Comparison of the Antioxidant Activity of Aspalathin with That of Other Plant Phenols of Rooibos Tea (*Aspalathus linearis*), α-Tocopherol, BHT, and BHA

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The antioxidant activity of aspalathin, the major flavonoid of unfermented rooibos tea, was compared with that of other polyphenols present in rooibos tea, α-tocopherol, BHT, and BHA using the β-carotene bleaching, α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging, and automated Rancimat methods. The phenolic compounds include the flavonoids vitexin, rutin, quercetin, luteolin, isoquercitrin, (+)-catechin, and the phenolic acids protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, syringic acid, and vanillic acid. (+)-Catechin had the longest induction period according to the Rancimat method, while BHT was the most effective inhibitor of β-carotene bleaching. Compounds with the highest degree of DPPH radical scavenging and highest rate of scavenging were caffeic acid and aspalathin, respectively. Aspalathin offered less protection against lipid oxidation than BHT and α-tocopherol according to the Rancimat and β-carotene methods, but it displayed a higher ability to scavenge the DPPH radical.

Keywords: *Antioxidant; aspalathin; polyphenols; rooibos; Rancimat; β-carotene bleaching; DPPH; BHA; BHT; α-tocopherol*

INTRODUCTION

Rooibos tea is made from the leaves and fine stems of *Aspalathus linearis*, which is indigenous to South Africa (Morton, 1983). Flavonoids (Figure 1) isolated from rooibos tea include aspalathin (Koeppen and Roux, 1966), orientin, iso-orientin (Koeppen and Roux, 1965), rutin, isoquercitrin (Koeppen *et al.*, 1962), vitexin, isovitexin, chrysoeriol, quercetin, luteolin (Rabe *et al.*, 1994), nothofagin, and (+)-catechin (Ferreira *et al.*, 1995). The phenolic acids (Figure 2) in rooibos tea consist of protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid (Rabe *et al.*, 1994), and syringic acid (Ferreira *et al.*, 1995). Most of these compounds are widely distributed in nature and have been shown to possess antioxidative properties (Pratt and Hudson, 1990; Ho *et al.*, 1992; Onyeneho and Hettiarachchy, 1992; Kanner *et al.*, 1994). Aspalathin, the major flavonoid of unfermented rooibos tea and constituting approximately 15 g/kg of the dry plant material (Joubert, 1996), is, however, unique to rooibos tea (Koeppen and Roux, 1966). Since there are no previous data on the antioxidant activity of this C−C-linked dihydrochalcone glycoside, determination of its activity in relation to that of other polyphenols and synthetic antioxidants is of interest.

Flavonoids have recently come under investigation for use as natural antioxidants in food preservation (Pratt and Hudson, 1990; Shahidi and Wanasundara, 1992) because of consumer resistance to synthetic antioxidants (Smith, 1991; Würtzen, 1993), as well as for their health-promoting properties in humans (Namiki, 1990; de Whalley *et al.*, 1990; Hertog *et al.*, 1993a,b).

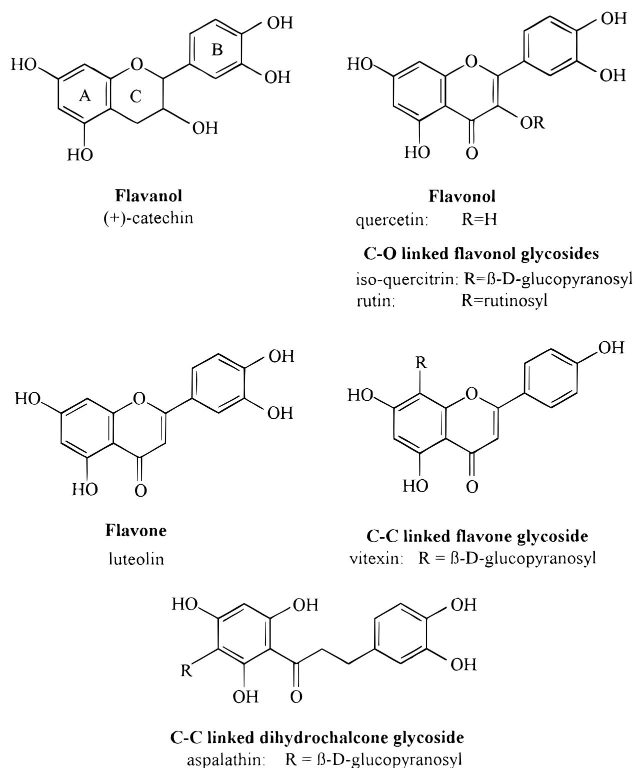

Figure 1. Structures of flavonoids present in rooibos tea.

The antioxidant activity of phenolic compounds is determined by their molecular structure and, more specifically, by the position and degree of hydroxylation of the ring structure. This influences the ability of the delocalization of unpaired electrons to stabilize the formed phenoxyl radical after reaction with the lipid radical (Gordon, 1990).

All flavonoids with the 3′,4′-dihydroxy configuration possess marked antioxidant activity (Dziedzic and Hud-

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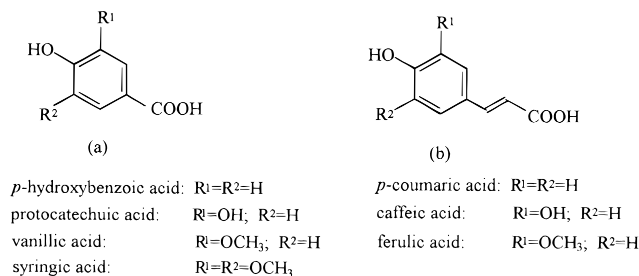

Figure 2. Hydroxy derivatives of benzoic acid (a) and cinnamic acid (b) present in rooibos tea.

son, 1983; Bermond, 1990; Herrmann, 1993). This activity increases with the number of hydroxyl groups substituted on the A- and B-rings (Kühnau, 1976; Husain *et al.*, 1987). Flavanones and flavones are less active than their corresponding chalcones, while the dihydrochalcones are more effective than their corresponding chalcones (Dziedzic and Hudson, 1983; Pratt and Hudson, 1990). There is as yet no certainty about the effect of the presence of a double bond between C2 and C3 on the antioxidant activity of flavonoids (Hudson and Lewis, 1983; Husain *et al.*, 1987; Pratt and Hudson, 1990; Herrmann, 1993). The presence of phenolic hydroxyl groups increases the antioxidant activity of phenolic acids, while methoxylation of the hydroxyl groups causes a decrease in activity (Marinova and Yanishlieva, 1992).

The aim of this study was to determine the antioxidant activity of phenolic acids and flavonoids found in rooibos tea with the emphasis on aspalathin in relation to the antioxidant activity of synthetic antioxidants. The β-carotene bleaching and α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging methods were used to determine antioxidant activity of equimolar concentrations of the compounds. Comparison of their relative efficiencies as antioxidants in an edible fat was made using the Rancimat method.

MATERIALS AND METHODS

Plant Material and Chemicals. Suppliers of chemicals were Sigma Chemical Co., St. Louis, MO [Tween 40, *trans*-β-carotene, linoleic acid (purity *ca.* 99%), protocatechuic acid, (+)-catechin, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, α-tocopherol, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and DPPH radical]; Fluka AG, Buchs, Switzerland (caffeic acid and ferulic acid); Carl Roth GmbH, Karlsruhe, Germany (isoquercitrin, vitexin, and luteolin); Merck, Darmstadt, Germany (rutin and quercetin); and Dr. Theodore Schuchardt, München, Germany (vanillic acid). Lard was purchased from Eskort Bacon Co-operative, Ltd., Natal, South Africa.

Aspalathin (purity *ca.* 95%) isolated from unprocessed rooibos tea was prepared and supplied in kind collaboration with Prof. D. Ferreira, Research Unit for Polyphenol and Synthetic Chemistry, University of the Orange Free State, Bloemfontein, South Africa.

Determination of Antioxidant Activity with the β-Carotene Bleaching Method. Antioxidant activity of aspalathin, vitexin, rutin, quercetin, luteolin, isoquercitrin, (+)-catechin, protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, syringic acid, vanillic acid, α-tocopherol, BHT, and BHA was determined according to a modified version of the β-carotene bleaching method of Pratt (1980). β-Carotene (0.1 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg), all dissolved in chloroform. After evaporation to dryness under vacuum at room temperature with a rotary evaporator, oxygenated distilled water (50 mL) was added and the mixture was emulsified for 1 min with a Branson Sonifier cell disruptor B15 at 16% duty cycle of a continuous output control of 3 to form emulsion A. Sonification of the emulsion was done to improve its stability at high temperatures (Frankel *et al.*, 1994). A stock solution of 1.3×10^{-3} M antioxidant in methanol was prepared, of which 200 μL was mixed with 5 mL of emulsion A in screw-capped vials, giving a final concentration of 5×10^{-5} M of antioxidant in the reaction mixture. A control consisting of 200 μL of water and 5 mL of emulsion A was prepared. A second emulsion (B) consisting of 20 mg of linoleic acid, 100 mg of Tween 40, and 50 mL of oxygenated water was also prepared. Water (200 μL), to which 5 mL of emulsion B was added, was used to zero the spectrophotometer. Blanks of the antioxidants were prepared using emulsion B instead of emulsion A. Readings of all samples were taken immediately (t = 0) and at 15 min intervals for 2 h (t = 120 min) on a Beckman DU-65 spectrophotometer at 470 nm. The vials were placed in a water bath at 50 °C between measurements. All determinations were performed in duplicate. The antioxidant activity coefficient (AAC) was calculated from the data with the formula (Mallet *et al.*, 1994)

$$\mathrm{AAC} = [(A_{\mathrm{A}(120)} - A_{\mathrm{C}(120)})/(A_{\mathrm{C}(0)} - A_{\mathrm{C}(120)})] \times 1000 \quad (1)$$

where $A_{\mathrm{A}(120)}$ is the absorbance of the antioxidant at t = 120 min, $A_{\mathrm{C}(120)}$ is the absorbance of the control at t = 120 min, and $A_{\mathrm{C}(0)}$ is the absorbance of the control at t = 0 min.

Determination of Antioxidant Activity with the DPPH Radical Scavenging Method. The antioxidant activity of aspalathin, vitexin, rutin, quercetin, luteolin, isoquercitrin, (+)-catechin, protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, syringic acid, vanillic acid, α-tocopherol, BHT, and BHA was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams *et al.*, 1995). A methanolic solution (50 μL) of the antioxidant was placed in a cuvette, and 2 mL of a 6×10^{-5} M methanolic solution of DPPH (at either 0.25 or 0.5 mol of antioxidant/mol of DPPH depending on strength of antioxidant) was added. Absorbance measurements commenced immediately. 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 absorbance stabilized (±16 min). Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant, *i.e.* the control, 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 percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994)

$$\%\ \mathrm{inhibition} = [(A_{\mathrm{C}(0)} - A_{\mathrm{A}(t)})/A_{\mathrm{C}(0)}] \times 100 \quad (2)$$

where $A_{\mathrm{C}(0)}$ is the absorbance of the control at t = 0 min and $A_{\mathrm{A}(t)}$ is the absorbance of the antioxidant at t = 16 min.

Determination of Antioxidant Activity with the Rancimat Method. The induction periods of lard as affected by the addition of aspalathin, rutin, quercetin, isoquercitrin, (+)-catechin, protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, α-tocopherol, and BHT were determined according to the method of Ho *et al.* (1992) with slight modifications. A Rancimat Model 679 (Metrohm AG, Switzerland) was used for the oxidation of lard with and without addition of antioxidants. Oxidation was carried out at 90 °C with an air flow rate of 20 L/h. A methanolic solution (100 μL) of the antioxidants was added to the lard (2.5 g), giving a final concentration of 0.02% (mass/mass) of antioxidant.

RESULTS AND DISCUSSION

Antioxidant Activity of Compounds According to the β-Carotene Bleaching Method. Figures 3 −6 show the decrease in absorbance of β-carotene in the presence of different antioxidants with the coupled

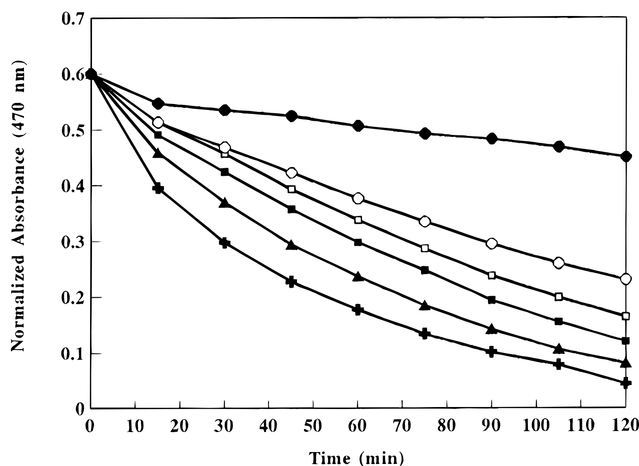

Figure 3. Antioxidant activity of pure compounds (5 × 10^{-5} M) found in rooibos tea as assessed with the β-carotene bleaching method. BHT was used as a reference compound. (+) Control; (■) *p*-coumaric acid; (▲) (+)-catechin; (○) ferulic acid; (□) syringic acid; (●) BHT.

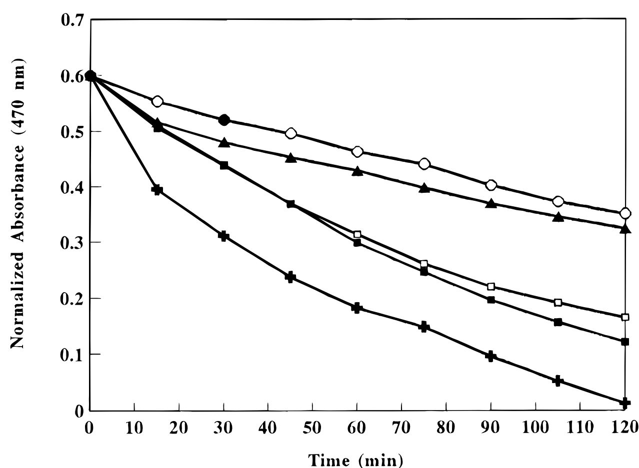

Figure 4. Antioxidant activity of pure compounds (5 × 10^{-5} M) found in rooibos tea as assessed with the β-carotene bleaching method. α-Tocopherol was used as a reference compound. (+) Control; (■) *p*-hydroxybenzoic acid; (△) α-tocopherol; (○) luteolin; (□) isoquercitrin.

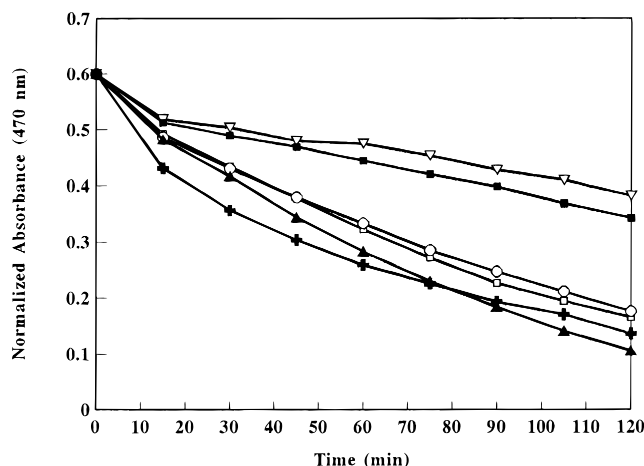

Figure 5. Antioxidant activity of pure compounds (5 × 10^{-5} M) found in rooibos tea as assessed with the β-carotene bleaching method. BHA was used as a reference compound. (+) Control; (■) quercetin; (▲) caffeic acid; (○) rutin; (□) protocatechuic acid; (▽) BHA.

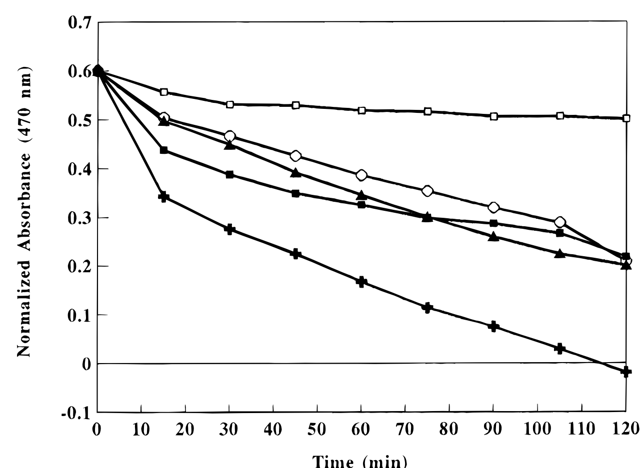

Figure 6. Antioxidant activity of pure compounds (5 × 10^{-5} M) found in rooibos tea as assessed with the β-carotene bleaching method. BHT was used as reference compound. (+) Control; (■) aspalathin; (▲) vitexin; (○) vanillic acid; (□) BHT.

oxidation of β-carotene and linoleic acid. Different bleaching rates were demonstrated for the antioxidants, with BHT noticeably the most effective antioxidant in this model system. In all cases, except for caffeic acid, it was demonstrated that the control sample without addition of antioxidant oxidized most rapidly. Caffeic acid showed antioxidant activity up to 75 min of reaction time, thereafter showing pro-oxidant activity as indicated by absorbance lower than that of the control. The AAC values calculated from data given in Figures 3−6 allow comparisons of the relative activity of the different antioxidants to be made (Table 1).

Antioxidant activity of the phenolic acids as determined by the β-carotene bleaching method decreased in the order vanillic acid > ferulic acid > syringic acid > *p*-hydroxybenzoic acid > *p*-coumaric acid > protocatechuic acid > caffeic acid (Table 1). In all cases the benzoic acid derviatives were more effective as antioxidants than their cinnamic acid analogues (vanillic acid > ferulic acid; *p*-hydroxybenzoic acid > *p*-coumaric acid; protocatechuic acid > caffeic acid). However, Pratt (1980), using the β-carotene bleaching method, demonstrated the relative efficiencies in the sequence caffeic acid > ferulic acid > *p*-coumaric acid, which is in agreement with a test carried out with bulk oil (Cuvelier *et al.*, 1992). A possible explanation for the difference in efficiencies found in this study may be the sonification of the emulsion, which was not used by Pratt (1980). However, according to Porter (1980), small oil droplets and the lipophilic nature of antioxidants would improve their efficiencies in oil-in-water emulsions. On the other hand, a high surface to volume ratio, where the surface is nearly the whole phase (Porter, 1980), and poor solubility of the lipophilic antioxidant in the aqueous phase may explain the contradictory results. The reverse order was shown not only by the more lipophilic cinnamic acids compared with their benzoic acid analogues but also by syringic acid in comparison with vanillic acid. Syringic acid with two *o*-methoxy moieties, which would increase the lipophilic nature of the molecule, was less effective than vanillic acid with one *o*-methoxy moiety. Since linoleic acid was used as a substrate for the β-carotene bleaching method, the greater acidity of the benzoic acid derivatives could account for their greater antioxidant activity in the weak acidic medium (pH ≈5). The carboxylate anion would associate strongly with the linoleic acid carboxylic acid group.

Within a group, *i.e.* benzoic acids or cinnamic acids, further distinction in terms of antioxidant activity could

Table 1. Antioxidant Activity of Pure Compounds Found in Rooibos Tea As Assessed with the β-Carotene Bleaching, DPPH Radical Scavenging, and Rancimat Methods

compound	AAC[a]	% inhibition[b]	induction time[c] (h)
control[d]			0.63
α-tocopherol	491.83 (14)[e]	75.10* (9)[e]	3.16 (6)[e]
aspalathin	357.14 (12)	91.74* (13)	2.55 (4)
BHA	509.65 (15)	69.16 (7)	
BHT	698.45 (17)	45.58 (5)	4.08 (7)
caffeic acid	−66.67 (1)	93.65* (17)	18.85 (10)
(+)-catechin	65.41 (3)	92.65* (15)	27.23 (12)
ferulic acid	328.16 (10)	64.67 (6)	1.26 (2)
isoquercitrin	224.14 (8)	91.99* (14)	4.17 (8)
luteolin	522.69 (16)	90.85* (11)	
p-coumaric acid	135.26 (5)	8.04 (2)	1.08 (1)
p-hydroxybenzoic acid	175.14 (6)	8.52 (3)	1.29 (3)
protocatechuic acid	52.63 (2)	79.80* (10)	15.50 (9)
quercetin	455.26 (13)	93.27* (16)	26.93 (11)
rutin	80.70 (4)	91.18* (12)	3.02(5)
syringic acid	202.88 (7)	85.80 (8)	
vanillic acid	350.65 (11)	20.66 (4)	
vitexin	306.28 (9)	7.26 (1)	

[a] AAC, antioxidant activity coefficient as determined with β-carotene bleaching method; concentration of antioxidant in emulsion = 5×10^{-5} M. [b] Determined by DPPH radical scavenging method; mole ratio of antioxidant/DPPH = 0.5, except * = 0.25. [c] Determined by Rancimat method; concentration of antioxidant = 0.02% (mass/mass) of lard. [d] Pure lard. [e] Figure in parentheses denotes rank in ascending order of antioxidant activity.

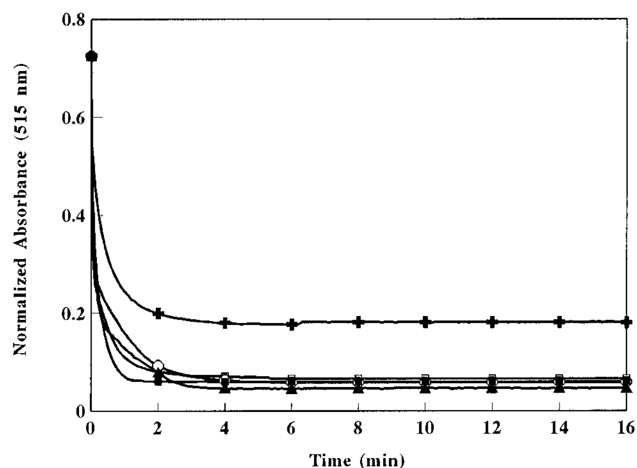

Figure 7. Hydrogen donating ability of pure compounds (0.25 mol/mol DPPH) found in rooibos tea. α-Tocopherol was used as a reference compound. (+) α-Tocopherol; (■) aspalathin; (▲) caffeic acid; (○) isoquercitrin; (□) luteolin.

be made on the basis of the number of hydroxyl and methoxy groups. Addition of *o*-methoxy groups in the case of syringic, ferulic, and vanillic acid increased antioxidant activity due to improved stabilization of the phenoxyl radical (Cuvelier *et al.*, 1992). However, addition of an *o*-hydroxyl group decreased antioxidant activity (protocatechuic acid < *p*-hydroxybenzoic acid; *p*-coumaric acid < caffeic acid), which is contrary to previous results (Pratt, 1980; Cuvelier *et al.*, 1992). Here affinity of the antioxidant for the lipid and thus the lipophilic nature of the molecules proved to be the determining factor.

Antioxidant activity of flavonoids as determined with the β-carotene bleaching method decreased in the order luteolin > quercetin > aspalathin > vitexin > isoquercitrin > rutin > (+)-catechin (Table 1). Vitexin does not possess the 3′,4′-dihydroxy configuration or *m*-5,7-dihydroxylation of the A-ring, which provides good electron delocalization and stabilization of the phenoxyl

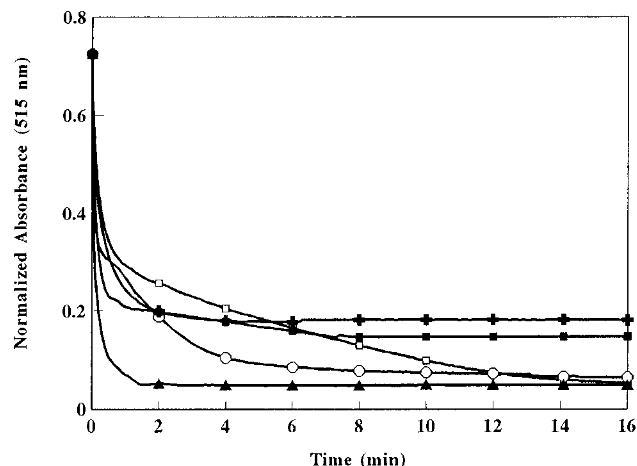

Figure 8. Hydrogen donating ability of pure compounds (0.25 mol/mol DPPH) found in rooibos tea. α-Tocopherol was used as a reference compound. (+) α-Tocopherol; (■) protocatechuic acid; (▲) quercetin; (○) rutin; (□) (+)-catechin.

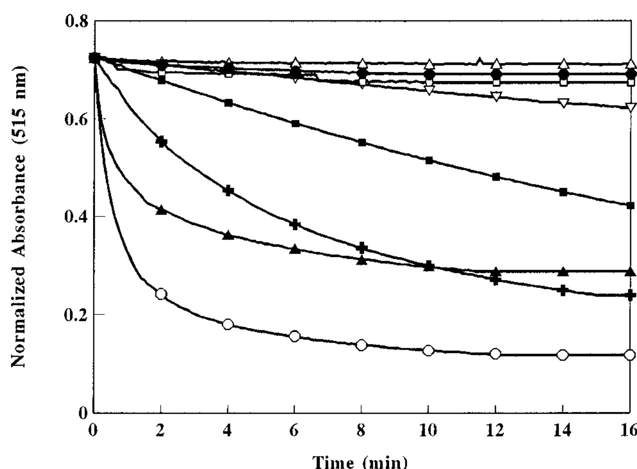

Figure 9. Hydrogen donating ability of pure compounds (0.5 mol/mol DPPH) found in rooibos tea. BHT and BHA were used as reference compounds. (+) BHA; (■) BHT; (▲) ferulic acid; (●) *p*-coumaric acid; (□) *p*-hydroxybenzoic acid; (○) syringic acid; (▽) vanillic acid; (△) vitexin.

radical. The presence of the C2−C3 double bond and C4 keto group seems to be essential for high antioxidant activity (quercetin > (+)-catechin). Vitexin, with only one hydroxyl group on the B-ring (Figure 1), was found to be a weaker antioxidant than luteolin, quercetin, and aspalathin (Figure 9). The relatively high activity of aspalathin may be due to the 2′,4′,6′-hydroxyl groups of the A-ring, as well as the keto−enol equilibrium (Rabe *et al.*, 1994).

Quercetin, which is an aglycon, is a more potent antioxidant than both its glycosides, isoquercitrin and rutin (Table 1), which possess a monosaccharide and disaccharide substituent, respectively (Figure 1). According to Pratt (1980), C3 glycosides possess approximately the same antioxidant activity as the corresponding aglycon when substitution is with a monosaccharide. In the case of a disaccharide, antioxidant activity is reduced. It was, however, also noted that considerable importance is attached to the free 3-hydroxyl by some researchers such as is found in quercetin. It was postulated that the 3-hydroxyl and the C2−C3 double bond would allow the molecule to undergo isomer changes to diketo forms, which would possess a highly reactive CH group at position 2. The presence of the 3-hydroxyl in the case of quercetin did

not increase its antioxidant activity compared to luteolin, which is in agreement with findings by Lee *et al.* (1995).

Antioxidant activity of all compounds tested using the β-carotene bleaching method decreased in the order BHT > luteolin > BHA > α-tocopherol > quercetin > aspalathin > vanillic acid > ferulic acid > vitexin > isoquercitrin > syringic acid > *p*-hydroxybenzoic acid > *p*-coumaric acid > rutin > (+)-catechin > protocatechuic acid > caffeic acid (Table 1). BHT acted as the best inhibitor of β-carotene bleaching, while caffeic acid was the poorest. Aspalathin was more potent than all of the phenolic acids but less effective than any of the reference standards. On the whole, the reference standards BHT, BHA, and α-tocopherol were better inhibitors of β-carotene bleaching than the phenolic acids and flavonoids present in rooibos tea that were tested (Table 1).

Antioxidant Activity of Compounds According to the DPPH Radical Scavenging Method. In Figures 7–9 the decrease in absorbance of the DPPH radical (DPPH$^•$) due to its reduction by different antioxidants is illustrated. Absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H. The more rapidly the absorbance decreases, the more potent the antioxidant activity of the compound in terms of hydrogen donating ability (Yen and Duh, 1994). Almost no decrease in absorbance occurred with the addition of *p*-coumaric acid, *p*-hydroxybenzoic acid, and vitexin, while vanillic acid showed only a slight decrease (Figure 9). Brand-Williams *et al.* (1995) also found that *p*-coumaric acid and vanillic acid react poorly with the DPPH radical. Other compounds, including protocatechuic acid, luteolin, rutin, aspalathin, isoquercitrin, (+)-catechin, quercetin, and caffeic acid, resulted in such a rapid decrease in absorbance when present at 0.5 mol/mol DPPH radical that analysis was performed at 0.25 mol/mol DPPH radical to discriminate between these compounds (Figures 7 and 8). The percentage inhibition of the DPPH radical by the compounds tested is given in Table 1.

Antioxidant activity of phenolic acids as determined by the DPPH radical scavenging method decreased in the order caffeic acid > protocatechuic acid > syringic acid > ferulic acid > vanillic acid > *p*-hydroxybenzoic acid > *p*-coumaric acid (Table 1). This is in agreement with results obtained by Brand-Williams *et al.* (1995), who demonstrated that the number of DPPH radical molecules reduced by the phenolic acids decreased in the order caffeic acid > protocatechuic acid > ferulic acid > vanillic acid ~ *p*-coumaric acid. From this sequence it can be concluded that cinnamic acid derivatives are better antioxidants than their benzoic acid counterparts (ferulic acid > vanillic acid; caffeic acid > protocatechuic acid). This can be explained in terms of the CH=CHCOOH group, which participates in stabilizing the radicals of cinnamic acid derivatives by resonance (Cuvelier *et al.*, 1992). Brand-Williams *et al.* (1995) also found cinnamic acid derivatives to be better scavengers of the DPPH radical than their benzoic acid analogues.

The percentage inhibition increased with ortho substitution of monophenols with a methoxy group, which acts as electron donor. Substitution with a hydroxyl group was more effective than with a methoxy group (protocatechuic acid > vanillic acid > *p*-hydroxybenzoic acid; caffeic acid > ferulic acid > *p*-coumaric acid). Syringic acid has a higher percentage inhibition than vanillic acid, since two methoxyl groups are better for electron delocalization than one (Gordon, 1990).

Antioxidant activity of flavonoids as determined by the DPPH radical scavenging method decreased in the order quercetin > (+)-catechin > isoquercitrin > aspalathin > rutin > luteolin > vitexin. All flavonoids except vitexin were analyzed at 0.25 mol/mol DPPH radical, indicating potent hydrogen donating abilities. The absence of the 3′,4′-dihydroxy configuration in vitexin explains the lower hydrogen donating capacity of this flavonoid. The absence or presence of the C2–C3 double bond and C4 keto group does not seem essential for high DPPH radical scavenging ability [(+)-catechin ~ quercetin]. The presence of a glycoside resulted in a decrease in antioxidant activity (quercetin > isoquercitrin > rutin) (Table 1), as was also noted from the results of the β-carotene bleaching method.

Antioxidant activity of all compounds tested using the DPPH radical scavenging method decreased in the order caffeic acid > quercetin > (+)-catechin > isoquercitrin > aspalathin > rutin > luteolin > protocatechuic acid > α-tocopherol > syringic acid > BHA > ferulic acid > BHT > vanillic acid > *p*-hydroxybenzoic acid > *p*-coumaric acid > vitexin (Table 1). Caffeic acid had the highest hydrogen donating capacity toward the DPPH radical, while vitexin had the poorest. Aspalathin was found to be a more potent DPPH radical scavenger than all of the reference standards and all of the phenolic acids except caffeic acid.

Antioxidant Activity of Compounds According to the Rancimat Method. Table 1 gives the induction times of lard as affected by the addition of different antioxidants. The higher the induction period of the lard with antioxidant added, compared to the control (pure lard), the better the antioxidant activity of that compound. All compounds tested resulted in an increase in induction time. Compounds were tested on a mass equivalent basis to determine their efficiency as antioxidants in edible fats at the maximum level of 0.02% (mass/mass) allowed.

Antioxidant activity of phenolic acids as determined with the Rancimat method decreased in the order caffeic acid > protocatechuic acid > *p*-hydroxybenzoic acid > ferulic acid > *p*-coumaric acid. These data correlate well with those obtained by Marinova and Yanishlieva (1992), who also used a mass equivalent basis.

A longer induction time was obtained for caffeic acid than for protocatechuic acid, its benzoic acid analogue, due to better electron delocalization (Gordon, 1990). Polyhydroxylation of phenolic acids results in an increase in antioxidant activity (caffeic acid > *p*-coumaric acid; protocatechuic acid > *p*-hydroxybenzoic acid) (Table 1). This is substantiated by research of Marinova and Yanishlieva (1992). Methoxylation of a *m*-hydroxyl group adjacent to a *p*-hydroxyl group resulted in a shorter induction time (caffeic acid > ferulic acid), since fewer hydrogens are available for radical scavenging. However, addition of a methoxyl group in the meta position of the *p*-hydroxyl group increases the antioxidant activity (ferulic acid > *p*-coumaric acid) due to the strong electron delocalizing effect of the methoxy substituent (Gordon, 1990).

Antioxidant activity of flavonoids as determined with the Rancimat method decreased in the order (+)-catechin > quercetin > isoquercitrin > rutin > aspalathin. Hudson and Lewis (1983) found quercetin to have a much longer induction period than (+)-catechin

in lard at 100 °C when present in equimolar concentrations. Aspalathin compared poorly with quercetin as antioxidant of lard.

Antioxidant activity of compounds according to the Rancimat method decreased in the order (+)-catechin > quercetin > caffeic acid > protocatechuic acid > isoquercitrin > BHT > α-tocopherol > rutin > aspalathin > *p*-hydroxybenzoic acid > ferulic acid > *p*-coumaric acid. (+)-Catechin and *p*-coumaric acid had the longest and shortest induction periods, respectively. Both reference compounds, BHT and α-tocopherol, were poor antioxidants in terms of induction time, which compares well with the data obtained from the DPPH radical scavenging method. BHT is volatile at the high temperature of the test (90 °C) and is rapidly swept from the lipid. It is only able to exert its influence in the first stage of the test (Rossell, 1989), which would account for its short induction time. Aspalathin had a shorter induction time than the reference compounds, but was again found to be a better antioxidant than all of the phenolic acids, except caffeic acid. When the molecular mass is taken into account, the concentration of caffeic acid was 2.5 times that of aspalathin, while the induction time of caffeic acid was more than 7 times that of aspalathin, which means that caffeic acid is a better antioxidant than aspalathin even when molecular mass is taken into account.

Aspalathin and the other polyphenols present in rooibos tea were found to be potent antioxidants, comparable in activity with α-tocopherol and the widely used synthetic antioxidants BHT and BHA. This should merit further investigation of the use of enriched rooibos tea extracts as natural antioxidants in food.

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