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Major production areas of rooibos (*Aspalathus linearis*) deliver herbal tea of similar phenolic and phenylpropenoic acid glucoside content

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ABSTRACT

A large sample set (n = 209) of fermented, unpasteurised rooibos, spanning the production years 2011–2013, was collected from the two major production areas (Western Cape and Northern Cape, South Africa). Hot water infusions, as prepared for grading, were analysed to quantify the content of ten flavonoid glycosides, the enolic phenylpropenoic acid glucoside, phenylpyruvic acid-2-*O*-glucoside (PPAG) and the phenolic acid, ferulic acid (FA), using a previously validated reversed phase high performance liquid chromatography with diode array detection (RP-HPLC-DAD) method. Principal component analysis showed no clear grouping of samples according to production area and/or production year, based on the content of individual compounds or sub-classes, i.e. dihydrochalcone, flavonol, flavone and PPAG + FA. Discriminant analysis indicated grouping according to year, but not according to production area. ANOVA showed significant production area × year interactions ($P < 0.05$) for the flavonol and PPAG + FA sub-classes. The dihydrochalcone sub-class was only significantly ($P < 0.05$) affected by production area with samples from the Western Cape having significantly higher aspalathin and nothofagin contents than those from the Northern Cape. The flavone sub-class was not affected by production area or year. The major flavones, orientin and isoorientin, were the predominant constituents of the rooibos tea infusions, followed by quercetin-3-*O*-robinobioside, PPAG and aspalathin. These compounds were present at levels >9 mg/L, while the other compounds were present at <5 mg/L.

1. Introduction

Aspalathus linearis, used for production of rooibos, a well-known herbal tea, grows mainly in the Cederberg area of the Western Cape Province, South Africa with the majority of commercial production centred in the Clanwilliam and Citrusdal areas. The rooibos plant is also naturally found, as well as cultivated, in the Nieuwoudtville area of the Northern Cape Province, specifically the Bokkeveld plateau on the border with the Western Cape. The Clanwilliam and Citrusdal areas have higher minimum and maximum daily temperatures, on average, than the Nieuwoudtville area (data supplied by ARC Institute for Soil, Climate and Water, South Africa), which is situated at higher elevation. These temperature differences could affect product quality as processing takes place in the open air under uncontrolled conditions (Joubert, 1996). Furthermore, environmental factors such as ambient temperature have been demonstrated to affect biomass yield and physiological processes (Hasanuzzaman et al., 2013), including flavonoid biosynthesis (Petrussa et al., 2013) of plants. Flavonoids are important secondary plant metabolites, offering protection against environmental stressors such as drought, UV-B irradiation and heat. In legumes such as *A. linearis*, they may induce rhizobial nodulation as demonstrated for other plant species (Falcone Ferreyra et al., 2012). Rooibos flavonoids play an integral role in the quality of the product, given the importance of the dihydrochalcone, aspalathin, in the development of the distinctive red-brown colour of the traditional, "fermented" product (Joubert, 1996; Heinrich et al., 2012), as well as the association of its flavonoids with taste and astringency (Koch et al., 2013), and the beneficial bioactive properties of rooibos such as anti-diabetic properties (Kawano et al., 2009; Muller et al., 2012; Son et al., 2013; Mazibuko, 2014). The global demand for rooibos and the need for rural development in the Northern Cape Province, in particular through agro-processing, have led to an initiative of the Northern Cape Department of Agriculture, Land Reform and Rural Development to find defining product characteristics enabling promotion of Northern Cape rooibos as an origin-based product for niche markets. A link between production area and product characteristics, including composition, has been established for many agricultural products, most notably wine. "Terroir", embodying the

Abbreviations: ANOVA, analysis of variance; DA, discriminant analysis; PCA, principal component analysis; FA, ferulic acid; PPAG, phenylpyruvic acid-2-*O*-glucoside; RP-HPLC-DAD, reversed-phase high-performance liquid chromatography with diode-array detection.

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interaction between the environment and the vine, is an entrenched concept in the wine industry and central to the South African "Wine of Origin" system, used judiciously in the marketing of wine (Ponte, 2009).

At this stage it is not known to what extent climatic differences and thus microclimate may have an effect on the quality of rooibos grown in these areas. Of greater concern is that modelling of climate change due to global warming indicates a future scenario of substantial contraction in the area suitable for growing rooibos with a general shift southwards and to higher altitudes of the traditional rooibos production area such as Nieuwoudtville, with the western areas along the coast becoming less suitable (Lötter and le Maitre, 2014).

In view of the future scenario, i.e. favouring rooibos production at higher elevations, it would be timely to obtain data that could indicate whether substantial differences exist in phenolic composition and the health-promoting potential of rooibos produced in the Western and Northern Cape Provinces. This could provide a basis for differentiating rooibos in the market. For the present study a comprehensive sample set, comprising unpasteurised rooibos samples of different grades to accommodate the effect of grade on the phenolic composition of rooibos (Joubert et al., 2012; Stanimirova et al., 2013), was selected. Unpasteurised rooibos samples were used as pasteurisation, a post-production process, significantly decreases the phenolic content (Koch et al., 2013; Stanimirova et al., 2013), which may obscure sample differences. Sampling occurred over three consecutive production years from the two major tea processing and marketing companies, located in the Western Cape and Northern Cape Provinces.

2. Materials and methods

2.1. Chemicals

All chemicals were analytical grade and sourced from either Sigma-Aldrich (St. Louis, MO, USA) or Merck Millipore (Darmstadt, Germany), unless otherwise specified. HPLC gradient grade acetonitrile and formic acid (98–100%) were obtained from Merck Millipore. Authentic reference standards with purities >95% were obtained from Extrasynthese (Genay, France; isovitexin, hyperoside, orientin, luteolin and chrysoeriol), Roth (Karlsruhe, Germany; vitexin, isoorientin and luteolin-7-*O*-glucoside), Sigma-Aldrich (ferulic acid, rutin and isoquercitrin), ARC Infruitec-Nietvoorbij compound library (Stellenbosch, South Africa; enolic phenylpyruvic acid-2-*O*-glucoside (PPAG)) and the Medical Research Council (PROMEC Division, Bellville, South Africa: aspalathin and nothofagin). Deionised water, prepared using an Elix water purification system (Merck Millipore), was further purified to HPLC grade using a Milli-Q Academic water purification system (Merck Millipore).

2.2. Rooibos samples and preparation of infusions

Different production batches of fermented rooibos were sampled during the 2011, 2012 and 2013 harvest seasons. The samples were randomly selected from each of the quality grades, A, B and C. The sample set of the Western Cape represented 18 grade A, 58 grade B and 41 grade C samples, while 16 grade A, 38 grade B and 38 grade C samples were obtained from the Northern Cape. All samples were sieved in accordance with industry practice to obtain the fraction >40 mesh and <10 mesh (Joubert et al., 2012).

A standardised preparation protocol for the infusion, based on the procedure used when rooibos is graded, was followed as described by Koch et al. (2013). Briefly, freshly boiled, distilled water (900 g) was poured onto 17.4 g dry tea leaves and stems and stirred for about 5 s whereafter the leaves and stems were infused for 5 min without stirring. The infusion was decanted through a fine-mesh sieve strainer. After cooling to room temperature, 200 mL of the infusion was filtered through Whatman No. 4 filter paper, and aliquots stored in 2 mL microfuge tubes at ca −20 °C until HPLC-DAD analysis.

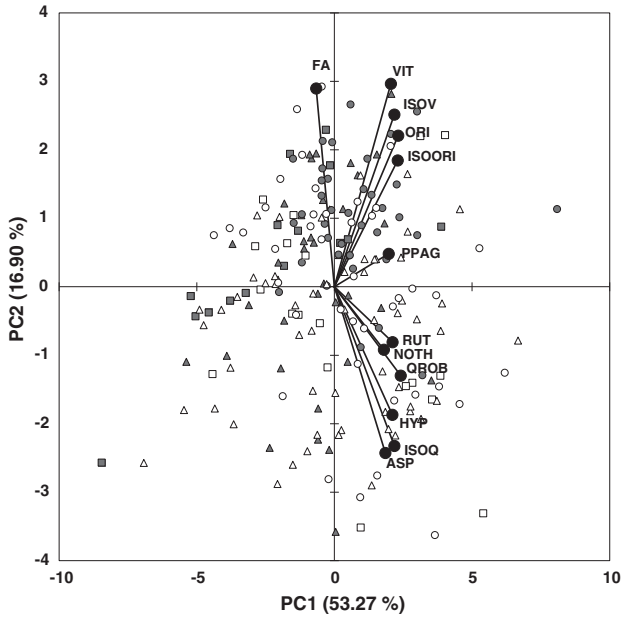

Fig. 1. Principal component analysis bi-plot of the first two principal components (PC1 and PC2) for the individual compounds present in infusions of rooibos samples from the Northern Cape (grey markers) and Western Cape (white markers) provinces collected during 2011 (squares), 2012 (triangles) and 2013 (circles). Abbreviations: PPAG, enolic phenylpyruvic acid-2-*O*-glucoside; FA, ferulic acid; ASP, aspalathin; NOTH, nothofagin; ORI, orientin; ISOORI, isoorientin; VIT, vitexin; ISOV, isovitexin; RUT, rutin; QROB, quercetin-3-*O*-robinobioside; ISOQ, isoquercitrin; HYP, hyperoside.

2.3. Quantification of phenolic compounds and phenylpyruvic acid-2-O-glucoside

HPLC-DAD analysis of rooibos infusions (Beelders et al., 2012) was performed on an Agilent 1200 system (Agilent Technologies, Waldbronn,

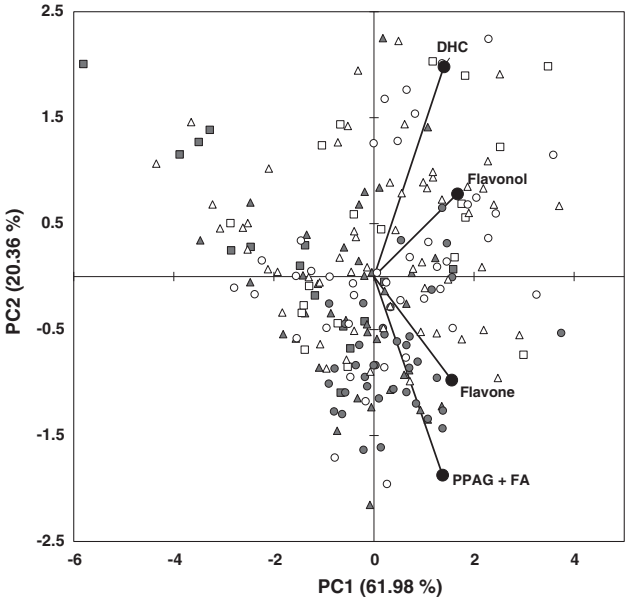

Fig. 2. Principal component analysis bi-plot of the first two principal components (PC1 and PC2) for compound sub-classes present in infusions of rooibos samples from the Northern Cape (grey markers) and Western Cape (white markers) provinces collected during 2011 (squares), 2012 (triangles) and 2013 (circles). Abbreviations: PPAG + FA sub-class comprising PPAG (enolic phenylpyruvic acid-2-*O*-glucoside) and ferulic acid (FA); DHC (dihydrochalcone) sub-class comprising aspalathin and nothofagin; Flavone sub-class comprising orientin, isoorientin, vitexin and isovitexin; Flavonol sub-class comprising rutin, quercetin-3-*O*-robinobioside, isoquercitrin and hyperoside.