Original Article

Use of plant extracts in summer and winter season butter oxidative stability improvement

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Edible fats and fat containing products undergo oxidation, both during production and storage, causing a sequence of unfavorable changes. Enrichment of lipids with plant polyphenols can profitably influence their oxidative stability, additional introduction to human body can also decrease the degenerative diseases morbidity. Two seasons butter quality were analysed: winter and summer season. Oxidative stability of butter was conducted on Rancimat and Oxidograph test conditions (110°C). To evaluate antioxidant activity of different plant extracts lipid samples were enriched with green tea and rosemary extracts, α-tocopherol and BHT at concentration of 0.02%, counted over lipid content. It was found that pure winter butter was more stable than pure butter from summer season in Rancimat test conditions (p<0.05). No statistical differences between samples in Oxidograph test were found. Summer season butter oxidative stability was highest in sample with addition of green tea extract: 71.22h for Rancimat and 81.23h for Oxidograph test. Best antioxidative activity in winter butter showed green tea extract, where induction period was 66.5 h for Rancimat and 64.0 h for Oxidograph test. Also rosemary extract and tocopherol showed strong antioxidative activity, weaker however than green tea extract. BHT, strong synthetic antioxidant showed much lower activity. Study indicated strong antioxidant activity of examined plant extracts in lipid systems.

Key Words: tea leaves, *Camellia sinensis*, tea extracts, polyphenols, butter, Rancimat, Oxidograph, lipid stability

Introduction

Lipids undergo the oxidation processes, causing a sequence of unfavorable changes, deterioration products sensory properties (rancidity, change of texture and colour) and decrease in nutritious value.[1, 2] Autoxidation is a free radical chain reaction, leading to increase in reactive radicals and hydroxides, which initiate further transmutations.[3, 4] It is desirable from technological and nutritional point of view to control oxidation process by adding inhibitory substances – antioxidants, providing suitable food quality.

Research results did not show antioxidant which could be active in all food products. Antioxidants can be divided into groups of natural (tocopherol, rosemary extract) and synthetic ones (BHA, BHT, TBHQ, GP).[5, 6] Last years bring out results, suggesting synthetic antioxidants use limitation, with regard to their potential toxicity and cancerogenicity.[7-9] There is a need of looking for new sources of those substances, which could influence food stability, would be harmless for human and show high antioxidant activity in case of their addition to food.

Polyphenols, plant compounds possessing strong antioxidative activity are the last years "discovery".[10-13] One of polyphenol sources are tea leaves (*Camellia sinensis L.*).[11, 14] Tea polyphenols - flavanols are six major catechins: (+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG).[11, 15] Catechins form nearly 30% of green tea dry weight and their content is higher in young leaves.[16] Tea extracts and constituents are also well known for their antioxidant activity in different in vitro systems.[11] Possibility of its utilization in food technology can contribute to improvement of quality and safety of food products. Enrichment of lipids with plant polyphenols can profitably influence their oxidative stability, additional introduction to human body can also decrease the degenerative diseases morbidity.

Objective of the present studies was to investigate antioxidative properties of green tea (*Camellia sinensis*) and rosemary (*Rosemarinus officinalis*) ethanol extracts in different season butter in comparison to other strong antioxidants.

Materials and methods

Plant extract

Tea leaves (*Camelia sinensis*.) and rosemary (*Rosemarinus officinalis*) were plants chosen for the research. Green tea and rosemary ethanol extracts were prepared according to method presented by Gramza *et al*.[17]

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Ethanol extracts were prepared after 24 hours maceration of leaves (100 g) in 95% ethanol (100 ml), at ambient conditions (procedure was repeated three times). Collected extracts were centrifuged after filtration (4500 rpm, 15 min). Ethanol was evaporated on rotary evaporator (RVO 200A, INGOS). The powdered extracts were kept frozen (-18°C) until further use. Rate of production yield was as follows: green tea ethanol - 12,2%, rosemary ethanol extract - 29,3%.[17] The range of the extracts concentration was determined at level of 0.02%, according to lipid content.

Butter

Experiment included two kinds of butter: winter and summer season: "table" (73% of fat) and "extra" (82% of fat). Oxidative stability of selected lipid substrate was conducted on Rancimat and Oxidograph test conditions (110°C). To evaluate antioxidant activity of different plant extracts lipid samples were enriched with tea and rosemary extracts, α-tocopherol and BHT at concentration of 0.02% counted over lipid content, control sample was pure butter with no additives. Rancimat test (Metrohm, Switzerland), based on conductometric measure of volatile acids dissociation products, which are being formed during the oxidation process.[18] In a reaction vessel lipid sample was oxidized at 110°C; air flow (20L/h), the end of induction period characterized sudden increase of water conductivity, as a result of volatile carboxylic acids dissociation.[19] Oxidograph test (Mikrolab Aarhus, Denmark)[20] is based measuring the consumption of oxygen by means of pressure transducers, as a result of lipid oxidation. The samples were heated (110°C) to accelerate the process and shorten the analysis time. The induction period end is evaluated graphically. On the ground of received induction periods reprints antioxidant effectivity of applied extracts was evaluated. Protection coefficient Pc was determined as the relation of sample with antioxidant addition induction period to control sample induction period.

$$Pc = \frac{\text{Induction period of sample}}{\text{Induction period of control}}$$

Statistical analysis

The results were obtained from a minimum of four 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 and discussion

Lipid stability analysis in Rancimat test conditions allowed to evaluate induction period on the basis of water conductivity increase, resulted by oxidation process. Tables 1 and 2 shows the lipid stability in Rancimat test. Higher the induction period value of sample with antioxidant in comparison to control, higher antioxidative activity of additive. According to season of butter production it was found that there were significant differences between samples (Table 1). Summer season butter (34.30h) was less stable than winter season butter (43.83h). Butter as milk product, consists of mainly triacylglycerols and natural prooxidans or antioxidants. It is water-in-oil emulsion containing an aqueous phase of phospholipids dispersed in fat. Phospholipids oxidize more readily than the triacylglycerol components, acting as antioxidants.[2]

Differences in lipids stability depends also on many other factors, including the cow's breed and feeding season. Summer milk is very rich in strong antioxidant and metal ions chelator: β-carotene and ascorbic acid, provided with greens eaten by animals. From the other point of view winter feeding is also enriched with many antioxidants, added into a silage for cow's feeding. With this informations it still difficult to explain or percieve butters succeptibility for oxidation, it must be remembered, that different season milk consists of different substances, enhancing or lowering butters stability.

In Rancimat test conditions longest induction period was stated in summer lipid sample with addition of green tea ethanol extract (71.22 h), what allowed to increase lipids stability for nearly 340% in comparison to control sample. Rosemary extract was also active antioxidant allowing to stabilize lipid for two times longer (Table 2a). Winter butter analysis showed similiar results, green tea extract and α-tocopherol were most active in examined conditions. The protection coefficiency in sample with green tea ethanol extract was higher than others antioxidants (Table 2a). According to its protection coefficient it was found that green tea extract protected lipid from oxidation for nearly three times and rosemary extract two times longer than control sample. Activity of rosemary extract was also high, comparable to synthetic BHT.

Analysis of lipid stability in Oxidograph test conditions allowed to evaluate induction period on the basis of oxygen consumption by the lipid sample. Table 2b shows the lipid stability in Oxidograph test. Higher the induction period value of sample with antioxidant in comparison to control, higher antioxidative activity of additive. According to butter production season it was found that there were no significant differences between samples (Table 1). Summer season butter (28.66h) was as stable as winter season butter (27.63h).

In Oxidograph test conditions longest induction period

Table 1. Pure summer and winter "table" butter stability in Rancimat and Oxidograph test (x±SD).

Butter	Induction period [h]	
	Rancimat	Oxidograph
Summer	$34.30 \pm 0.06_a$	$28.66 \pm 0.05_a$
Winter	$43.83 \pm 0.07_b$	$27.63 \pm 0{,}09_a$

a, b – mean values without common letters in the same row are significant at $p<0{,}05$. Results expressed as mean values of three replicates per two treatments.

Table 2. Tea extracts influence on "extra" butter stability in Rancimat (a) and Oxidograph (b) tests (x±SD)

a)

Butter	Additive	Rancimat induction period [h]	Protection coefficient
Summer	Control	21.23 ± 0.07*	1.00
	BHT	36.11 ± 0.02*	1.70
	Green tea extract	71.22 ± 0.09*	3.35
	Rosemary extract	42.01 ± 0.1**	1.97
	α-tocopherol	62.11 ± 0.05	2.92
Winter	Control	26.53 ± 0.04*	1.00
	BHT	43.66 ± 0.04**	1.64
	Green tea extract	66.46 ± 0.09*	2.51
	Rosemary extract	38.26 ± 0.05*	1.44
	α-tocopherol	60.86 ± 0.10	2.29

Mean values significant at: $p<0.05$; * $p<0.01$; ** $p<0.001$. Results expressed as mean values of three replicates per two treatments. Additives amount 0.02% counted over a lipid content. Protection coefficient Pc is the relation of sample with antioxidant induction period to control sample induction period.

b)

Butter	Additive	Oxidograph induction period [h]	Protection coefficient
Summer	Control	30.33 ± 0.09	1.00
	BHT	49.43 ± 0.06*	1.63
	Green tea extract	81.23 ± 0.17	2.68
	Rosemary extract	61.60 ± 0.08**	2.03
	α-tocopherol	63.60 ± 0.04**	2.09
Winter	Control	24.20 ± 0.10	1.00
	BHT	36.90 ± 0.06	1.52
	Green tea extract	64.03 ± 0.04**	2.65
	Rosemary extract	45.50 ± 0.14*	1.88
	α-tocopherol	64.76 ± 0.16**	2.67

Mean values significant at: $p<0.05$; * $p<0.01$; ** $p<0.001$. Results expressed as mean values of three replicates per two treatments. Additives amount 0.02% counted over a lipid content. Protection coefficient Pc is the relation of sample with antioxidant induction period to control sample induction period.

was stated in summer lipid sample with addition of green tea ethanol extract (81.23 h), what allowed to increase lipids stability for 270% in comparison to control sample. Rosemary extract was also active antioxidant allowing to stabilize lipid for two times longer (Table 2b). Winter butter analysis showed similiar results, green tea extract and α-tocopherol were most active in examined conditions. The protection coefficiency in sample with green tea ethanol extract was higher than others antioxidants. According to its protection coefficient it was found that green tea extract protected lipid from oxidation for nearly two and a half times and rosemary extract two times longer than in control sample. Activity of α-tocopherol was also high similiarly to green tea extract. Activity of rosemary extract was slightly lower, but higher than synthetic BHT.

Ho *et al*.[21] have examined the activity of tea extracts: green, black and oolung in Rancimat test conditions. They have stated highest activity of green tea extract addition, lowest of oolung extract. Research of Chen and Chan [22] showed high antioxidant activity of tea catechins in heated rapeseed oil. Results of present research are in consistence with results of Chen *et al*[23, 24], who found that green tea extract possessed higher antioxidant activity than BHT and rosemary extract in rapeseed oil (incubated at 100ºC). Also the research of antioxidant activity measured as oxygen consumption by the lipid showed high antioxidative activity of non-fermented tea extract. Those activity authors have connected with polyphenol contents in the extracts. Ethanol extract of black tea showed slender activity, what could be a result of partial destruction of polyphenols during leaves fermentation process. Other research showed high antioxidant activity of green tea's main polyphenol-catechin.[25] Its antioxidant activity in Rancimat test conditions (90ºC) was higher than BHT and α-tocopherol. Protection coefficients were for α-tocopherol 5.01; BHT 6.47 and catechin 43.22.

Other research of the authors showed that tea leaves can be the source of polyphenolic compounds with strong antioxidative properties in lipid systems. [10, 18] Antioxidant properties of Yunan tea aqueous and ethanol extracts appearred to be governed by the total polyphenol and ECG, EC and C content. Highest antioxidant activity of green tea ethanol extract was comparable to α-tocopherol activity in sunflower oil and lard. Statistical analysis of tea extracts antioxidant activity in lipids confirmed essential influence of catechin contents.

Conclusions

The present study indicated strong antioxidant activity of examined plant extracts in lipid systems. It was found that examined antioxidants were similiarly stable in Rancimat and Oxidograph tests conditions. Lipid stablity analysis showed significant influence of green tea extract. Higher activity of natural extracts (green tea, rosemary extracts) could be explained by high polyphenol content. BHT was significantly less active antioxidant in examined conditions.

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