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## Production and quality aspects of rooibos tea and related products.

### A review

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#### Summary

Use of the herbal tea, rooibos, made from the indigenous South African fynbos plant, *Aspalathus linearis* spp. *linearis*, has shown tremendous growth on the international markets since the 1990s. From a small beginning in 1904, solely depended on wild harvesting, the industry has developed out of the selected and cultivated Nortier type, leading to improved quality. Traditional rooibos is processed, entailing an oxidation („fermentation“) step, essential to develop the characteristic sweetish flavour and red-brown colour. Higher anti-oxidant levels for unfermented rooibos resulted in the development of green rooibos and extracts enriched in aspalathin, a potent anti-oxidant unique to rooibos. Major markets for rooibos extracts are ready-to-drink iced teas and cosmetic products.

#### Introduction

The use of rooibos tea (*Aspalathus linearis*) spans at least more than 230 years and is still enjoyed by an ever-increasing market. In 1772, the botanist, Carl Thurnberg, during a visit to Africa, reported that indigenous people of the Cape, the Khoi, used the rooibos plant to make a beverage. The plant is indigenous to South Africa and forms part of the fynbos biome, one of six floral kingdoms. In the early 1900s the tea came to the attention of Benjamin Ginsberg, a merchant of Clanwilliam. He bought the tea for marketing from descendants of the Khoi in the region who, during the summer months, harvested the rooibos plants growing wild in the mountains.

In the early 1930s the first experiments with cultivation of rooibos took place. By the Second World War, the demand for rooibos tea had escalated due to a shortage of Oriental tea. However, after the war, the rooibos tea market collapsed; poor and inconsistent quality tea, and overproduction made rooibos as crop uneconomical by 1953/54. These factors prompted the establishment of the „Rooibos Tea Control Board“ to stabilize the industry through orderly marketing and standardization of the tea quality. This one-channel marketing system was abolished in 1997, which opened the way for several marketing companies to enter the rooibos industry.

The worldwide demand for rooibos tea grew from 524 tons in 1955 to 10,600 tons in 2003, with exports comprising 6,400 tons. Most of the tea is exported to Germany (previously West Germany), where it was first sold in 1961 as „Rotbuschtee“ or „Massai-Tee“. Some tea was also exported to the USA and sold under the trade name ‘Kaffree tea’ (MORTON, 1983). Since 1984 rooibos was sold in Japan where its „anti-ageing“ properties were an important selling aspect. Recently, the opportunities, provided by a changing herbal tea market, stimulated the development of unfermented („green“) rooibos. Most of its production is sold as herbal tea or is used to prepare extracts for the food, beverage and cosmetic industries.

#### Botanical classification

The rooibos tea plant, *Aspalathus linearis* (Brum.f.) Dahlg., is unique to South Africa. *Aspalathus linearis* ssp. *linearis* is the most common

of the subspecies and includes those types used for tea production. The plants are erect or prostrate shrubs, growing up to 2 m tall in nature, with yellow flowers and needle-like leaves. DAHLGREN (1968) first described the variation in this species, giving a detailed account of the morphology and geographical distribution of the various wild forms and different types. The size, density of branching, development of short shoots, leaf size and flowering time of the species *A. linearis* vary considerably.

It is only the red type, divided into the selected and improved Nortier type (cultivated), and the wild-growing Cederberg type, with its broader and coarser leaves (MORTON, 1983) that is normally used for processing. Other types, i.e. the grey, black and red-brown types, were in the early years harvested in the wild for tea processing, and also today a small quantity of wild tea finds its way to the processors. Marketing of the grey and black types was discontinued by 1966 due to their poor quality (ANON., 1967). The black type gives an infusion that is not typically red-brown, nor is the flavour characteristic (COETZEE et al., 1953).

#### Cultivation

Commercial cultivation of the Nortier type rooibos occurs mainly in the Cederberg mountain region, but some tea is also cultivated in areas as far as Darling and Nieuwoudtville. The plants grow in deep, well-drained, sandy, acid soil (NOLTE, 1968). Being a legume, the plant has a well-nodulated root system for fixing elemental nitrogen from soil water. *In vitro* propagation was investigated for producing large numbers of homogenous plants (LE ROUX et al., 1992), but due to poor survival when planted out, plants are still propagated from seeds. Recent attempts to propagate rooibos from cuttings were also not successful (J. Brand, Rooibos Ltd, Clanwilliam, pers. comm., 2006). Seedlings, 100 to 150 mm high, are planted between June and August at approximately 8,000 to 10,000 plants per hectare. Branching is stimulated by topping the plant after about 8 months to a height of 30 cm. The first harvest takes place after 18 months during summer, but full production is only realized after 3 years. A fully-grown bush yields about 70 to 125 g of dry tea (J. Brand, Rooibos Ltd, pers. comm., 2006). CHENEY and SCHOLZ (1963) documented that the best harvest occurs in the fourth and fifth years after planting and that some of the bushes start to die after the seventh harvest. However, the present situation has changed drastically, with bushes already dying after the second harvest. This problem is caused amongst other by the two fungus species, *Diaporthe phaseolorum* and *Neocosmospora vasinfecta* (SMIT and KNOX-DAVIES, 1989). Insects that could cause considerable damage to the plants, if not effectively controlled, are the clearwing moth (Sesiidae), looper (*Isturgia exerraria*) and leafhopper (*Molopoterus theae* Theron). Chemical spaying is used to control these insects, but the demand for organically produced tea has prompted research into development of an integrated pest management programme. Biological control mechanisms such as the use of pheromone for the clearwing moth are investigated (J. Brand, Rooibos Ltd, pers. comm., 2006).

For the production of traditional rooibos („fermented“ tea) harvesting takes place in the summer, from December, until early autumn. Tea is harvested mechanically or manually with sickles by cutting the whole bush a few centimeter above the topping height. No or little flowers should be present since it gives an unpleasant flavour to rooibos tea. After harvesting the branches are bound in bundles for transport to the processing yard.

### Processing

The processing of traditional rooibos tea entails the following: Tea shoots are shredded into 3 to 4 mm lengths, placed in a „fermentation“ heap and bruised by late afternoon, followed by addition of water, further bruising and mixing. Bruising and addition of water are necessary to accelerate „fermentation“, which is initiated during shredding. The water also serves to extract polyphenols, acting to colour the stems when absorbed. Fermentation takes place during the night and the heap is spread open to dry in the sun the following morning. Fermentation can take from 8 to 24 h, with an average fermentation time of 12 to 14 h, depending on the climatic conditions, the composition of the plant material and processing conditions. During fermentation the aroma of the wet tea changes from resinous, hay-like and grassy to sweet, apple-like or honey-caramel to sour depending on the stage of fermentation. As soon as the characteristic sweet, honey-like aroma and red-brown colour of the tea are fully developed, the product is spread open in a thin layer (15-20 mm) to dry in the sun. The development of a sour aroma is indicative of over-fermentation. Brushing is done to break up any lumps and to accelerate drying, but excessive quantities of tea dust are formed which have to be separated later on. Before packaging the tea is

sieved to obtain the required fine cut, which is then steam-pasteurized to ensure that the tea meets the required microbial standards.

Since no control over individual processing parameters is possible with the traditional open-air fermentation method, controlled fermentation and drying of rooibos have been investigated as alternatives to ensure optimum and consistent quality (Joubert and de Villiers, 1997). However, this progress is resisted by industry due to cost implications in terms of capital expenditure and energy requirements.

Research showing unfermented rooibos to have higher antioxidant capacity than its traditionally processed counterpart (von Gadow et al., 1997a; Standley et al., 2001), stimulated the development of green rooibos (de Beer and Joubert, 2002) as an alternative rooibos product for the international market. With the processing of green rooibos, the oxidative changes should be kept to a minimum to obtain tea with a green colour. This is achieved, either by inactivation of enzymes by subjecting the tea to a steaming process or drying whole shoots at low temperatures and air humidity to a critical moisture content before shredding, followed by further drying to the required moisture content.

Other processing includes the preparation of extracts and powders. First developed in the 1980s (Joubert, 1988, 1990), it is only the past few years that aqueous extracts and extract powders, prepared from fermented tea, find application in the food and beverage industries (Anon., 2005a,b). A large selection of ready-to-drink beverages, either natural or flavoured, is offered on the market in South Africa, and has recently been introduced also to the European market. Other applications include candies, flavouring of a carob-based „chocolate“ and coating of honeybush tea leaves (*Cyclopia* spp.) with a rooibos extract to enhance its antioxidant activity.

**Tab. 1:** Comparison of the antioxidant activity of aqueous soluble solids of green and fermented rooibos

Assay	Endpoint	Green rooibos	Fermented rooibos	Reference(s)
DPPH*	% Scavenging <sup>a</sup>	86.6	83.4	VON GADOW et al., 1997b
	% Scavenging <sup>b</sup>	87.3	83.0	JOUBERT et al., 2004
	EC <sub>50</sub> <sup>c</sup>	2.33	3.62	adapted from STANDLEY et al., 2001
	EC <sub>50</sub> <sup>c</sup>	3.24	3.87	JOUBERT et al., 2004
	Rate of scavenging <sup>d</sup>	8.3 x 10 <sup>-4</sup>	7.35 x 10 <sup>-4</sup>	WINTERTON, 1999
O <sub>2</sub> <sup>e</sup>	IC <sub>50</sub> <sup>c</sup>	44.4	60.5	adapted from STANDLEY et al., 2001
	IC <sub>50</sub> <sup>c</sup>	69.4	78.3	JOUBERT et al., 2004
Linoleic acid emulsion	% Inhibition (CD) <sup>f</sup>	28.6	28.0	JOUBERT et al., 2005
β-Carotene-linoleic acid oxidation	AAC <sup>g</sup>	557	607	VON GADOW et al., 1997b
Sunflower oil-in-water emulsion	% Inhibition (peroxides) <sup>h</sup>	90.0	80.9	WINTERTON, 1999
	Induction time (PV) <sup>i</sup>	35	31	
	% Inhibition (CD) <sup>j</sup>	58.1	54.5	
Methyl linoleate micelles	% Inhibition of TBARS <sup>k</sup>	22.8	30.3	WINTERTON, 1999

<sup>a</sup> Scavenging (%) of DPPH\* (6 x 10<sup>-5</sup> M) after 2 h

<sup>b</sup> Scavenging (%) of DPPH\* (3.04 x 10<sup>-5</sup> M) after 20 min

<sup>c</sup> Effective concentration of soluble solids (mg) per ml reaction mixture to scavenge 50% of DPPH\*

<sup>d</sup> DPPH\* rate of scavenging (s<sup>-1</sup>), calculated during unsteady state conditions (time 0 – 3 min), expressed as the change in the absorbance at 515 nm over time.

<sup>e</sup> Concentration of soluble solids (mg) per ml reaction mixture required to inhibit 50% of NBT reduction

<sup>f</sup> Inhibition (%) of conjugated diene (CD) formation after 21 h incubation at 40°C

<sup>g</sup> Antioxidant activity

<sup>h</sup> Inhibition of peroxides after 35 days incubation at 30°C

<sup>i</sup> Time required for oxidation to reach a peroxide value (PV) of 10 meq/kg oil with incubation at 30°C

<sup>j</sup> Inhibition of CD formation after 31 days incubation at 30°C

<sup>k</sup> Inhibition of the formation of thiobarbituric reactive substances (TBARS) after 16 h incubation at 37°C

Extracts from green rooibos (TIEDTKE and MARKS, 2002; OTTO et al., 2003), containing high levels of the active principle, aspalathin, are produced for the nutraceutical and cosmetic industries. Green rooibos is preferably used because of its higher antioxidant activity (Tab. 1). Selective extraction of fermented rooibos also provides a product with enhanced antioxidant activity (VON GADOW et al., 1997b; JOUBERT et al., 2004), but the brownish dried extract is not suitable for the cosmetic industry.

### Quality standards and control

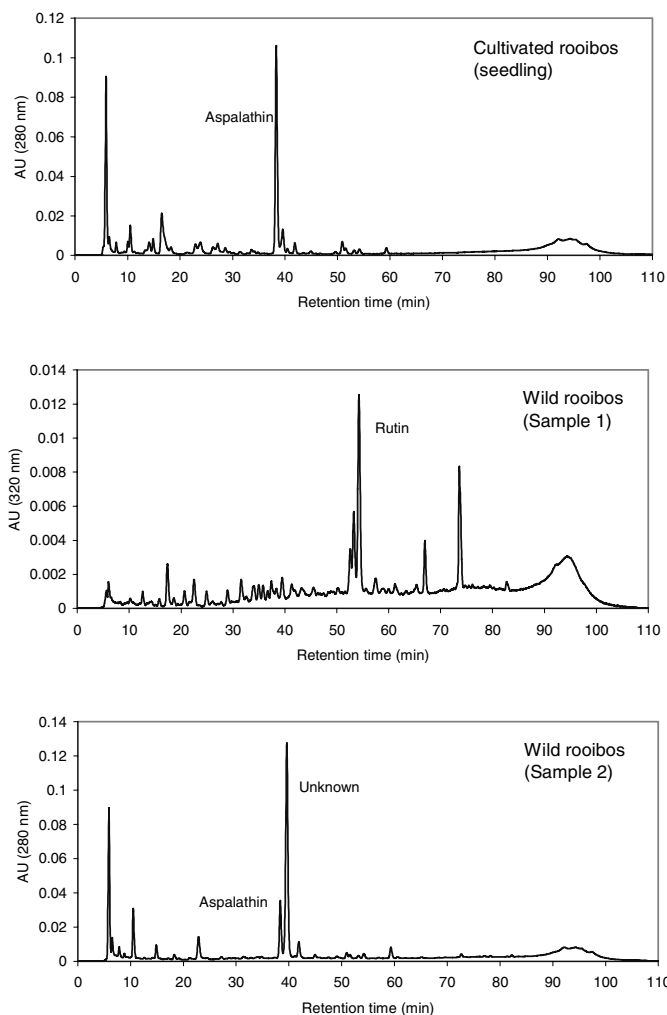
Apart from quality standards pertaining to microbial contamination and pesticide residue levels (ANON., 2000), no regulatory quality standards or requirements relating to composition, active compounds or antioxidant activity exist for traditional and green rooibos. Nevertheless, there is an increasing interest to offer green rooibos extracts with high levels of aspalathin for the cosmetic and functional food markets (B. Weinreich, Raps & Co, Kulmbach, Germany, pers. comm., 2003). However, the aspalathin content of green rooibos is not routinely determined by processors and presently visual inspection of colour serves as the only quality control parameter. The leaves should have a light green colour, giving a light yellowish to orange infusion. The development of a red-brown leaf colour is indicative of undesirable oxidation, and thus degradation of aspalathin in green rooibos. Usually less than 7% of the aspalathin content is retained after fermentation (JOUBERT, 1996). The lack of a rapid screening method for quantification of aspalathin content of green rooibos prompted the development of NIR (STEUER et al., 2000; SCHULZ et al., 2003; MANLEY et al., 2006), as well as RAMAN spectroscopy (unpublished data) prediction models. Their advantage is that measurement of aspalathin can be done directly on the dried, ground plant material.

Manufacturers of extracts have introduced product specifications such as minimum Total Polyphenol (TP) content and Total Antioxidant Activity (TAA) values. Green rooibos extract, standardized at 15% aspalathin content, and a fermented rooibos extract, standardized in terms of orientin content, have recently been introduced for the functional food market.

Another factor worth considering is authenticity. The increasing demand for organic rooibos has sparked renewed interest from farmers in rooibos growing in the wild. Some samples of wild rooibos, obtained from a processing yard, showed atypical HPLC profiles compared to the cultivated type (Fig. 1). Sample 1 contains rutin as major compound with little or no aspalathin whereas in sample 2 an unidentified dihydrochalcone or flavanone has been detected by HPLC-DAD as major compound. VAN HEERDEN et al. (2003) demonstrated large phenolic variation in wild populations of rooibos.

### Chemical composition

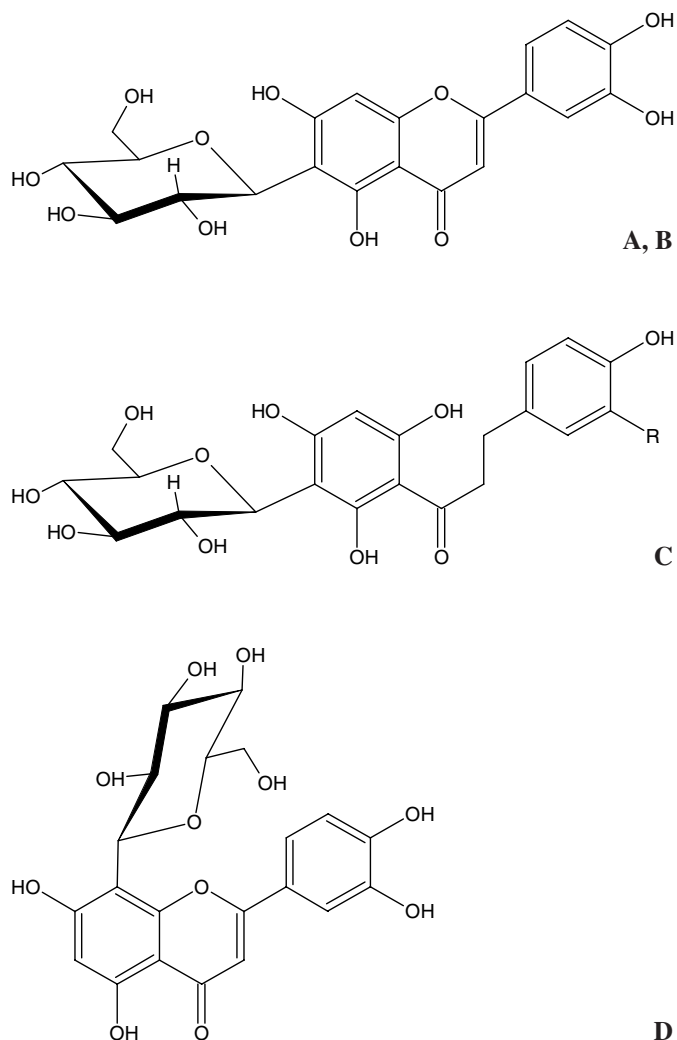
Rooibos tea is caffeine-free with a low tannin content compared to black tea (BLOMMAERT and STEENKAMP, 1978). Exhaustive extraction of the water extract with an organic solvent showed that it contains ca. 50% complex tannin-like substances (FERREIRA et al., 1998). The isolated tannin was shown to be an irregular heteropolymer of the procyanidin type with (+)-catechin and (-)-epicatechin as chain-extending units and only catechin as the terminal flavanol (FERREIRA et al., 1998; MARAIS et al., 1998). Other condensed tannin-type compounds are procyanidin B3 and the profisetidin triflavanoid, bis-fisetinidol- (4 $\beta$ ,6:4 $\beta$ B,8)-catechin which are present only at very low concentration (FERREIRA et al., 1995). Apart from the two major dihydrochalcones, aspalathin (structure elucidation by KOEPPEN and ROUX, 1966) and nothofagin (JOUBERT, 1996), the



**Fig. 1:** HPLC chromatogrammes of a rooibos seedling (sampled in the seed bed) and two samples of wild rooibos bushes obtained from a drying yard. HPLC analysis was performed according to the method described by JOUBERT et al. (2005)

flavones orientin, isoorientin (Fig. 2) (KOEPPEN and ROUX, 1965) vitexin, isovitexin, chrysoeriol (RABE et al., 1994) and luteolin (SNYCKERS and SALEMI, 1974) have been detected in rooibos, as well as the flavonols rutin, isoquercitrin (KOEPPEN et al., 1962), quercetin (SNYCKERS and SALEMI, 1974), hyperoside (BRAMATI et al., 2002), luteolin-7-*O*-glucoside (KAZUNO et al., 2005) and the flavanol (+)-catechin (FERREIRA et al., 1995) have been found in the plant material. A biomimetic study of the fermentation process showed oxidative conversion of aspalathin into the flavanones, (S)- and (R)-eriodictyol-6-C- $\beta$ -D-glucopyranoside (MARAIS et al., 2000). Traces of the flavanones, dihydroorientin and dihydro-isoorientin in fermented rooibos were noted by BRAMATI et al. (2002). The presence of the novel compound, 5,7-dihydroxy-6-C- $\beta$ -D-glucopyranosylchromone, suggests oxidative conversion of dihydro-isoorientin during fermentation (FERREIRA et al., 1995). The phenolic acids, isolated from fermented rooibos comprise the benzoic acids, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid, and the cinnamic acids, *p*-coumaric acid, ferulic acid and caffeic acid (RABE et al., 1994).

Whereas most of the flavonoids occur ubiquitously in the plant kingdom, until now aspalathin was only detected in rooibos, contributing to its novelty value. Analysis of 97 samples of rooibos,



**Fig. 2:** Structures of aspalathin (R = OH) and nothofagin (R = H) [A,B], orientin [C] and iso-orientin [D]

harvested from different bushes located in 24 plantations over the production area during winter time, showed large variation in the aspalathin and nothofagin content. Large variation was also noticed in the aspalathin content of 3-year-old bushes, planted on the same farm. This is mainly attributed to genetic variation of individual plants. The processed tea retains comparatively low quantities of aspalathin and nothofagin (Tab. 2), yet aspalathin, together with rutin, was shown to be one of the major flavonoids of an infusion prepared from fermented rooibos (BRAMATI et al., 2002). Quercetin, luteolin and chrysoeriol are often present in very low quantities in a cup of tea (TOYODA et al., 1997). The major compounds in tea powders prepared from fermented rooibos with a large percentage of stems comprised aspalathin, nothofagin, orientin, vitexin, isoquercitrin and rutin (Tab. 3).

#### Natural tea flavour and flavoured tea products

The aroma of green rooibos tea is grassy, hay-like, whereas the fermented tea has a sweet and sometimes pleasant caramel note. The typical flavour of fermented rooibos tea is influenced by numerous volatile constituents of different chemical groups such as hydrocarbons, alcohols, aldehydes, ketones, lactones, acids, esters, imides, phenols and furans (HABU et al., 1985; KAWAKAMI et al., 1993). Model

experiments applying dichloromethane extraction and simultaneous steam distillation and extraction (SDE) were carried out and the volatile substances were tentatively identified by GC-MS. The main components in the SDE extract were guaiacol, 2-phenylethanol, various ketones,  $\beta$ -damascenone and 6,10,14-trimethyl-2-pentadecanone, as well as acids such as acetic acid, 3-methylbutanoic acid, hexanoic acid and octanoic acid. While the dichloromethane tea extracts were found to contain many kinds of lactones such as 4-butanolide and dihydroactinidiolide, the SDE extracts lacked these aroma substances (KAWAKAMI et al., 1993). In spite of the fact that more than 120 volatile substances have been identified in rooibos tea extracts, no olfactory studies have been done to identify aroma substances responsible for the aroma profile of rooibos tea infusions.

Present available flavourings of rooibos tea are limited mainly to the aroma types „vanilla“ and „lemon“. Relating to the latest wellness trend, mixtures with some herbs such as rosemary or ginger are also offered. Beside this, rooibos tea flavoured with honey aroma is consumed in South Africa. This product should not be confused with honeybush tea, which is a herbal tea prepared from *Cyclopia* species.

#### Health value

The discovery by Annetjie Theron that rooibos tea contributes considerably to the convalescence of her 14-month-old, colicky baby, started off rooibos tea as a healthy drink. SNYCKERS and SALEMI (1974) attributed the anti-allergic effect of rooibos when administered to babies to the antispasmodic properties of quercetin and luteolin that would have a calming effect on the stomach. The indication that topical application of aqueous rooibos extract helps for skin problems such as eczema and nappy rash has resulted in development of special skin creams for babies and other natural cosmetic products. Previously, these products were limited to the South African market, but are now shipped worldwide (LEVY, 2004).

With the interest in natural antioxidants, attention also turned to rooibos. Antioxidant activity has been demonstrated for rooibos in a variety of *in vitro* test systems. Scavenging of the biologically relevant reactive oxygen species (ROS),  $O_2^{\cdot-}$ ,  $\cdot OH$  and  $H_2O_2$  (YOSHIKAWA et al., 1990; STANDLEY et al., 2001; LEE and JANG, 2004; JOUBERT et al., 2004), inhibition of lipid peroxidation (VON GADOW et al., 1997a, b; HITOMI et al., 1999; WINTERTON, 1999; JOUBERT et al., 2005) and scavenging of  $DPPH^{\cdot}$  and  $ABTS^{\cdot+}$  (BRAMATI et al., 2003; SCHULZ et al., 2003; JOUBERT et al., 2004; MANLEY et al., 2006) have been demonstrated. VON GADOW et al. (1997a) showed that green tea (*Camellia sinensis*) is more potent than green or fermented rooibos, applying the  $DPPH^{\cdot}$  and carotene-linoleic acid assays. This was confirmed by RICHARDS et al. (2001), using the  $ABTS^{\cdot+}$  and FRAP assays, taking the total polyphenol content of the aqueous extract into account. BRAMATI et al. (2003) showed that TAA of green rooibos leaves is ca. 50% of that of green and black tea. The TAA of green rooibos extracts correlates with their aspalathin content (BOTH, 2005). SCHULZ et al. (2003) find the apparent contribution of aspalathin to the TAA of green rooibos between 22 to 57%.

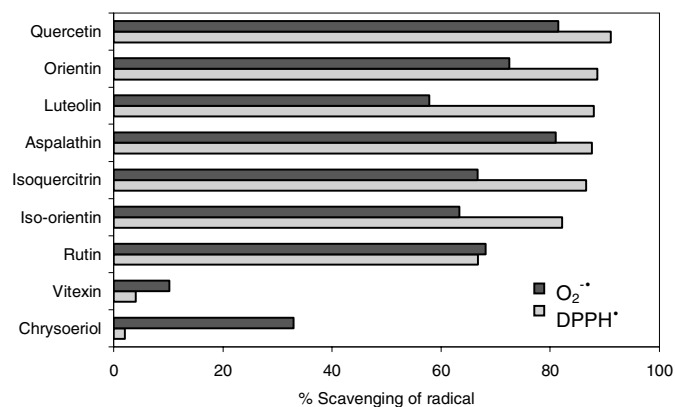
The relative antioxidant potency of aspalathin as the major flavonoid (VON GADOW et al., 1997c) is of special interest to the extract manufacturer. Fig. 3 summarises the relative potency of this dihydrochalcone compared to other rooibos flavonoids as determined in the  $DPPH^{\cdot}$  and  $O_2^{\cdot-}$  assays. Its flavone analogues, orientin and isoorientin (JOUBERT et al., 2004), which are present in comparable quantities in fermented rooibos, as well as nothofagin (SNIJMAN et al., 2003) are less potent. Aspalathin was found to be one of the major contributors to pro-oxidant activity of rooibos extracts (JOUBERT et al., 2005). However, it is postulated that ROS are key mediators of apoptosis which eliminates cancer cells (SALGANIK, 2001). The



**Tab. 2:** Aspalathin and nothofagin content of unfermented (green) and fermented (traditional) rooibos tea

Tea	Parameter	Aspalathin (g/100g)	Nothofagin (g/100g)
Unfermented <sup>1</sup>	Range	3.84-9.66	0.2-1.24
	Average (n = 97)	6.62	0.67
Fermented	Range	0.02-1.16	0-0.40
	Average (n = 89)	0.26	0.12

<sup>1</sup>Samples prepared by drying whole shoots at 40°C in a forced air circulation drying tunnel

**Fig. 3:** Comparison of the DPPH<sup>•</sup> and O<sub>2</sub><sup>•-</sup> scavenging ability of aspalathin with other rooibos flavonoids**Tab. 3:** The total polyphenol and major flavonoid content of fermented rooibos

Compound	Content (mg / g powder) <sup>1</sup>	Content (mg / 150 ml cup of tea) <sup>2</sup>	Content (mg / 150 ml cup of tea) <sup>3</sup>
Aspalathin	3.50 ± 1.33	1.05	3.09
Nothofagin	1.10 ± 0.18	0.33	nd <sup>4</sup>
Orientin	1.61 ± 0.16	0.48	2.51
Isoorientin	0.52 ± 0.09	0.16	2.08
Vitexin	2.36 ± 0.10	0.71	0.83
Isovitexin	0.89 ± 0.25	0.27	0.66
Isoquercitrin + rutin <sup>5</sup>	3.15 ± 0.87	0.95	–
Isoquercitrin + hyperoside <sup>6</sup>	–	–	1.07
Rutin	–	–	3.17
Luteolin	trace	trace	0.07
Quercetin	trace	trace	0.27
Chrysoeriol	trace	trace	0.06
Total polyphenols <sup>7</sup>	273 ± 28	82	88 <sup>8</sup>

<sup>1</sup> Rooibos extract powder prepared by aqueous extraction of waste material containing a high percentage of stems, followed by concentration and spray-drying. Number of analysed production batches: 9.

<sup>2</sup> Based on reconstituted tea powder (300 mg/150 ml).

<sup>3</sup> Data adapted from BRAMATI et al. (2002); data converted to represent the flavonoid content given by a 2.5 g teabag/150 ml (infused for 10 min).

<sup>4</sup> Not determined

<sup>5</sup> Co-eluted; expressed in terms of quercetin

<sup>6</sup> Co-eluted; expressed in terms of isoquercitrin

<sup>7</sup> Total polyphenol content expressed as gallic acid equivalents (determined with Folin-Ciocalteu reagent)

<sup>8</sup> Data adapted from BRAMATI et al. (2003).

oxidative status prevailing within a cell would therefore determine whether the biological response would be beneficial or deleterious (KLAUNIG and KAMENDULIS, 2004).

Presently, a study is in progress to investigate the bio-availability of aspalathin. Sufficient knowledge of its absorption and biotransformation in the human body would be necessary to evaluate the biological significance of this compound.

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