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Original Research Article

Phenolic content and antioxidant activity of rooibos food ingredient extracts

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ABSTRACT

Hot water extracts of the herbal tea, rooibos, are increasingly used as an ingredient in ready-to-drink beverages and a variety of food products. The quantity of extract solids used in the product is occasionally related to the equivalent number of one-cup-servings, yet to date no comprehensive data have been available to serve as guideline. The extent of variation in total polyphenol, aspalathin, orientin and isoorientin contents, as well as total antioxidant capacity (TAC) of hot water extract of fermented rooibos was determined and compared with that of the hot water soluble solids of infusions, prepared similar to a cup of tea. Extract preparation from a large number of individual rooibos production batches ($n = 74$) partly simulated industrial processing, while infusions were prepared from a sub-set of samples ($n = 20$). Based on the total polyphenol and aspalathin contents, rooibos extract and infusion were equivalent when compared on a soluble solids basis. The isoorientin and orientin contents of the soluble solids of the infusion were slightly higher than those of the extract. The TAC of the soluble solids of the infusion, measured with the oxygen radical absorbance (ORAC) assay, was slightly higher than that of the extract, while the opposite was observed for the TAC, measured with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

1. Introduction

Tea, especially green tea, and herbal teas have received much attention in recent years due to their health-promoting properties, in particular their antioxidant properties. The focus fell largely on phenolic compounds as the bioactive phytochemicals responsible for the antioxidant capacity of these beverages (Arthur et al., 2011; Krafczyk et al., 2009; Lambert and Elias, 2010).

Consumption of rooibos, a South African herbal tea produced from *Aspalathus linearis*, has grown substantially since its first introduction to the domestic market in 1904. In 2010 rooibos represented approximately 23% of the South African tea market with sales reaching more than 5000 tons. It is enjoying popularity among an estimated 10.9 million households. Germany, the Netherlands, the United Kingdom, Japan and the United States of America represent 86% of the export market of 6000 tons in 2010 (Joubert and De Beer, 2011). One of the contributing factors to the growing popularity of rooibos is its antioxidant activity and associated health-promoting properties (Joubert et al., 2008). It also led to the commercial production of hot water extracts for use as a food ingredient in a variety of food and beverage products, among others ready-to-drink iced teas, yogurt, drinking yogurt, jam and 'instant cappuccino' (Joubert and De Beer, 2011), thereby increasing dietary exposure to the secondary metabolites of *A. linearis*.

Recent research, demonstrating that daily consumption of six cups of fermented rooibos infusion over a 6-week period improved the lipid profile and redox status in adults at risk of developing cardiovascular disease (Marnewick et al., 2011), supports its potential as a health-promoting beverage. Villaño et al. (2010) demonstrated that the plasma antioxidant status of the volunteers peaked 1 h after consuming 500 mL of unfermented and fermented RTD rooibos beverages, confirming rooibos tea and its products are a source of dietary antioxidants in humans.

Other studies focussed specifically on the bioavailability of the rooibos dihydrochalcone *C*-glucoside, aspalathin (Breiter et al., 2011; Courts and Williamson, 2009; Stalmach et al., 2009), as rooibos is the only source to date of this bioactive flavonoid. The bioactivity of aspalathin relates to its antioxidant activity (Joubert et al., 2005; Krafczyk et al., 2009; Snijman et al., 2009; Von Gadow et al., 1997), antimutagenicity (Snijman et al., 2007) and glucose-lowering effect (Kawano et al., 2009; Mose Larsen et al., 2008). The presence of aspalathin and its metabolites in the plasma of volunteers after consumption of rooibos (Breiter et al., 2011; Stalmach et al., 2009) underscores its relevance as a rooibos bioactive compound. Apart from aspalathin, rooibos also contributes nothofagin, another rare *C*–*C* linked dihydrochalcone glucoside and antioxidant to the diet. The major rooibos flavones, orientin and isoorientin, are oxidation products of aspalathin.

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The aspalathin content of rooibos extracts depend on its concentration in the plant (Schulz et al., 2003), extent of fermentation of the plant material (Joubert, 1996) and processing conditions. Analysis of commercial RTD rooibos iced teas, containing hot water extracts of fermented rooibos, showed that some of the products contained no aspalathin or its oxidation products, orientin and isoorientin, suggesting either that very poor quality rooibos extract, or no rooibos extract, was used in the formulation of these products (Joubert et al., 2009). Recently, a food product claiming to provide the equivalent of 'six cups of rooibos tea' in a single portion was introduced to the South African market. This raises questions of batch-to-batch variation of the extract and the basis used to ensure that the required number of cups of rooibos tea is achieved in the product. Presently, no standardisation of fermented rooibos extract in terms of aspalathin is done, but a standardised extract, based on isoorientin and orientin content (>0.5% total), is available from an international ingredient company. Normally, South African extract producers use total polyphenol content and total antioxidant capacity, measured using the Folin-Ciocalteu and DPPH radical scavenging assays, respectively, as quality indicators.

Several antioxidant assays, each with their own advantages and disadvantages, are available to assess plant extracts (Karadag et al., 2009; Niki and Noguchi, 2000; Prior et al., 2005). The choice of assay for quality control depends to some extent on the intended market of products. The DPPH radical scavenging assay was selected for quality control purposes of rooibos extract as it employs a stable radical and is easy to execute (Prior et al., 2005). The ORAC assay, despite its many drawbacks, is often used to evaluate total antioxidant capacity of food products and ingredients destined for the American market (Bell and Ou, 2007). Ninfali et al. (2009), analysing different commercial herbal extracts, including rooibos extract, recommended the use of ORAC values, as well as a marker compound or group of similar compounds, for improving standardisation of herbal extracts. Rutin was chosen as the marker compound for rooibos, but they concluded that it is not representative of the antioxidant activity of the extract (Ninfali et al., 2009).

The aim of this study was to determine the extent of variation in total polyphenol, aspalathin, nothofagin, orientin and isoorientin contents, as well as total antioxidant capacity of simulated industrial hot water extracts of fermented rooibos, prepared from a large number of individual rooibos production batches. The same parameters were used to compare the hot water extract and infusion ('cup of tea') to determine whether the extract, when used at the same soluble solids content present in the infusion, would deliver the same phenolic content and antioxidant activity.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents were purchased from Sigma–Aldrich (Steinheim, Germany; 2,2′-azo-bis-(2-methylpropionamidine) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (≥97%), ascorbic acid), Merck (Darmstadt, Germany; HPLC gradient grade acetonitrile, Folin-Ciocalteu reagent), Fluka (Buchs, Switzerland; glacial acetic acid), Riedel de Haën (Seelze, Germany; sodium fluorescein), Extrasynthese (Genay, France; isoorientin (≥99%)) and Carl Roth (Karlsruhe, Germany; orientin (≥99%)). Aspalathin (≥97%) and nothofagin (≥97%) were obtained from the PROMEC unit of the Medical Research Council (Parow, South Africa). General analytical grade laboratory reagents were purchased from Sigma–Aldrich or Merck. A Modulab purifier (Continental Water Systems Corp., San Antonio, USA) was used to prepare laboratory grade deionised water, which was further purified to HPLC grade using a Milli-Q academic water purifier (Millipore, Bedford, USA).

2.2. Sourcing of rooibos samples

Fermented rooibos samples (n = 74; 500 g each), representing unrefined plant material (i.e. comminuted, but not sieved) of different production batches, were collected randomly over a six-month-period during the 2009 harvest year from the major rooibos processing and marketing company (Rooibos Ltd., Clanwilliam, South Africa). The samples originated from more than 55 producers spread over the production area and represented the most common quality grades, B and C, as determined by Rooibos Ltd. Grading of samples affects remuneration of producers and is based on leaf colour, infusion colour, infusion flavour and the percentage yield of refined rooibos.

2.3. Determination of yield of refined rooibos

The yield was determined according to industry practice by sieving 400 g of unrefined fermented plant material through 10 and 40 mesh sieves for 1.5 min at 190 rpm using a SMC Mini-sifter (JM Quality Services, Cape Town, South Africa). The fractions <40 mesh and >10 mesh represent dust and coarse tea, respectively, while the fraction >40 mesh and <10 mesh represents refined rooibos. The percentage yield of each fraction was determined gravimetrically.

2.4. Preparation of simulated industrial extracts

Duplicate extracts from each production batch were prepared by adding boiling water (100 mL) to unrefined rooibos (10 g) in a screw-cap glass extraction vessel, which was placed in a water bath at 93 °C for 30 min. The mixture was stirred every 5 min. The resulting extract was filtered through Whatman #4 filter paper while warm, followed by cooling to room temperature in a water bath. Extracts were frozen and freeze-dried using a VirTis Advantage Plus freeze-drier (SP Scientific, Warminster, PA, USA).

2.5. Preparation of 'cup of tea' infusions

Ten samples were randomly selected from each quality grade for preparation of 'cup of tea' infusions (n = 20). Refined rooibos (250 g), spread in a thin layer on 40 mesh stainless steel trays, was subjected to steam pasteurisation for 2 min at >96 °C. The steam pressure, generated with a THE 400 NS Electropac electrode boiler (John Thompson Boilers, Cape Town, South Africa) was maintained at 2.76 N/m^2 at the inlet to the cabinet. On removal from the cabinet, the trays were placed in a forced circulation drying tunnel for 20 min at 40 °C to remove superficial moisture and decrease the moisture content to <10%. Preparation of a 'cup of tea' infusion entailed adding 200 mL boiling deionised water to 2.5 g refined, pasteurised rooibos and infusing for 5 min before filtering through a tea strainer followed by filtration through Whatman #4 filter paper. Aliquots (1 mL) of each infusion, prepared in duplicate, were frozen at ca. −20 °C until total polyphenol content, flavonoid content and total antioxidant capacity analyses.

2.6. Determination of soluble solids content

Soluble solids content of simulated industrial extracts before freeze-drying, and of 'cup of tea' infusions were determined gravimetrically by evaporating duplicate aliquots of 5 mL and 20 mL, respectively, on a steam bath followed by drying in a forced air oven at 100 °C for 60 min.