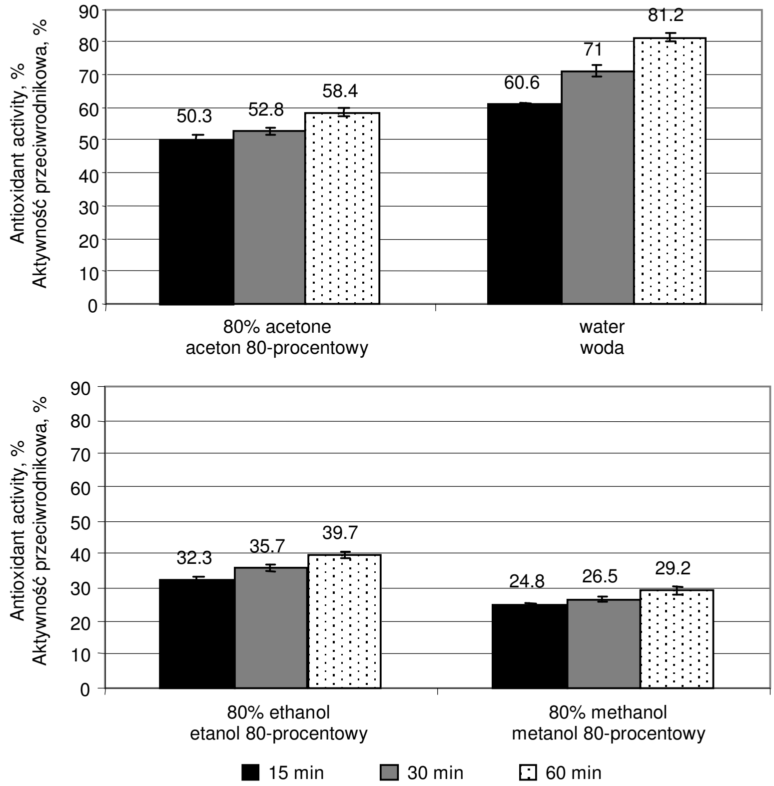

Fig. 4. Antioxidant activity of tea extracts against $ABTS^{\bullet+}$ radicals
Rys. 4. Aktywność przeciwrodnikowa ekstraktów zielonej herbaty wyrażona jako procent zmiatanych rodników $ABTS^{\bullet+}$

for the extracts obtained as a result of longer shaking of green tea with each examined extracting system. The highest level of scavenging radicals provided water extracts of green tea (ca. 70% reduced radicals) but the lowest deactivation level had methanol solutions (the average 27% scavenged radicals). Since water extracts included the biggest number of catechins, and methanol extracts had the smallest number of them, we may suppose that catechins gave green tea extracts the ability to scavenging $ABTS^{\bullet+}$ radicals

This supposedly seems to be confirmed by the results of the researchers applying both traditional assessment of the ability of compounds to reduce $ABTS^{\bullet+}$ cation radicals [Salah et al. 1995] and a modified method. Pannala et al. [2001] used the second method to compare natural polyphenol compounds, paying special attention to gallatte of catechins and especially to gallatte of epigalocatechin and gallatte of epicatechin occurring in big amounts in green tea.

The conducted regression analysis shows that there is a strong positive correlation between the contents of catechins and ability of extracts to scavenging cation radicals $ABTS^{\bullet+}$ (correlation at the significance level $\alpha = 0.05$). The calculated determination index shows that catechins present in the extracts in 98% decide on the ability of green tea extracts to inhibit ABTS$^{\bullet+}$ radicals.

Features to bind ions of transitional metals have a big share in the antioxidant activity of polyphenols, thus the study marked abilities of single green tea extracts to chelate iron ions (II; Fig. 5). Iron ions were bound by acetone extracts to the highest extent (% of chelated iron was ca. 65). These extracts had the greatest amount of catechins. Water solutions chelated iron ions to the weaker extent. According to Miller et al. [1996], compounds with gallate rests in their molecules (so gallatte of catechins and their polymers) show big activity in the range of chelating metal ions.

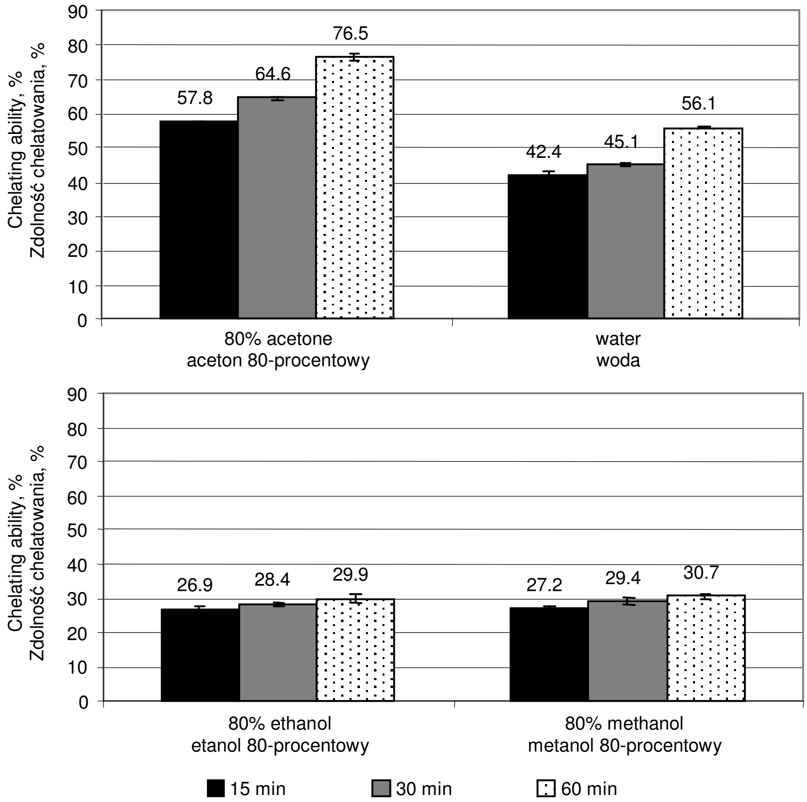

Fig. 5. The ability to chelate Fe(II) by green tea extracts
Rys. 5. Zdolność chelatowania jonów żelaza (II) przez ekstrakty zielonej herbaty

The conducted regression analysis shows that the correlation index between the contents of catechins and activity of extracts in chelating iron ions amounted to ca. 0.82. The correlation proved to be statistically important (at the significance level $\alpha = 0.05$). The determination indexes specified for single extraction times show that the ability of extracts to chelate iron ions (II) originates in 99% from the contents of catechins in them.

CONCLUSIONS

1. The best solvent for the extraction of total polyphenols was 80 % acetone, whereas for total catechins was water.

2. The increase of extraction of polyphenols with prolonged extraction time was observed.

3. All extracts have antioxidant properties against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ and abilities to chelate iron ions (II). Those specificities were correlated with contents of individual group of polyphenols in extracts.

REFERENCES

Baraniak B., Krzepiłko A., Stryjecka M., 2002. Aktywność antyutleniająca związków fenolowych ekstrahowanych różnymi rozpuszczalnikami z kalafiora [Antioxidant activity of polyphenols after different solvents extraction from cauliflower]. Żywn. Nauka Techn. Jakość 3 (32), 58-66 [in Polish].

Lai L.S., Chou S.T., Chao W.W., 2001. Studies on the antioxidative activities of Hsian-tsao leaf gum. J. Agric. Food Chem. 49, 963-968.

Manach C., Scalbert A., Morand Ch., Remesy Ch., Jimenez L., 2004. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr. 5, 727-747.

Miller N.J., Castelluccio C., Tijburg L., Rice-Evans C., 1996. The antioxidant properties of theaflavins and their gallatte esters – radical scavengers or metal chelators. FEBS Letters 1, 40-44.

Oszmiański J., 1995. Polifenole jako naturalne przeciwutleniacze w żywności [Polyphenols as natural antioxdants in food]. Przem. Spoż. 3, 94-96 [in Polish].

Pannala A.S., Chan T.S., O'Brien P.J., Rice-Evans C.A., 2001. Flavonoid B-Ring Chemistry and Antioxidant Activity: Fast Reaction Kinetics. Biochem. Biophys. Res. Commun. 1161-1168.

Perva-Uzunalic A., Skerget M., Knez Z., Weinreich B., Otto F., Grucher S., 2006. Extraction of active ingredients from green tea (*Camellia sinensis*). Extraction efficiency of major catechins and caffeine. Food Chem. 96, 4, 597-605.

Re R., Pellegrini N., Pannala A., Yang M., Rice-Evans C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 9, 1231-1237.

Robinson R., 2001. Green tea extract may have neuroprotective effects in Parkinson's disease. The Lancet 4, 358.

Saint-Cricq de Gaulejac N., Provost C., Vivas N., 1999. Comparative study of polyphenol scavenging activities assessed by different methods. J. Agric. Food Chem. 47, 425-431.

Salah N., Miller N.J., Paganga G., Tijburg L., Bolwell G.P., Rice-Evans C., 1995. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Archiv. Biochem. Biophys. 2, 339-346.

Scalbert A., Manach C., Morand Ch., Remesy Ch., Jimenez L., 2005. Dietary polyphenols and the prevention of diseases. Crit. Rev. Food. Sci. Nutr. 45, 287-306.

Singleton V.L., Rossi J. A., 1965. Colorimetry of total phenolics with phosphomolybolic – phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-158.

Song T.T., Hendrich S., Murphy P.A., 1999. Estrogenic activity of glycitein, a soy isoflavone. J. Agric. Food Chem. 47, 1607-1610.

Sun B., da Silva R.J.M., Springer I., 1998. Critical factors of vanillin assay for catechins and proanthocyanidinis. J. Agric. Food Chem. 46, 4267-4274.

Tura D., Robards K., 2002. Sample handling strategies for the determination of biophenols in food and plants. J. Chromat. A, 975, 71-93.

Turkmen N., Sari F., Velioglu Y.S., 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem. 4, 835-841.
Yokozawa T., Chen C.P., Dong E., Tanaka T., Nonaka G.-I., Nishioka I., 1998. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. Biochem. Pharm. 2, 213-222.

WPŁYW CZASU I RODZAJU ROZPUSZCZALNIKA NA EFEKTYWNOŚĆ EKSTRAKCJI POLIFENOLI Z ZIELONEJ HERBATY I WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCE OTRZYMANYCH EKSTRAKTÓW

Streszczenie. Celem pracy było zbadanie wpływu rodzaju rozpuszczalnika i czasu na efektywność ekstrakcji polifenoli oraz właściwości przeciwutleniające ekstraktów otrzymanych z zielonej herbaty. Ekstrakcję prowadzono w temperaturze pokojowej z użyciem czterech rozpuszczalników: wody oraz 80% roztworów wodnych etanolu, metanolu i acetonu (v/v) w czasie 15, 30 i 60 minut. W otrzymanych ekstraktach oznaczono zawartość polifenoli ogółem i katechin. Właściwości przeciwutleniające ekstraktów zbadano metodą z wykorzystaniem stabilnych rodników $DPPH^{\cdot}$ oraz metodą z użyciem kationorodników $ABTS^{\cdot+}$. Określono również zdolności ekstraktów do chelatowania jonów żelaza (II). Na podstawie otrzymanych wyników stwierdzono, że zarówno rodzaj rozpuszczalnika, jak i czas ekstrakcji miały istotny wpływ na wydobycie polifenoli z herbaty zielonej. Najskuteczniejszym ekstrahentem polifenoli ogółem był 80-procentowy roztwór acetonu, a katechin – woda. Zaobserwowano, że wraz z wydłużeniem czasu trwania ekstrakcji rosło wydobycie związków polifenolowych. Wszystkie ekstrakty wykazywały właściwości przeciwutleniające wobec rodników $DPPH^{\cdot}$ i $ABTS^{\cdot+}$, a także zdolności do wiązania jonów żelaza (II).

Słowa kluczowe: wydajność ekstrakcji, zielona herbata, polifenole, właściwości przeciwutleniające

Accepted for print – Zaakceptowano do druku: 12.01.2007

For citation – Do cytowania: Drużyńska B., Stępniewska A., Wołosiak R., 2007. The influence of time and type of solvent on efficiency of the extraction of polyphenols from green tea and antioxidant properties obtained extracts. Acta Sci. Pol., Technol. Aliment. 6(1), 27-36.