American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 2 (7): 662-670, 2007
ISSN 1557-4571

Polyphenols-Potential Food Improvement Factor

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Abstract: The aim of the research was to analyse the antioxidative properties of plant extracts; green tea (GTE) and rosemary (RE) ethanol extracts and comparison with other commonly used antioxidants. Lipid substrate consisted of three forms: bulk, on cellulose matrix and emulsified sunflower oil TAG. The level of lipid oxidation was investigated by different methods: bulk oil-peroxides, p-anisidine and Totox value, oil emulsion-peroxide value, malondialdehyde (TBARS) and Conjugated Linoleic Acid dienes (CLA) content. All forms of lipid were incubated at 40°C Schaal oven test in complete darkness. Addition of GTE and RE has efficiently increased bulk TAG stability. It was found that 1000 ppm of GTE inhibited lipid oxidation properly, lowering almost two times the peroxides content, RE almost three times. BHT was slightly weaker antioxidant. TAG on microcellulose matrix showed better stability with addition of GTE at concentration of 1000 than 200 ppm, lower however than BHT and RE. Analyses of emulsified TAG allowed to state that GTE did not protect lipid as well as in the other lipid forms. GTE (1000 ppm) showed significantly lower activity than BHT. Addition of 200 ppm GTE influenced prooxidatively lipid oxidation. Results showed that all antioxidants added stabilized sunflower oil triacyloglicerols, however they were not similarly active in all lipid forms.

Key words: Antioxidants, tea extract, rosemary extract, lipids, TAG, lipid oxidation

INTRODUCTION

Edible fats and oils undergo the oxidation processes, causing a sequence of unfavorable changes, mainly deterioration the sensory properties of product (rancidity, change of texture and colour), decrease in nutritious value (Frankel, 1998a; Wasowicz *et al*., 2004). Rate of triacyloglycerols oxidation process depends on saturation degree and their position in molecule (Yanishlieva-Maslarowa, 2001; Frankel, 1998c). It was stated that polyunsaturated fatty acids are more sensitive to oxidation than saturated one, what helps to predict the fatty acid mixture susceptibility on oxidation processes. Therefore it is desirable to control oxidation processes by addition of inhibitory substances providing suitable quality of food product. Results of many research did not show antioxidant, active in all food product. Activity depends from many factors, like solubility and mixing ability in different systems, stability in time of processing, as well as agreement with legal standards (Houlihan and Ho, 1985; Giese, 1996).

Lately special attention turned toward polyphenols, plant world compounds (Chan *et al*., 2007; Gramza and Korczak, 2005; Gramza *et al*., 2005, 2006; Parr and Bolwell, 2000, Prior and Cao, 2000a, b). Polyphenols are main topic for many researches. Those secondary metabolites from plant world are well known for its biological and pharmacological properties. Research showed that polyphenols are very potent antioxidant constituents, which stabiles food products. Correct nutrition is main factor ensuring degenerative diseases prevention, which is why addition of plant extracts, rich in polyphenols could enrich food products, claiming to nutraceutic name.

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There are known many substances possessing antioxidant properties, but not many of them are positively recognized by the consumers. That is why there is a need to find sources of natural substances which are more acceptable than synthetic ones. One of those substances with antioxidative character are polyphenols found in green tea leaf (*Camelia sinensis*) and rosemary (*Rosemarinus officinalis*) (Harbowy and Balentine, 1997; Korczak *et al*., 1998; Gramza *et al*., 2005; Chen *et al*., 2006; Gramza-Michalowska and Bajerska-Jarzebowska, 2007). Main polyphenols from tea are catechins and rosemary is rosemarinic acid. Plant extracts are mixtures of different polyphenols, known for their antioxidant activity. It was proved that their activity is governed by their chemical structures (Shahidi and Wanasundara, 1992; Atoui *et al*., 2005). Possibility of plant extracts utilization in food technology would contribute food quality and safety improvement.

The aim of this study was to compare potential antioxidative properties of selected plant extracts in different forms of lipid appearance and comparison with commonly used antioxidants.

MATERIALS AND METHODS

Chemical Reagents

All chemicals used were analytical grade: Tween (Sigma), $FeSO_4*7H_2O$ (Merck), NH_4SCN (Merck), TCA (Ubichem), TBA (Sigma), ddH_2O, KOH, K_2HPO_4, anhydrous sodium sulfate, activated alumina, ethanol, methanol, HCl (POCh), hexane and carbon (Merck), cellulose microcrystalline (Sigma), green tea (*Camelia sinensis* L.) were purchased at a local store, rosemary (*Rosemarinus officinalis* L.) was purchased from ZIOLI Polskie Pharma. Other antioxidants used were BHT-butylohydroxytoluene (Merck) and α-tocopherol (Sigma).

Plant Extracts Preparation

Yunan green tea leaves (*Camelia sinensis* L.) and rosemary (*Rosemarinus officinalis*) ethanol extracts were prepared according to method presented by Gramza *et al*. (2004). Ethanol extracts were prepared after maceration of tea leaves in 95% ethanol, than collected, filtered and ethanol evaporated. Range of extracts concentration was determined experimentally (200, 1000 ppm, according to lipid content). Powdered extracts were diluted in ethanol to assure of extracts dispersion in lipid emulsion. Rate of production yield was as follows: green tea ethanol extract (GTE) -12.2%, rosemary ethanol extract (RE) -29.3%.

Lipid Substrates

Sunflower oil (ZPT Kruszwica) containing no antioxidant was obtained according to the method by Chipault *et al*. (1955) with modifications. The sunflower oil triacyloglicerols (TAG) were free of antioxidants after removing treatment with adsorbents. Twenty five percentage hexane solution (Merck) of sunflower oil passed over a 2.5×50 cm column of anhydrous sodium sulfate (POCH), activated alumina (POCH) and carbon (Merck). The column was light protected. All these operations were performed in an atmosphere of nitrogen. The colorless percolate was evaporated under vacuum, yielding oil with peroxide value of 1.5 (meq O_2 kg^{-1} oil) was considered to be sufficiently free of antioxidants for the purpose of this study. TAG fatty acids composition was determined according to method by Wasowicz *et al*. (1984). TAG were introduced for the research in form of pure bulk TAG, emulsion and on cellulose microcrystalline matrix. TAG emulsion (5%) was prepared according to Lingnert *et al*. (1979). Emulsion was freshly prepared in: phosphate buffer (pH 7.0) with Tween 20 (Sigma). Microcrystalline cellulose was mixed with TAG (1:2), thickness of the layer was 1-1.2 cm.

Determination of Antioxidant Activity

Antioxidant activity of additives was tested on level of 0.02 and 0.1%, according to lipid phase. Extracts were dissolved in a 80% ethanol and added to the sunflower oil triacyloglycerols, mixed and

vacuum evaporated. Than the TAG in three differing forms with antioxidants were incubated at 40°C Schaal oven test in complete darkness (Pardun and Kroll, 1970). The rate of lipids oxidative stability was recorded periodically. After the incubation time the samples of TAG were recovered from cellulose by extraction according to the method by Pikul *et al.* (1983). Chemical analysis of pure TAG oxidation included determination of peroxide value (PV) (Anonymous, 2005), anisidine (AV) and total oxidation values (TV) (Anonymous, 2001). Oxidation stage of TAG emulsion was examined by periodical measurements of peroxides (Kawashima *et al*., 1981), malondialdehyde (TBARS) (Buege and Aust, 1978) and conjugated linoleic acid dienes content (CLA) (Lingnert *et al*., 1979).

Statistical Analysis

The results were obtained from a minimum of 4 independent experiments and averaged. Data were analyzed 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

Sunflower oil is very rich in different antioxidant, which is why the purification process was conducted. It was found that purified sunflower oil was rich in polyunsaturated and monounsaturated fatty acids. Evaluations showed highest content of linoleic and oleic acid, 62.5 and 25.1%, respectively. There are many lipid cleaning methods, most popular is chromatography with use of active carbon, helping to remove tocopherols, without changes in fatty acids content in purified lipid (Frankel, 1998b). Comparison of two sunflower oils before and after purification seems to confirm that thesis (Table 1). TAG of sunflower oil, rich in polyunsaturated fatty acids (62.7%) is very susceptible to oxidation and needs to be stabilized.

Research was conducted to show the activity differences of plant extracts in different lipid forms. Aiming that purpose different food products examples were used: bulk, emulsified and distributed in thin microcellulose layer sunflower oil TAG. Activity of two different Green Tea Extracts (GTE) concentrations were determined, 200 and 1000 ppm. Other antioxidants were introduced in concentration of 200 ppm.

Table 1: TAG fatty acids composition of sunflower oil (%) (Mean±SD)

	Fatty acid content (%)	
Type of fatty acid	Sunflower oil	Purified TAG of sunflower oil
12:00	nd	nd
14:00	0.07±0.00^{a}	0.07±0.00^{a}
15:00	nd	nd
16:00	6.41±0.06^{d}	6.51±0.10^{d}
16:01	0.09±0.00^{a}	0.09±0.00^{a}
17:00	0.05±0.00^{a}	nd
17:01	nd	nd
18:00	4.33±0.05^{c}	4.37±0.00^{c}
18:01	24.79±0.15^{e}	25.10±0.12^{e}
18:02	62.82±0.19^{f}	62.56±0.37^{f}
18:03	0.21±0.01^{a}	0.22±0.00^{ab}
20:00	0.29±0.00^{a}	0.26±0.01^{ab}
20:01	0.27±0.00^{a}	0.15±0.15^{a}
22:00	0.65±0.03^{b}	0.67±0.02^{b}
Fatty acids sum (%)		
Saturated	11.81±0.03^{a}	11.89±0.07^{a}
Monounsaturated	25.15±0.15^{b}	25.35±0.26^{b}
Polyunsaturated	63.04±0.18^{c}	62.77±0.36^{c}

Mean values with different letter(s) differ among them ($p<0.05$), nd: Not detected

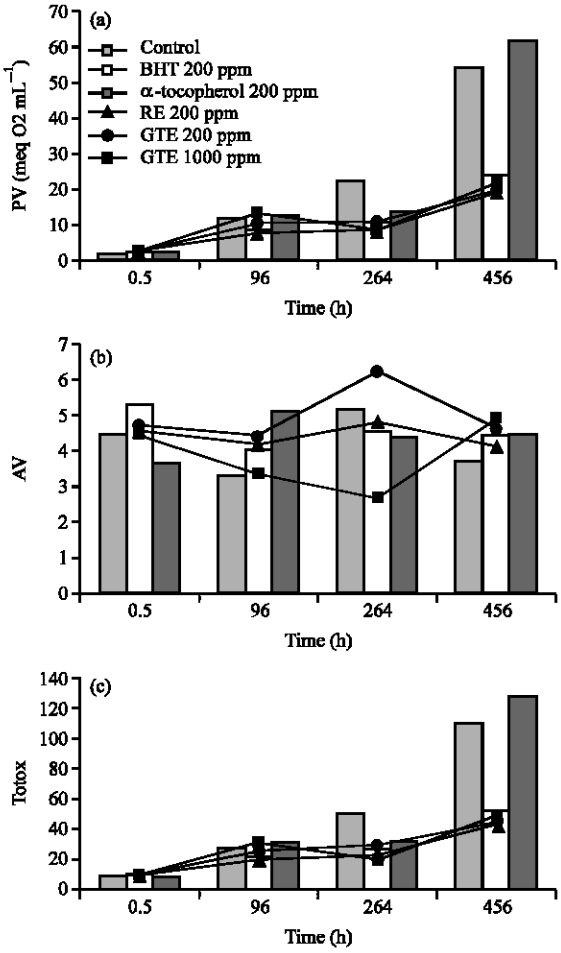

Fig. 1: Antioxidants effect on (a) peroxide, (b) p-anisidine and (c) Totox values of bulk sunflower oil TAG

During the 456 h of incubation period PV was lower in the samples with RE, GTE and BHT than in the control sample (Fig. 1). The highest antioxidant activity was found in RE and GTE (200 ppm), comparable to BHT. Also high antioxidant activity was found in GTE (1000 ppm), α-tocopherol showed the weakest activity as compared with other antioxidants. It is shown that natural antioxidants as RE and GTE can be as potent antioxidant as synthetic BHT. Measuring the secondary oxidation products by p-anisidine value allowed stating the differentiations between samples (Fig. 1). After 264 h AV level decreased only in sample with GTE 1000 ppm. On 456th h of incubation increase of samples AV have been noticed. Measuring secondary oxidation products it was shown that there was no antioxidant active. Others were similiar, causing almost 10% higher oxidation level than the control sample. Total oxidation value shows complete process of oxidation, being a sum of primary and secondary oxidation products, expressed as a Totox value. Among antioxidants tested RE and GTE (200 and 1000 ppm) showed best lowering oxidation products formation, similarly to BHT. Less active was α-tocopherol (200 ppm), which showed 120% weaker antioxidant activity than synthetic BHT.

Sunflower oil TAG on cellulose matrix stability changes were examined. Changes in Peroxide Value (PV) of incubated TAG on cellulose matrix as a function of time are shown on Fig. 2. During the

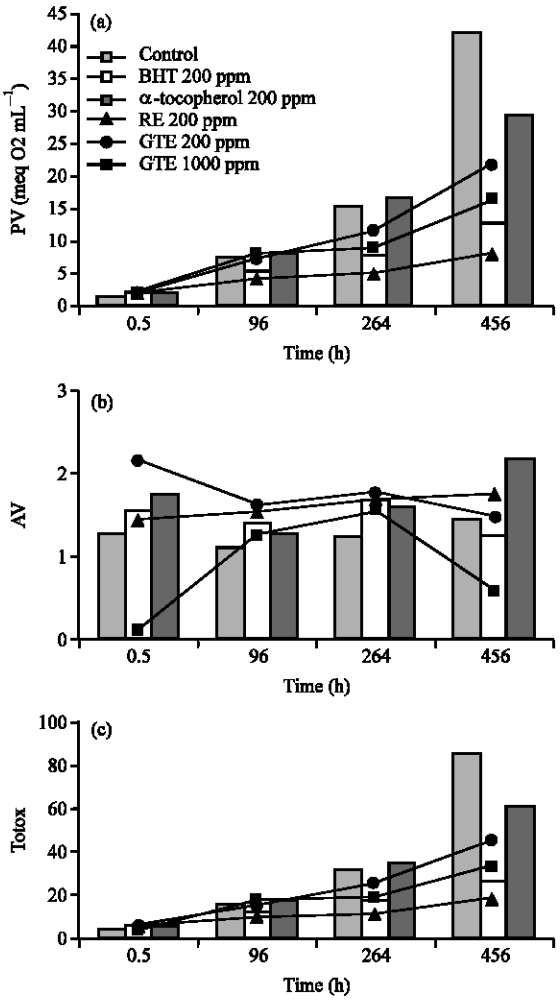

Fig. 2: Antioxidants effect on (a) peroxide, (b) p-anisidine and (c) Totox values of sunflower oil TAG on cellulose matrix

456 h of incubation period PV was lower in the samples with RE, BHT and GTE than in the control sample. Best antioxidant activity measured by peroxide content was stated in sample with RE. After 456 h the differentiation has been noticed. The lowest antioxidant activity was found in lipid sample with addition of α-tocopherol. It is shown that natural antioxidants as RE and GTE can be as active antioxidant as synthetic BHT in cellulose bounded lipid. Secondary oxidation products showed also differentiations between samples. Again GTE was best antioxidant, much more active than RE or BHT. The 456th incubation hour showed prooxidative activity of α-tocopherol. Totox value as sum of primary and secondary oxidation products content shows complete process of oxidation. Among antioxidants tested RE and BHT (200 ppm) showed best activity, GTE was less active, but higher than α-tocopherol.

Sunflower oil TAG emulsion stability was measured. It was stated, that peroxides content increased rapidly after 5 days of incubation (Fig. 3). During the 19 days of incubation period peroxides content was lowest in samples with BHT. Other additives showed lower activity. GTE (1000 ppm)

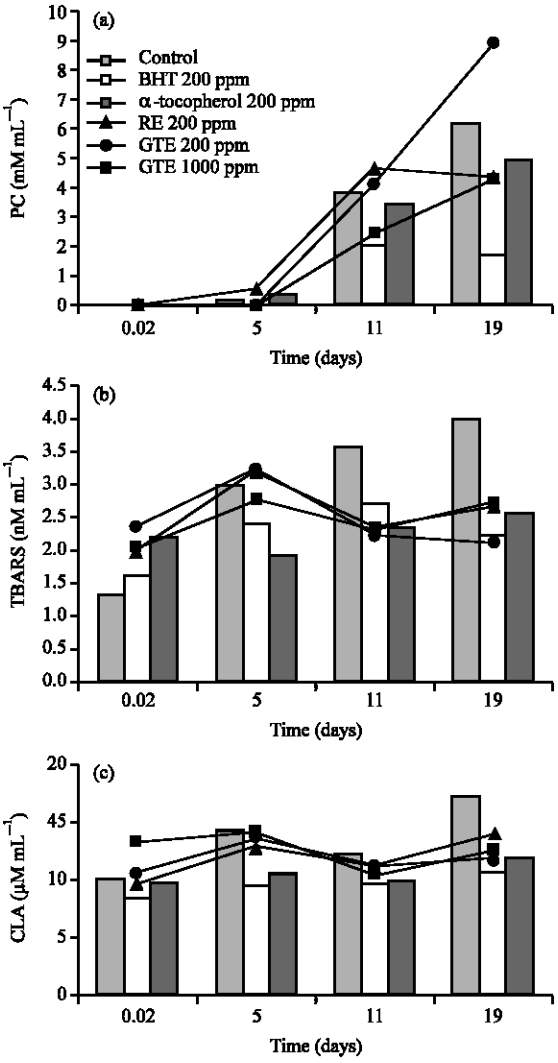

Fig. 3: Antioxidants effect on (a) peroxide, (b) TBARS and (c) CLA content of sunflower oil TAG emulsion

was as active as RE, higher than α-tocopherol and GTE (200 ppm). It showed that natural antioxidants as RE and GTE can be 50% as active antioxidant as synthetic BHT in lipid emulsion. Measures of the secondary oxidation products by TBARS showed also differentiations between samples. After 19 days TBARS level increased, accept GTE 200 ppm sample. The 19 days showed that best antioxidant activity measured by secondary oxidation products was in samples with addition of BHT and GTE 200 ppm. Others were similiar to α-tocopherol, lowering oxidation level in comparison to control sample.

CLA-conjugated linoleic acid dienes content is a standard method for measurement of lipid stability. Eleventh day showed that among antioxidants tested BHT was the strongest one, similarly but less active were GTE, RE and α-tocopherol.

At the end of incubation time it was found, that all plant origin extracts were less active than BHT, but inhibited oxidation process for about 35%.

Results showed that all antioxidants added stabilized sunflower oil triacyloglicerols; however they were not similarly active in all lipid forms. During the incubation best antioxidant activity in comparison to synthetic BHT showed 200 ppm of rosemary extract and 1000 ppm green tea extract. In examined conditions weakest antioxidant properties were found in α-tocopherol sample.

DISCUSSION

Addition of GTE and RE has efficiently increased bulk TAG stability. It was found that 200 ppm of RE inhibited lipid oxidation properly, lowering almost three times the peroxides content. BHT was slightly weaker antioxidant. TAG on microcellulose matrix showed better stability with addition of GTE at concentration of 1000 than 200 ppm, lower however than BHT and RE. Analyses of emulsified TAG allowed to state that GTE did not protect lipid as well as in the other lipid forms. GTE (1000 ppm) showed significantly lower activity than BHT. Addition of 200 ppm GTE influenced prooxidatively lipid oxidation.

Roedig-Penman and Gordon (1997) research showed that aqueous extract of green tea was very active antioxidant in 30% sunflower oil emulsion. Antioxidant affectivity analysis of pure tea polyphenols EGCG and ECG did not show statistically important activity, myricetine however was very strong antioxidant in concentration of 10^{-4} M. Another important conclusion was that tea extract (0.03%) showed comparable activity to BHT (0.02%). Rosemary extract showed moderated activity, similarly to green tea methanol extract.

Other research results showed that emulsions with tea extracts addition were more stabile, than the pure one and dependent on its concentration in examined system (Gramza-Michalowska *et al*., 2007a). Highest antioxidant activity, comparable to BHT and rosemary extract was found in lipid sample with addition of yellow tea ethanol extract. It was also found, that tea extracts purification process did not result in decreased antioxidant activity in emulsified lipid systems (Gramza-Michalowska *et al*., 2007b).

Research of Ho *et al*. (1997) confirmed that green tea leaves phenolic compounds exhibit strong antioxidative activity in lipid systems. One of the extracts constituents, gallic acid shows affinity to air-oil phase in the emulsion (Frankel *et al*., 1994). Other investigations stated strong antioxidative activity of green tea aqueous extracts in bulk corn oil, however prooxidative in emulsified corn oil system (Frankel *et al*., 1997; Huang and Frankel, 1997; Huang *et al*., 1997). Plant extracts are the mixture of constituents representing different chemical properties, which is why it is important to pay attention to lipids form, which is stabilized by antioxidant. There is a possibility of polarity paradox appearance. Porter *et al*. (1989) found that polar antioxidants were much more active in non-polar medium, while non-polar antioxidants were more active in polar systems. This phenomenon was explained by the constituents distribution into aqueous phase, where they are less efficient in protection from oxidation, than in lipid phase. Polar antioxidants are more active in bulk oil, being concentrated on the oil-air phase boundary. On the other hand non-polar antioxidants were more active in emulsions, protecting lipid on the oil-water boundary (Coupland and McClements, 1996; Decker, 1998; Frankel *et al*., 1994).

Research of Gramza *et al*. (2006) showed strong antioxidative properties of green tea extract in lipid systems. The antioxidant potential of Yunan tea aqueous and ethanol extracts appeared to be governed by the total polyphenol and ECG, EC and C content. Also strong correlation between antioxidant activity and polyphenol content indicates that monomeric polyphenols are likely to have strong impact in the overall lipid stability status. Green tea ethanol extract showed highest antioxidant activity, comparable to α-tocopherol in sunflower oil and lard.

It must be underlined that activity of antioxidative compounds in oil in water and water in oil emulsions differs significantly from activity in bulk oil (Pokorny, 1999). In multiphase systems antioxidants localization depends from their polarity and solubility. Many authors found that antioxidant activity of some antioxidants in bulk oil was inversely proportional to activity in emulsified lipid (Frankel *et al*., 1994; Huang *et al*., 1994). Above mentioned facts were confirmed in present

research, where green tea ethanol extract showed high antioxidant activity separate in bulk oil, significantly lower however emulsified lipid system. Antioxidant activity analysis showed that among examined antioxidants RE and GTE caused best lipid stability, protecting from lipid oxidation in bulk and cellulose layer TAG. Emulsified lipids showed lower oxidative stability and addition of 200 ppm GTE exhibited prooxidative activity. Research showed that plant origin ethanol extracts, used as food additives, could play preventive role in lipid oxidation process, but only in selected forms of lipid appearance.

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