

Antioxidant activity of extracts from old tea leaves

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Abstract

The effect of methanolic extracts of old tea leaves (OTL) (leaves numbers 5–8 of the shoots) on the stability of rapeseed oil during heating at 60°C and deep-fat frying of potato crisps at 180°C was determined. The OTL extract was effective in retarding oil deterioration at 60°C, with activity increasing with concentration in the range 0.02–0.25%. At a concentration of 0.25% the OTL extract was similar in activity to a rosemary extract added at 0.1%. The OTL extract (0.1%) was as active in retarding the deterioration of oil as a rosemary extract (0.1%) during repeat frying of potato crisps. Hence it is clear that old tea leaves, which at present are often considered as agricultural waste, contain antioxidants that may usefully be extracted and added to foods. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

There have been increasing efforts in recent years to develop effective natural antioxidants for edible oils in order to retard lipid oxidation, which may lead to off-flavours, the formation of toxic products and the reduction of nutritional quality (Frankel, 1993; Jadhav et al., 1996). Tea extracts are powerful antioxidants owing mainly to the presence of the flavanols epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) (Salah et al., 1995; Zandi and Gordon, 1995). These compounds are believed to have physiological effects by acting as free radical scavengers (Zhao et al., 1989; Quartley et al., 1994). Tea is considered a major dietary contributor in reducing mortality from coronary heart disease in elderly men (Hertog et al., 1993). Green tea extracts have the potential for large-scale application as natural antioxidants. Zandi and Gordon (1995) reviewed the main literature published prior to 1995 on tea as a source of natural antioxidants. More recently, it was reported that ethanolic extracts of green tea strongly inhibit oxidation of canola oil (Chen et al., 1996), and tea catechins retard oxidation of marine oils with a

similar effectiveness to synthetic antioxidants (Wanasundara and Shahidi, 1996).

Tea catechins are effective scavengers of free radicals (Salah et al., 1995), with more effective catechins having a galloyl moiety at C3 (Rice-Evans and Miller, 1996) and a trihydroxy structure in the B ring (Nanjo et al., 1996). Catechins are also effective by metal chelation (Shahidi et al., 1992). Chlorophyll present in organic extracts from green tea also affects the antioxidant activity of the extracts (Gutierrez-Rosales et al., 1992).

Extracts of green tea are becoming increasingly important as functional ingredients in the diet and are being added to a range of foods and beverages (Anon, 1997). Both green and black tea are manufactured from young shoots, mainly the first 2–4 leaves and a bud. Old tea leaves, which are not used in tea manufacture, are considered as agricultural waste. In this study, the effect of concentration of old tea leaf extracts on the stability of rapeseed oil during heating and frying has been compared with that of a rosemary extract.

2. Materials and methods

Samples of old tea leaves (*Camellia sinensis* (L.) O. Kuntze) were collected from a tea garden in Ramsar (northern Iran). Old leaves comprised leaves numbers 5–8 of the shoots. Polyphenol oxidase was inactivated by plunging the leaves into boiling water for 3 min. The

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leaves were then drained, dried at room temperature and ground to a fine powder.

Refined, bleached, deodorised low erucic acid rapeseed oil containing citric acid but no added antioxidants was supplied by Van den Bergh and Jurgens (Purfleet, UK). Ascorbyl palmitate (AP) (96%) was purchased from Sigma Chemical Co. (Poole, UK). Tertiary butyl hydroquinone (TBHQ) (>98%) was supplied by Fluka AG (Buchs, Switzerland). A commercial rosemary extract (Herbor 021) was supplied by Food Ingredients Specialties (Hayes, UK). All other chemicals were ACS grade or better quality.

2.1. Preparation of methanolic extract

The ground sample of old tea leaves (OTL) was heated with methanol (5 times volume) under reflux for 2 h. After filtration, the residue was washed with methanol (2×50 ml). Solvent was evaporated under vacuum at 50°C, leaving a residue of 21.0% of the initial weight.

2.2. Schaal oven test

Duplicate samples of rapeseed oil (50 g) with added antioxidants were heated at 60°C for 20 days. Samples (5 g) were removed periodically for analysis. The peroxide value (PV), conjugated dienes (% CD) and *p*-anisidine value (AV) were determined by the methods of the American Oil Chemists' Society (AOCS, 1989).

2.3. Frying procedure

Rapeseed oil (1000 g) was heated in a domestic fryer (Model 7122A, Tefal Super 500 deluxe, France) to 180°C. Moris Piper potato slices (100 g, 5 cm diameter×0.2 cm thickness) were added and fried for 5 min. After each frying operation, the oil was left to cool to room temperature and a sample was removed for analysis. The same oil sample was used to fry 12 successive batches of potato crisps over a period of 3 days without

further addition of oil. Deterioration of the frying oil was monitored by analysis of *p*-anisidine value (AOCS, 1989) and polar components by column chromatography (IUPAC, 1991).

2.4. Statistical analysis

Statistical analysis to determine significant differences in antioxidant activity at 60°C involved plotting PV against time to determine times to PV=20 and 50 meq kg⁻¹, and then applying ANOVA one-way analysis to determine the pooled standard deviation. The individual means were compared to a two-sample *t*-test using the pooled standard deviation to determine differences significant at the 5% level.

3. Results and discussion

Fresh low erucic acid rapeseed oil of good quality was used for the experiments. Analytical characteristics were PV 0.2 meq kg⁻¹, iodine value 115, and the contents of fatty acids 18:1, 18:2, 18:3 and 22:1 were 61, 21, 9 and 0.3%, respectively (Zandi and Gordon, 1995). The OTL extract clearly contains some useful antioxidant components. All samples with OTL extract level added at 0.02–0.25% were more stable on heating at 60°C than the control, when assessed by the change in peroxide value (Fig. 1). The oil stability increased significantly ($p < 0.05$) with the addition of 0.05% OTL extract when assessed by the time to peroxide values of 20 meq kg⁻¹ and 50 meq kg⁻¹, and 0.02% OTL extract had a significant effect when assessed by the time to 50 meq kg⁻¹ (Table 1). The conjugated diene measurements (Fig. 2), and the *p*-anisidine values on day 20 (Table 2) confirmed the antioxidant effect of OTL extract. The antioxidant effect of OTL extract increased with concentration and, at a concentration of 0.25%, the antioxidant activity was not significantly different ($p > 0.05$) from that of the rosemary extract (0.1%). TBHQ (0.02%) was the most active antioxidant studied.

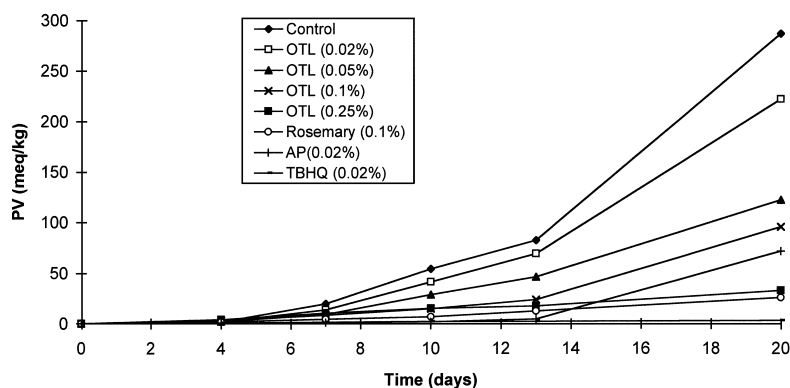


Fig. 1. Effect of additives on the oxidation of rapeseed oil at 60°C, assessed by the change in peroxide value.

Table 1
Times (days) to peroxide values of 20 meq kg⁻¹ and 50 meq kg⁻¹ for rapeseed oil heated for 20 days at 60°C

| Additive | Time to PV = 20 | Time to PV = 50 |
|----------------------------|--------------------|--------------------|
| None | 7.01 ^a | 9.61 ^a |
| OTL (0.02%) | 7.66 ^{ab} | 10.93 ^a |
| OTL (0.05%) | 8.7 ^b | 13.11 ^b |
| OTL (0.1%) | 11.73 ^c | 15.54 ^c |
| OTL (0.25%) | 13.98 ^d | > 20 ^e |
| Rosemary (0.1%) | 16.83 ^e | > 20 ^e |
| Ascorbyl palmitate (0.02%) | 14.62 ^d | 17.76 ^d |
| TBHQ (0.02%) | > 20 ^f | > 20 ^e |

^{a-f} Values that are significantly different according to the Student *t*-test ($p < 0.05$).

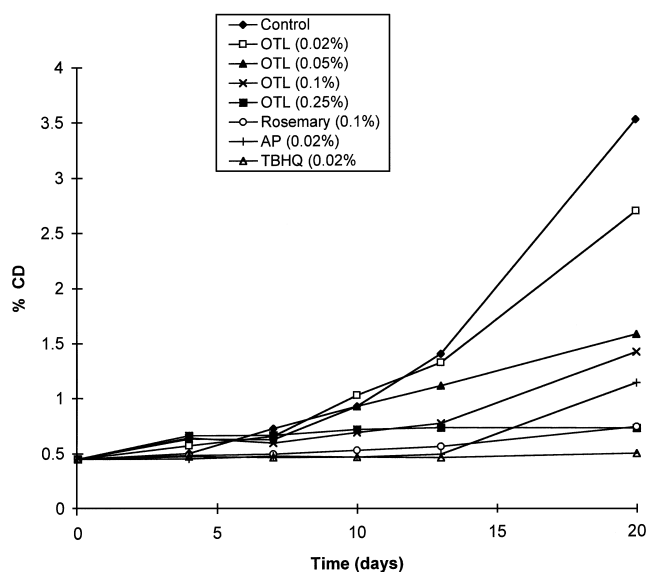


Fig. 2. Effect of additives on the oxidation of rapeseed oil at 60°C, assessed by the change in conjugated diene content.

Ascorbyl palmitate (0.02%) was a very effective antioxidant for 13 days, but then the oil deteriorated rapidly so that this sample was significantly less stable than the OTL extract (0.25%) sample after 16 days.

The OTL extract (0.1%) was at least as active as the rosemary extract (0.1%) when assessed as an antioxidant in rapeseed oil during the repeat frying of batches of potato crisps. This is clearly seen in the changes in *p*-anisidine values in the frying oil (Fig. 3). The *p*-anisidine value measures mainly unsaturated aldehydes formed during hydroperoxide decomposition. The antioxidant effect of the extracts was also shown by a reduction in the formation of polar compounds, which changed during 12 frying operations from 2.4 ± 0.19% for the fresh oil to 10.0 ± 0.06% for the control, but only reached 9.3 ± 0.17% for the sample with added rosemary extract (0.1%) and 9.3 ± 0.21% for the sample with added OTL extract (0.1%). The control was sig-

Table 2
p-Anisidine values (AV) of rapeseed oil heated for 20 days at 60°C

| Additive | AV ^a |
|----------------------------|-----------------|
| None | 80.7 ± 1.5 |
| OTL (0.02%) | 55.0 ± 0.1 |
| OTL (0.05%) | 25.5 ± 0.1 |
| OTL (0.1%) | 17.7 ± 0.0 |
| OTL (0.25%) | 9.5 ± 0.1 |
| Rosemary (0.1%) | 4.8 ± 0.1 |
| Ascorbyl palmitate (0.02%) | 15.6 ± 0.3 |
| TBHQ (0.02%) | 2.7 ± 0.0 |

^a Mean and range of duplicate analyses.

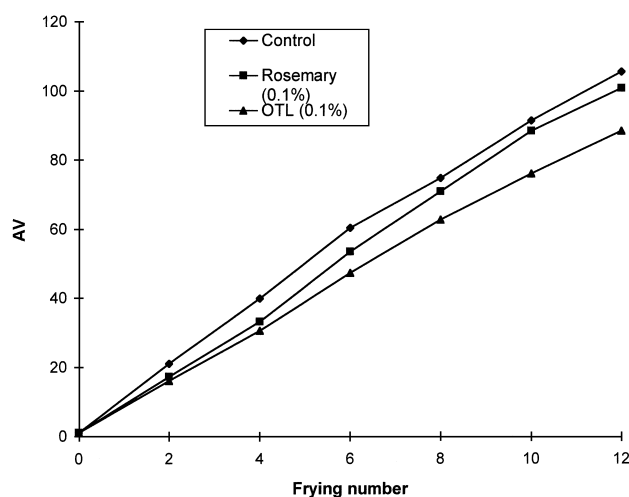


Fig. 3. Effect of additives on the deterioration of rapeseed oil used for repeat frying at 180°C, assessed by the change in *p*-anisidine value.

nificantly different ($p < 0.01$) from the samples with added rosemary or OTL extract, based on four replicate analyses.

The antioxidant activities of extracts from old and young tea leaves are not significantly different when assessed in rapeseed oil at 60°C (Zandi and Gordon, 1995). Hence it is clear that old tea leaves, which at present are considered as agricultural waste, contain antioxidants that may usefully be extracted and added to foods.

References

- Anon (1997) The benefits of green tea. *Food Ingredients and Analysis International* **19**(1), 16–17.
- AOCS (1989) *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn, ed. D. Firestone, AOCS, Champaign.
- Chen, Y. Z., Chan, P. T., Ma, H. M., Fung, K. P. and Wang, J. (1996) Antioxidant effect of ethanol tea extracts on oxidation of canola oil. *Journal of the American Oil Chemists' Society* **73**, 375–380.

- Frankel, E. N. (1993) In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends in Food Science and Technology* **4**(7), 220–225.
- Gutierrez-Rosales, F., Garrido-Fernandez, J., Gallardo-Guerrero, L., Gandul-Rojas, B. and Minguez-Mosquera, M. I. (1992) Action of chlorophylls on the stability of virgin olive oil. *Journal of the American Oil Chemists' Society* **69**, 866–871.
- Hertog, M. J. L., Fresken, E. J. M., Hollman, P. C. H., Katan, M. B. and Kromhout, D. (1993) Dietary antioxidative flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **342**, 1007–1011.
- IUPAC (1991) *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn, 1st supplement, Blackwell Scientific Publications, Oxford.
- Jadhav, S. J., Nimbalkar, S. S., Kulkarni, A. D. and Madhavi, D. L. (1996) Lipid oxidation in biological and food systems. In *Food Antioxidants*, eds. D. L. Madhavi, S. S. Deshpande and D. K. Salunkhe, pp. 5–63. Marcel Dekker, New York.
- Nanjo, F., Goto, K., Suzuki, M., Sakai, M. and Hara, Y. (1996) Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radicals in Biology and Medicine* **21**, 895–902.
- Quartley, B. J. P., Clifford, M. N., Walker, R. and Williams, C. M. (1994) Antioxidant activity of green tea *in vivo*. SCI lecture paper 0029, pp. 1–8. Society of Chemical Industry, London.
- Rice-Evans, C. A. and Miller, N. J. (1996) Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Society Transactions* **24**, 790–795.
- Salah, N., Miller, N. J., Parganga, G., Tifburg, L., Bolwell, G. P. and Rice-Evan, C. (1995) Polyphenolic flavonols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics* **322**, 339–346.
- Shahidi, F., Ke, P. J., Zhao, X., Yang, Z. and Wanasundara, P. K. (1992) Antioxidative activity of green and black tea in meat model systems. In *Proceedings of the 38th International Congress of Meat Science and Technology*, Aug 23–28, pp. 599–602. Anonymous, Clermont-Ferrand, France.
- Wanasundara, U. N. and Shahidi, F. (1996) Stabilisation of seal blubber and menhaden oils with green tea catechins. *Journal of the American Oil Chemists' Society* **73**, 1183–1190.
- Zandi, P. and Gordon, M. H. (1995) Stabilisation of rapeseed oil by green tea extracts. In *Proceedings of the International Tea Symposium*, pp. 216–227. Anonymous, Shanghai, China.
- Zhao, B., Li, X., He, R., Cheng, S. and Wenjuan, X. (1989) Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophysics* **14**, 175–185.