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Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea

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The antioxidant activity of aqueous extracts of rooibos tea (unfermented, semi-fermented and fermented) was compared with that of green, oolong and black teas. The α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging and β-carotene bleaching methods were used to determine the antioxidant activity of extracts prepared in a similar manner and diluted to the same amount of soluble solids. All the tea extracts were strong inhibitors of β-carotene bleaching as well as highly active hydrogen donors to the DPPH radical. Antioxidant activity as assessed with the β-carotene bleaching method decreased in the order: green > black > oolong > fermented rooibos > unfermented rooibos > semifermented rooibos. However, antioxidant activity as assessed by the DPPH radical scavenging method decreased in the order: green > unfermented rooibos > fermented rooibos > semifermented rooibos > black > oolong. © 1997 Elsevier Science Ltd

INTRODUCTION

The consumption of tea, especially green tea, has been found to reduce the risk of various cancers in humans (Worthy, 1991). Interest has now moved to other 'teas' such as maté (Gugliucci & Stahl, 1995) and rooibos tea (*Aspalathus linearis*) (Ito *et al.*, 1991; Komatsu *et al.*, 1994; Yoshikawa *et al.*, 1990), which are also believed to have health-giving properties. The health aspects of rooibos tea are mainly linked to its phenolic content and associated antioxidant activity (Niwa & Miyachi, 1986).

Leafy materials, such as tea, are well known as rich sources of flavonoids and phenolic acids and are recognized as a major source of flavonoids in the diet (Hertog *et al.*, 1993). The phenolic composition of a tea extract is greatly influenced by processing or, more specifically, fermentation. Catechins, the major phenolic group in green tea, undergo oxidation to form theaflavins and thearubigens in the manufacture of black tea (Ho *et al.*, 1992; Yen & Chen, 1995; Yeo *et al.*, 1995; Xie *et al.*, 1993). Rooibos tea contains only very small quantities of (+)-catechin (Marais, 1996). Aspalathin, a dihydrochalcone, constitutes the major flavonoid of unfermented rooibos tea, while most of it is oxidized during fermentation (Joubert, 1996) to flavanones and unknown polymeric substances (Marais, 1996).

The aims of this study were to determine whether fermentation affects the antioxidant activity of rooibos tea and to compare its antioxidant activity to that of green, oolong and black teas.

MATERIALS AND METHODS

Plant material and chemicals

Tween 40, *trans*-β-carotene, linoleic acid (purity approx. 99%) and α,α-diphenyl-β-picrylhydrazyl (DPPH) radical were purchased from Sigma Chemical Co. (St Louis, USA). Five samples of green tea—Gunpowder, Japanese Sen-cha, Japanese Green Leaf, Kokeisha Japanese Green Leaf (slightly smokey), Sen-cha Ban-cha (Japanese Green)—were bought at speciality tea shops. Four different brands of oolong tea, including two samples of Formosa oolong, were also purchased from speciality tea shops. Seven samples of popular brand names of black tea and rooibos tea (Super, Choice and Standard grade) each were purchased at a local supermarket. Five batches of fresh green rooibos tea were harvested at

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different plantations in the tea-producing area and freeze-dried without comminution. The freeze-dried material was ground with a Retch mill. Harvesting of seven batches of tea for the manufacture of semifermented rooibos tea took place at the same plantation, but at different locations within the plantation. The tea was comminuted to approx. 4 mm lengths to initiate oxidation. Oxidation was allowed to take place for approximately 3 h before freezing at −30°C, followed by freeze-drying.

Preparation of aqueous tea infusions

Tea extracts were prepared by pouring boiling distilled water (1000 ml) onto the dry leaves (50 g), followed by stirring with a magnetic stirrer (Heidolph) for 10 min and additional steeping for 30 min at room temperature. The extracts were strained and filtered through nylon mesh (120 μm), followed by filter paper (Whatman No. 54) with a Buchner filter under vacuum, and cooled to room temperature. Aliquots of the extracts were kept frozen (−18°C) until further use.

Determination of total polyphenol, flavonoid and total water-soluble solids contents of aqueous infusions

Polyphenol analysis of extracts according to Singleton & Rossi (1965) before and after precipitation of flavonoids (Kramling & Singleton, 1969) gave total polyphenol, non-flavonoid phenols and, by difference, flavonoid contents of the extracts. Results were expressed as gallic acid equivalents per 100 g water-soluble solids. The total water-soluble solids content of an aliquot (20 ml) of extract was determined in duplicate (Joubert, 1988).

Determination of antioxidant activity with the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging method

The DPPH radical scavenging method of Brand-Williams *et al.* (1995) was modified as follows. The soluble solids content of the extracts was standardized to give stock solutions containing 50 mg soluble solids per 100 ml water. A methanolic solution (50 μl) of the antioxidant was placed in a cuvette and a 6×10^{-5} M methanolic solution of DPPH (2 ml) was added. The decrease in absorbance at 515 nm was determined continuously with data capturing at 6 s intervals with a Beckman DU-65 spectrophotometer and Data Capture Software, until the absorbance stabilized (±70 min). The absorbance of the DPPH radical stock solution was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution as recommended by Blois (1958). All determinations were performed in duplicate. The inhibition (%) of the DPPH radical was calculated from absorbance data according to Yen and Duh (1994).

Determination of antioxidant activity with the β-carotene bleaching method

Antioxidant activity of the tea extracts and the control was determined using a modified version of the β-carotene bleaching method of Pratt (1980). Sonification of the emulsion was carried out for 1 min with a Branson Sonifier Cell Disruptor B15 (16% duty cycle; continuous output control of 3) to improve its stability at high temperatures (Frankel *et al.*, 1994). Tea extract (200 µl) containing 500 mg soluble solids per 100 ml water was used for analysis. Readings of all samples were taken immediately ($t = 0$) and at 15 min intervals for 2 h ($t = 120$) on a Beckman DU-65 spectrophotometer at 470 nm. The vials containing the reaction mixture were placed in a waterbath at 50°C between measurements. All determinations were performed in duplicate. The antioxidant activity coefficient (AAC) was calculated from the data according to Mallet *et al.* (1994).

Statistical analysis

One-way analysis of variance was performed on the data using SAS (Release 6.04). Student's *t*-LSD (least significant difference) ($P = 0.05$) was calculated to compare means for the different teas.

RESULTS AND DISCUSSION

Antioxidant activity of tea extracts as determined by the DPPH radical method

Figure 1 illustrates the decrease in absorbance of the DPPH radical due to the scavenging ability of the soluble solids in the extracts of the different teas. All teas showed a rapid decrease in absorbance, with green and oolong teas exhibiting the fastest and slowest rate over

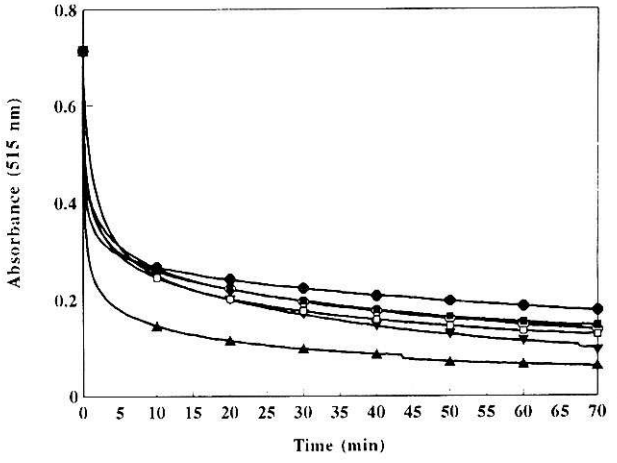

Fig. 1. Hydrogen-donating ability of different tea extracts (50 mg soluble solids per 100 ml water) as assessed by the DPPH radical method. ●, black; ■, oolong; ▲, green; ○, fermented rooibos; □, semifermented rooibos; ▼, unfermented rooibos.

the first 5 min, respectively (Fig. 1). The percentage inhibition of the tea extracts also followed this trend (Table 1). The higher the percentage inhibition, the greater the hydrogen-donating ability, and thus the antioxidant activity, of the tea soluble solids.

Hydrogen-donating ability of the tea extracts decreased in the order green > black > oolong ($P < 0.05$) (Table 1). Ho *et al.* (1992) found the same trend for the antioxidant activity of these teas with the Rancimat method. However, Yeo *et al.* (1995) found that oolong tea scavenged the DPPH radical better than green and black teas. Oolong tea used for their study contained the highest level of epigallocatechin gallate (Yeo *et al.*, 1995), which has been shown to be the most potent antioxidant of the flavanols of oolong tea (Xie *et al.*, 1993). The oxidation of the flavanols of green tea would greatly contribute to the difference in percentage inhibition between green, oolong and black teas. The gallocatechins, i.e. (+)-epigallocatechin and (+)-epigallocatechin gallate, which are potent antioxidants (Lunder, 1992; Xie *et al.*, 1993), are the first to be oxidized by polyphenol oxidases in the leaves because of their high oxidation potential and high concentration in green tea (Robertson, 1992). They are oxidized to form thearubigens and theaflavins, the major phenolic compounds of black tea (Robertson, 1992), which are less effective antioxidants than the flavanols (Xie *et al.*, 1993). This change in phenolic composition explains the lower inhibition of the DPPH radical by black tea compared with green tea (Table 1). Oolong tea, which is semifermented, no longer contains the major antioxidative gallocatechins but also does not yet contain a great amount of thearubigens and theaflavins which are found in fully fermented black tea (Ho *et al.*, 1992). Black tea also contains gallic acid (Ho *et al.*, 1992), a potent hydrogen donator to the DPPH radical (Brand-Williams *et al.*, 1995).

The water-soluble solids of oolong tea contained significantly less total polyphenols and flavonoids than green and black tea, with the latter teas having approximately the same total polyphenol and flavonoid contents (Table 2). This alone could explain the low DPPH radical scavenging ability of oolong compared with green and black teas.

Antioxidant activity of the different rooibos teas as determined by the DPPH radical scavenging method decreased in the order: unfermented rooibos > fermented rooibos > semifermented rooibos (Table 1). No significant difference in the percentage inhibition ($P > 0.05$) was detected. This was also reflected by the total polyphenol and flavonoid contents of the soluble solids (Table 2). However, the same trend in change of percentage inhibition of the DPPH radical was noted for both tea types with fermentation, although rooibos tea extracts were not affected to the same extent as those of *Camellia sinensis*.

Aspalathin, an active scavenger of DPPH (Von Gadow, 1996), is the major flavonoid of unfermented rooibos tea (Koeppen & Roux, 1966), but less than 7% of the aspalathin originally present in unfermented rooibos survives the fermentation process (Joubert, 1996). However, fermented rooibos tea was found to possess considerable antioxidant activity with a inhibition of 83.43% (Table 1). This antioxidant activity can thus be attributed to the presence of other active components such as oxidation products of aspalathin in the fermented tea. Aspalathin undergoes cyclization during oxidation to form the flavanones dihydro-orientin and dihydro-iso-orientin (Koeppen & Roux, 1966). According to Dziedzic *et al.* (1985), the dihydrochalcones are more effective as antioxidants than their corresponding flavanones, which could offer an explanation for this decrease in hydrogen-donating ability with fermentation. On the other hand, further oxidation of the flavanones to their corresponding flavones, orientin and

Table 1. Antioxidant activity of different teas as assessed with the DPPH radical scavenging and β-carotene bleaching methods

Type of tea	Inhibition[a] (%)	AAC[b]
Green	90.8a	695a[c]
Oolong	71.2c	597bc
Black	81.7b	650ab
Unfermented rooibos	86.6b	557cd
Semifermented rooibos	81.9b	522d
Fermented rooibos	83.4b	605bc
LSD ($P = 0.05$)	5.97	—

[a]Determined by DPPH radical scavenging method; mass ratio of tea solids to DPPH = 0.52.
[b]AAC, antioxidant activity coefficient as determined by β-carotene bleaching method; concentration of stock solution = 500 mg soluble solids per 100 ml solution.
Means within a column followed by the same lowercase letter are not significantly different at $P = 0.05$.
[c]Data have been log-transformed to obtain normal data. The means in this column are those derived from the normal data.
LSD, least significant difference.

Table 2. Total water-soluble solids (TWSS), total polyphenol and flavonoid contents of different teas

Type of tea	TWSS (g per 100 ml)	Total polyphenols[a] (%)	Flavonoids[a] (%)
Green	1.56a	34.9a	33.0a
Oolong	1.23b	24.9b	21.7b
Black	1.67a	33.9a	32.0a
Unfermented rooibos	1.09c	36.2a	32.6a
Semifermented rooibos	0.64d	35.6a	31.5a
Fermented rooibos	0.69d	35.6a	30.5a
LSD ($P = 0.05$)	0.11	2.76	2.75

[a]Expressed as % of TWSS.
Means within a column followed by the same lowercase letter are not significantly different at $P = 0.05$.
LSD = least significant difference.

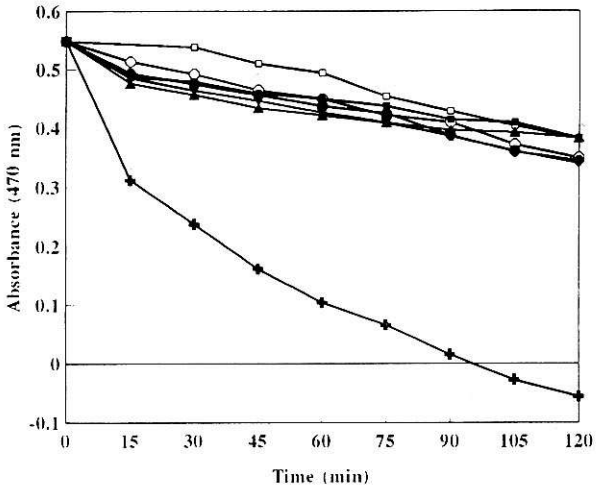

Fig. 2. Antioxidant activity of different tea extracts (500 mg soluble solids per 100 ml water) as assessed by the β-carotene bleaching method. ●, black; ■, oolong; ▲, green; ○, fermented rooibos; □, semifermented rooibos; ▼, unfermented rooibos; +, control).

iso-orientin, and polymeric products (Marais, 1996) could contribute to the slight increase in radical scavenging ability of fermented rooibos compared to semifermented rooibos tea (Table 1). The formation of an unsaturated bond between C-2 and C-3 in the case of the flavones would enhance the antioxidant activity (Herrmann, 1993).

The antioxidant activity of all the tea extracts tested using the DPPH radical scavenging method decreased in the order: green > unfermented rooibos > fermented rooibos > semifermented rooibos > black > oolong. Green tea was more potent than all the other teas tested ($P < 0.05$), while oolong was the poorest hydrogen donator of the six teas tested ($P < 0.05$). The antioxidant activity of the remainder of the teas did not differ statistically from each other.

Antioxidant activity of different teas using the β-carotene bleaching method

Figure 2 indicates the decrease in absorbance of β-carotene in the presence of different tea extracts with the coupled oxidation of β-carotene and linoleic acid. The control sample without the addition of tea extract oxidized most rapidly, while all extracts followed a similar strong inhibition of bleaching of β-carotene and subsequent decrease in absorbance. The antioxidant activity coefficients calculated from data given in Fig. 2 give the antioxidant activity of all the extracts tested in relation to one another (Table 1).

The antioxidant activity of tea extracts decreased in the order: green > black > oolong (Table 1). The decrease in antioxidant activity between green and oolong teas was statistically significant ($P < 0.05$), although antioxidant activity did not change significantly between green and black, and between black and oolong, tea. These results correlate well with those obtained using the DPPH radical method.

Antioxidant activity of rooibos tea extracts decreased in the order: fermented rooibos > unfermented rooibos > semifermented rooibos. There was a statistically significant increase in antioxidant activity between semifermented and fully fermented rooibos tea ($P < 0.05$), although unfermented rooibos tea did not differ significantly from either fermented or semifermented rooibos tea ($P > 0.05$).

Antioxidant activity of all the tea extracts tested using the β-carotene bleaching method decreased in the order: green > black > fermented rooibos > oolong > unfermented rooibos > semifermented rooibos. Green tea was thus significantly more potent in terms of antioxidant activity on a mass equivalent basis of soluble solids than all the other teas. Semifermented rooibos tea extract was the weakest inhibitor of β-carotene bleaching. In general, rooibos tea extracts seem to be weaker inhibitors of β-carotene bleaching than extracts of green, oolong and black teas, while they were found to be stronger DPPH radical scavengers than both black and oolong teas on a mass equivalent basis. Although the water-soluble solids of the different tea extracts contained approximately the same amount of total polyphenols and flavonoids, rooibos extracts contained fewer soluble solids than the green, oolong and black teas (Table 2). The water-soluble matter of rooibos tea leaves amount to less than half of that of black tea (Joubert, 1988). Thus, cup for cup, fermented rooibos tea would contain less antioxidant activity than green and black teas. However, preparation procedures can be manipulated (Von Gadow, 1996) to increase the antioxidant activity of rooibos tea. This also has implications for the use of the water-soluble solids of rooibos tea in therapeutic preparations.

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