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TEA EXTRACTS ANTIOXIDATIVE POTENTIAL IN EMULSIFIED LIPID SYSTEMS*

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Abstract. Tea leaves (*Camelia sinensis* L.) extracts are source of polyphenols, i.e. antioxidant components. Research showed possible tea extracts use in food technology, influencing contained lipids stability improvement. The aim of the research was comparison of different teas extracts activity in emulsified lipid system. The present research examined different teas: white, green, yellow, oolong and black aqueous and ethanol extracts. To evaluate the most potent addition level different tea extracts concentrations were chosen. Linoleic acid oxidative stability was measured by linoleic acid conjugated dienes production monitoring. Emulsions with additives were incubated 19 hours at 37°C in darkness. Results showed different tea extracts antioxidant activity, dependent on its concentration in examined system. Highest antioxidant activity, comparable to BHT and rosemary extract was found in lipid sample with addition of yellow tea ethanol extract.

Key words: tea, *Camelia sinensis* L., antioxidants, lipid emulsion, linoleic acid

INTRODUCTION

Lipid oxidation is free radical chain reaction which leads to reactive radicals and hydroperoxides content increase, initiating further deterioration reactions [Frankel 1985, Min and Boff 2002, Wąsowicz et al. 2004]. It is a process causing a sequence of unfavourable changes in lipids and lipid containing food products, mainly deterioration of the sensory properties (rancidity, change of colour and texture), decrease in nutritious value and increase in health risk [Frankel 1998, Gray 1978, Drozdowski 1996]. There are many factors influencing lipid stability. Oxidation chain reaction can be influenced by antioxidants presence, allowing balancing the chemical reactions or developing profitable changes in technological processes, leading to the increase of process efficiency or limitation of unfavourable changes influencing the quality and stability of a food product.

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Antioxidants addition is an efficient way to protect value of lipid-containing food products. Food market is interested in giving the consumers new, highly desired products, which apart from strong antioxidative activity would implicate health promoting constituents like polyphenols. There are numerous investigations showing that plant extracts, rich in polyphenols, might exhibit strong antioxidative properties in lipid systems [Giese 1996, Szukalska 1999, Gramza and Korczak 2005 a, Gramza et al. 2005 a]. Tea leaves (*Camelia sinensis* L.) are one of polyphenol sources, commonly known as flavonols or catechins [Graham 1992, Chu and Juneja 1997, Gramza-Michałowska and Bajerska-Jarzębowska 2007]. Antioxidant properties of tea are due to presence of polyphenols, namely catechins: (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)--catechin (C), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG).

The objective of this study was evaluation of the most effective extract's concentration and comparison between the examined extracts in emulsified linoleic acid.

MATERIAL AND METHODS

Reagents. All chemicals used were analytical grade: linoleic acid (Nu-Chek Prep), Tween (Sigma), ddH_2O, KOH, K_2HPO_4, ethanol, methanol (POCh), BHT (Merck), Rosemary ethanol extract, Phytrox (Jan Dekker Int.).

Antioxidants – tea extracts preparation. Five kinds of tea leaves were chosen for the research: white, green, yellow, oolong and black. Tea extracts were prepared applying two different methods. Aqueous extracts were prepared by triple boiling of grinded tea leaves for 15 min at 80°C, collected extracts were filtered and lyophilized under vacuum (HETO). Ethanol extracts were prepared after triple 24 hours maceration of tea leaves in 80% ethanol. Collected extracts were filtered and ethanol was evaporated on rotary evaporator (RVO 200A, INGOS). The powdered aqueous and ethanol extracts were kept frozen until further use. Range of extracts concentration was as follows: 100; 200; 500; 1000 ppm. Powdered extracts were diluted before addition into emulsion (0.2 mL) to assure of extracts dispersion in lipid emulsion. Results were compared to strong antioxidants activity of BHT, Rosemary ethanol extract and Phytrox at standard technological concentration of 200 ppm.

Lipid substrates and antioxidants. Tea extracts antioxidant activity was examined in 10 mM emulsions of linoleic acid. Emulsions were freshly prepared according to method previously presented by Gramza et al. [2006 a]. Samples were incubated in darkness in temperature of 37°C for 19 hours. Oxidation stage was examined by initial and final measurements of conjugated linoleic acid dienes content (CLA) [Lingnert et al. 1979]. The antioxidant efficiency of studied samples was expressed as *antioxidant 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 added. Results expressed as Aec > 0 antioxidative properties, Aec < 0 prooxidative properties of the additive.

Statistical analysis. The results were obtained from a minimum of two 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.

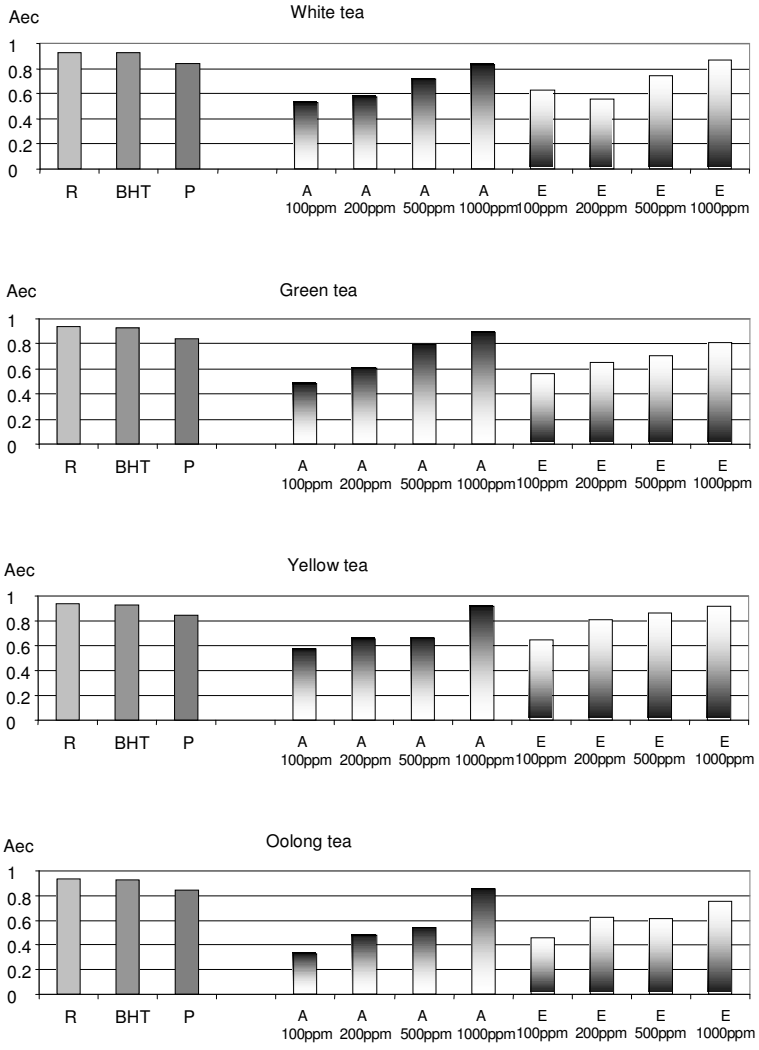
RESULTS AND DISCUSSION

After 19 h of emulsions incubation all tea extracts showed antioxidative properties (Fig. 1). Addition of tea extracts resulted in lower increase of oxidation indicator in comparison to control sample with no antioxidants added. Best antioxidant activity in linoleic acid emulsion expressed as antioxidant effectivity coefficient (Aec), was found in sample with addition of rosemary ethanol extract (0.94) and BHT (0.93). Phytrox – commercial rosemary extract showed lower activity (Aec = 0.84). It was found that antioxidant activity of examined tea extract was dose dependent and higher with its concentration increase.

Aqueous extracts were weaker antioxidants as compared to ethanol extracts ($p < 0.05$). Among aqueous extracts highest antioxidant activity at concentration of 1000 ppm was evaluated in yellow (0.92), green (0.89) and black (0.89) tea extracts. White and oolong tea showed significantly lower activity. Weakest antioxidant activity was found in oolong tea aqueous extract 100 ppm (Aec = 0.33).

Ethanol extracts showed higher antioxidative activity as compared to similar concentrations of aqueous extracts. It was found that highest antioxidant activity showed ethanol extract of yellow tea, where Aec accounted for: 100 ppm (0.65); 200 ppm (0.81); 500 ppm (0.86) and 1000 ppm (0.92). Other ethanol extracts showed lower activity. Extracts concentration of 500 and 1000 ppm was evaluated to be the best additives concentration showing highest antioxidative activity in examined linoleic acid emulsion conditions. Comparison of tea extracts antioxidant activity with well-known commercial antioxidants proved tea extracts high activity. Antioxidant effectivity coefficient Aec of yellow tea extracts (1000 ppm), green and black tea aqueous extracts (1000 ppm) was comparable to BHT and rosemary extract, higher however than Phytrox's activity. Multifactor analysis of variance did not show significant influence of tea leaves kind on tea extracts antioxidant activity.

Antioxidants partition in emulsified lipid systems could significantly influence its activity. It is important to remember that in dispersed systems like lipid emulsions, antioxidants mobility is high, what does not occur in food emulsions like margarine, mayonnaise, etc. [Bondet et al. 2000]. In multiphase systems antioxidants localization depends on their solubility and polarity [Huang et al. 1994]. According to Frankel et al. [1994] hydrophilic antioxidants (catechin, gallic acid, rosmarinic acid) are more active in bulk oils, orientated on o/w layer, than lipophilic (BHT, α-tocopherol, which are oriented in lipid phase). Roedig-Penman and Gordon [1997] found that EGC and EC are less solubilized in water than EGCG i ECG, being more active in emulsions, according to higher concentration on o/w layer. Catechin and gallic acid hydrophilic character antioxidants could be less effective in emulsions [Huang et al. 1997]. Non-fermented tea leaves (white, green tea) mainly consist of catechins, partially fermented (yellow, oolong tea) and fully fermented teas (black tea) consists of theaflavins and higher amounts of gallic acid as a result of fermentation process [Gramza and Korczak 2005 b]. All of tea polyphenols possess antioxidant activity; however it is dependent on its proportions, and higher in catechins rich extracts [Gramza et al. 2006 a]. Results of Gramza et al. [2005 a, b] showed best antioxidative activity of green tea ethanol extract in emulsified linoleic acid. It was found that ethanol extracts constituting of higher amount of lipophilic fraction showed higher antioxidative activity than aqueous extracts, constituting of higher amount of hydrophilic fraction. Research proved the incidence of polar


White tea
Aec
1
0.8
0.6
0.4
0.2
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R BHT P A 100ppm A 200ppm A 500ppm A 1000ppm E 100ppm E 200ppm E 500ppm E 1000ppm
Green tea
Aec
1
0.8
0.6
0.4
0.2
0
R BHT P A 100ppm A 200ppm A 500ppm A 1000ppm E 100ppm E 200ppm E 500ppm E 1000ppm
Yellow tea
Aec
1
0.8
0.6
0.4
0.2
0
R BHT P A 100ppm A 200ppm A 500ppm A 1000ppm E 100ppm E 200ppm E 500ppm E 1000ppm
Oolong tea
Aec
1
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R BHT P A 100ppm A 200ppm A 500ppm A 1000ppm E 100ppm E 200ppm E 500ppm E 1000ppm

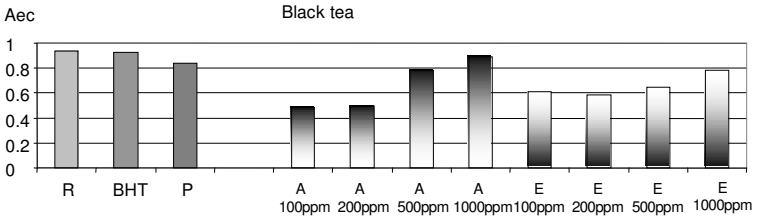

Fig. 1. Antioxidant effectivity coefficient (Aec) of different tea extracts in linoleic acid emulsion: R – rosemary, P – Phytrox, A – aqueous, E – ethanol extracts

paradox in emulsified lipid systems. Huang et al. [1997] showed high antioxidative activity of green tea aqueous extracts in corn oil, however prooxidant activity in emulsified corn oil. Plant extracts are the mixture of different properties constituents, which is why its activity could be different in kinds of lipid occurrence, because of possible "polar paradox" phenomenon. This occurrence is a result of antioxidants partition into aqueous phase, where its activity is lower than in lipid phase [Porter et al. 1989]. Polar antioxidants are more active in bulk oils, being concentrated on oil layer. Non-polar antioxidants however are more effective in lipid emulsions, protecting lipid in a oil/water phase layer [Frankel et al. 1994, Coupland and McClements 1996, Decker 1997].

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OCENA AKTYWNOŚCI PRZECIWUTLENIAJĄCEJ EKSTRAKTÓW HERBAT W UKŁADACH TŁUSZCZU ZEMULGOWANEGO

Streszczenie. Ekstrakty z liści herbaty (*Camelia sinensis* L.) są źródłem składników o charakterze przeciwutleniającym, czyli polifenoli. Wyniki badań wskazały na nowe możliwości wykorzystania tych ekstraktów w technologii żywności, oddziałujących pozytywnie na stabilność oksydacyjną tłuszczów. Celem badań było porównanie aktywności eks-

traktów różnych herbat w układzie zemulgowanego kwasu linolowego. Do badań wykorzystano ekstrakty wodne i etanolowe herbaty białej, zielonej, żółtej, czerwonej i czarnej. Celem wyznaczenia najaktywniejszego ekstraktu analizowano różne stężenia. Stabilność emulsji kwasu linolowego oznaczano testem na zawartość dienów skoniugowanych kwasu linolowego. Wyniki badań wskazały na zróżnicowaną aktywność ekstraktów herbat, uzależnioną od ich stężenia w układzie. Najwyższą aktywność przeciwutleniającą, porównywalną z BHT i ekstraktem rozmarynu, stwierdzono w próbie emulsji z dodatkiem etanolowego ekstraktu herbaty żółtej.

Słowa kluczowe: herbata, *Camelia sinensis* L., przeciwutleniacze, emulsja tłuszczowa, kwas linolowy

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