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A landscape photograph showing a green field in the foreground, with a range of mountains in the background under a clear blue sky.

Use of Near Infrared Spectroscopy in quality control of green Rooibos and Honeybush

The growing interest in natural plant products by the nutraceutical and cosmetic industries resulted in a large number of polyphenol-enriched extracts on the market. Due to their high antioxidant activity, two indigenous plants, rooibos and honeybush are increasingly being used by the South African industry for the development of new products.

Authors: Marena Manley and Mariza Botha, Department of Food Science, Stellenbosch University, South Africa
Elizabeth Joubert, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa

Use of Near Infrared Spectroscopy in quality control of green Rooibos and Honeybush

Abstract

Near infrared spectroscopy (NIRS) was used to develop calibration models to predict aspalathin, nothofagin and dihydrochalcone contents of dried, green rooibos, and mangiferin and hesperidin contents of dried, green *Cyclopia genistoides* plant material. NIRS can effectively predict the aspalathin content of dried, green rooibos with a standard error of prediction (SEP) and correlation coefficient (r) of 0.45 g.100 g⁻¹ and 0.92, respectively, and the dihydrochalcone content of rooibos with an SEP of 0.49 g.100 g⁻¹ and r of 0.93. Extending the aspalathin content range of the sample set with samples manipulated with a rooibos extract powder containing high concentrations of aspalathin (15.95 g.100 g⁻¹) and nothofagin (1.94 g.100 g⁻¹), slightly less accurate models were obtained for the aspalathin (SEP = 0.53 g.100 g⁻¹; r = 0.93) and dihydrochalcone (SEP = 0.57 g.100 g⁻¹; r = 0.94) contents. The NIRS calibration model developed for nothofagin content (SEP = 0.10 g.100 g⁻¹; r = 0.84) (extended range: SEP = 0.10 g.100 g⁻¹; r = 0.88) of dried, green rooibos, as well as the calibration models developed for mangiferin (SEP = 0.46 g.100 g⁻¹; r = 0.86) and hesperidin (SEP = 0.38 g.100 g⁻¹; r = 0.85) contents of dried, green *C. genistoides* can be used for screening purposes.

Introduction

The growing interest in natural plant products by the nutraceutical and cosmetic industries during the past number of years resulted in a large number of polyphenol-enriched extracts on the market. Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) are two indigenous South Af-

rican herbal plants containing high quantities of the polyphenolic compounds, aspalathin and mangiferin, respectively, contributing to their antioxidant activity.

Rooibos tea is the common name for the plant, *Aspalathus linearis* spp. *linearis*, and its infusion. The absence of caffeine and its low tannin content compared to black tea, make it a popular herbal tea, both locally and internationally. The phenolic composition of rooibos is unique in that it contains aspalathin, a rare CC dihydrochalcone glycoside.

Honeybush is low in tannins and contains no or traces of caffeine. Until the 1990's, honeybush tea was only consumed locally to a limited extent. The phenolic composition differs among *Cyclopia* species as well as types within species.

The antioxidant properties of green (unoxidised) rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) have led to an increasing interest in the development of polyphenol-enriched extracts for the nutraceutical and cosmetic industries.

The objectives of this study were to:

- develop NIRS calibration models for the prediction of aspalathin, nothofagin and dihydrochalcone contents of dried, green rooibos plant material and the mangiferin and hesperidin contents of dried, green *C. genistoides* plant material.

Materials and Methodology

Green rooibos plant material

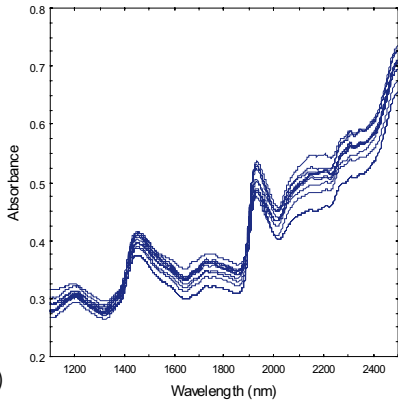
A large selection of dried, green rooibos plant material ($n = 340$) harvested during different years (1984, 1999, 2002 and 2004) and from different production areas (Citrusdal and Clanwilliam, South Africa) was obtained from the sample collection of ARC Infruitec-Nietvoorbij. Fresh plant material was dried at 40°C to ca. 8-10% moisture content and ground with a Retsch mill (1 mm sieve). The aspalathin and nothofagin contents of nine samples were increased by adding varying amounts of green rooibos extract powder with high aspalathin (15.95 g 100 g⁻¹) and nothofagin (1.94 g 100 g⁻¹) contents to extend the range of the aspalathin and nothofagin contents.

Green *C. genistoides* plant material

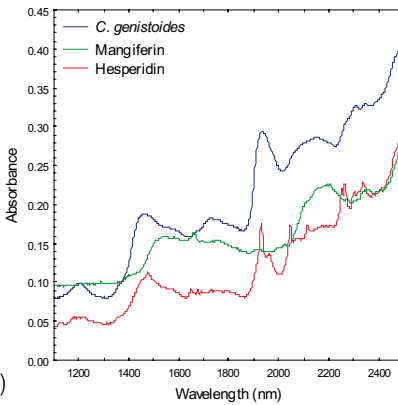
The dried, green *C. genistoides* plant material ($n = 240$) was obtained from ARC Infruitec-Nietvoorbij (harvested during 2001, 2003, 2004 and 2005). The fresh plant material was dried at 40°C to ca. 8-10% moisture content and ground with a Retsch mill (1 mm sieve).

Near infrared spectroscopy measurements

Dried, green rooibos and *C. genistoides* plant material: A Buchi NIRLab N-200 Fourier transform near infrared (FT-NIR) spectrophotometer with NIRLabWare (version 3.0) near infrared (NIR) measurement software was used to perform the NIRS measurements in diffuse reflectance mode. The ground, dried plant material was presented to the instrument in rotating glass petri-dishes and the NIR spectra collected from 1000-2500 nm at a resolution of 12 cm⁻¹ resulting in 1557 data points.



a)



b)

a) Typical NIR spectra of ground, dried, green rooibos and b) *C. genistoides* plant material.

NIRS calibration model development:

Buchi NIRCAl (version 4.21) was used for calibration model development. The pre-treatments, multivariate calibration methods and number of samples in the calibration and validation sets used for each respective compound are summarised in Table 1. The calibration models were validated by means of independent validation and no outliers were removed.



Conclusions

Near infrared spectroscopy showed promise as a rapid method to quantify the aspalathin and dihydrochalcone contents in green rooibos plant material, since an accuracy of ca. 0.5 g.100 g⁻¹ would be acceptable for the prediction of these compounds by the industry. It could be used to standardise green rooibos plant material at an early stage of processing. Nothofagin, mangiferin and hesperidin NIRS calibration models could be used for screening purposes. Nothofagin is present in much smaller quantities than aspalathin which could have contributed to the slightly less accurate calibration models. The lower accuracy of the mangiferin and hesperidin calibration models could be due to the difference in solubility of these compounds.

Preparation of different extracts using different solvents for optimum extraction of each compound might improve the accuracy of the reference data and therefore the calibration models. All the calibration models developed for the green rooibos and *C. genistoides* plant material could be improved by adding more samples to give a more even distribution of the aspalathin, nothofagin, dihydrochalcone, mangiferin and hesperidin contents.

References

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2. Manley, M., Joubert, E. & Botha, M. (2006). Quantification of the major phenolic compounds, soluble solid content and total antioxidant activity of green rooibos (*Aspalathus linearis*) by means of near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* (in print).

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BÜCHI Labortechnik AG
Postfach
9230 Flawil 1
Schweiz
Tel. +41 71 394 63 63
Fax +41 71 394 65 65
buchi@buchi.com
www.buchi.com

BÜCHI Labortechnik GmbH
Postfach 10 03 51
45003 Essen
Deutschland
Freecall 0800 414 0414
Tel. +49 201 747 490
Fax +49 201 237 082
deutschland@buchi.com
www.buechigmbh.de

BÜCHI Labortechnik GmbH
Branch Office Netherlands
Postbus 142
3340 AC Hendrik-Ido-Ambacht
The Netherlands
Tel. +31 78 684 94 29
Fax +31 78 684 94 30
netherlands@buchi.com
www.buchi.nl

BÜCHI Italia s.r.l.
Centro Direzionale, Milano Fiori
Pal. A-4, Strada 4
20090 Assago (MI)
Italia
Tel. +39 02 824 50 11
Fax +39 02 57 51 28 55
italia@buchi.com
www.buchi.it

BUCHI (THAILAND) Ltd.
ASEAN Competence Center
300 Phaholyothin Road
Samsennai, Phayathai
Bangkok 10400
Thailand
Tel. +66 2 278 54 95
Fax +66 2 279 05 48
bacc@buchi.com
www.buchi.com

BUCHI SMP
Services Private Ltd.
201, Magnum Opus
Shantinagar Industrial Area
Vakola, Santacruz (East)
Mumbai 400 055,
India
Tel. +91 22 56 98 94 50
Fax +91 22 56 98 94 52
smplisp@vsnl.com
www.buchi.com

BUCHI Analytical Inc.
19 Lukens Drive
New Castle
Delaware 19720
USA
Tel. +1 302 652 3000
Fax +1 302 652 8777
us-sales@buchi.com
www.buchi-analytical.com

BUCHI Hong Kong Ltd.
1305, Hang Seng Mongkok Bldg.
677 Nathan Road
Kowloon, Hong Kong
China
Tel. +852 2389 2772
Fax +852 2389 2774
china@buchi.com
www.buchi.com

Nihon BUCHI K.K.
3F IMON Bldg.,
2-7-17 Ikenohata, Taito-ku,
Tokyo 110-0008
Japan
Tel. +81 3 3821 4777
Fax +81 3 3821 4555
nihon@buchi.com
www.nihon-buchi.co.jp

BUCHI UK Ltd
5 Whitegate Business Centre
Jardine Way
(off) Broadway
Chadderton
Oldham OL9 9QL
United Kingdom
Tel. +44 161 633 1000
Fax +44 161 633 1007
uk@buchi.com
www.buchi.co.uk

BUCHI Sarl
5, rue du Pont des Halles
Z.A. du Delta
94656 Rungis Cedex
France
Tél. +33 1 56 70 62 50
Fax +33 1 46 86 00 31
france@buchi.com
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Table 1:
Summary of pretreatments, multivariate calibration methods and number of samples in the calibration and validation sets used during calibration model development of the respective compounds in dried, green rooibos and *C. genistoides*.

Compound	Pretreatment(s)	Multivariate calibration method	Calibration set (n)	Validation set (n)
Green rooibos plant material				
Aspalathin, nothofagin and dihydrochalcone contents	Db1 ^a	PLS ^b	220	111
Aspalathin, nothofagin and dihydrochalcone contents (extended aspalathin and nothofagin contents)	n01 ^c , db1	PLS	227	113
Green <i>C. genistoides</i> plant material				
Mangiferin content	MSC ^d	PLS	160	80
Hesperidin content	Db1	PLS	160	80

^a First derivative

^b Partial least squares regression

^c Normalisation (between 0 and 1)

^d Multiplicative scatter correction

The accuracy of the calibration models was expressed by means of the standard error of prediction (SEP), the correlation coefficient (*r*) and the ratio of SEP to standard deviation of the validation set (RPD), which is an indication of the efficiency of

a calibration. The goal of model development is to obtain a calibration model with a low SEP, a high *r*, preferably above 0.91 and a RPD higher than 5. The SEP should also be as close as possible to the standard error of laboratory (SEL).

Results and discussion

Summary of the NIRS validation results for the prediction of aspalathin, nothofagin and dihydrochalcone contents of dried, green rooibos and mangiferin and hesperidin contents of dried, green *C. genistoides* plant material, is given in Table 2.

Table 2:

	Aspalathin		Dihydrochalcone		Nothofagin		Mangiferin	Hesperidin
	1	2 ^a	1	2 ^a	1	2 ^a		
SEP (g.100g⁻¹)	0.45	0.53	0.49	0.57	0.10	0.10	0.46	0.38
r	0.92	0.93	0.93	0.94	0.84	0.88	0.86	0.85
Bias	0.01	0.01	0.01	-0.002	-0.01	-0.01	-0.04	0.02
PLS factors^b	5	4	5	4	5	4	4	6
RPD	2.62	2.70	2.71	2.89	1.8	2.10	1.96	1.90

^a Including 9 samples with added green rooibos extract powder with high aspalathin and nothofagin contents.

^b Number of PLS factors used.



Validation plots of the predicted aspalathin values versus the measured (HPLC) aspalathin values for calibration models for green rooibos plant material with different aspalathin content ranges: a) aspalathin content range = 0.60-8.61 g.100 g⁻¹ (calibration set) and b) aspalathin content range = 0.60-10.59 g.100 g⁻¹ (calibration set).

Validation plots of the predicted dihydrochalcone values versus the measured (HPLC) dihydrochalcone values for calibration models for green rooibos plant material with different dihydrochalcone content ranges: a) dihydrochalcone content range = 0.66-9.61 g. 100 g⁻¹ (calibration set) and b) dihydrochalcone content range = 0.66-11.83 g.100 g⁻¹ (calibration set).

Validation plots of the predicted nothofagin values versus the measured (HPLC) nothofagin values for calibration models for green rooibos plant material with different nothofagin content ranges: a) nothofagin content range = 0.07-1.09 g. 100 g⁻¹ (calibration set) and b) nothofagin content range = 0.07-1.24 g.100 g⁻¹ (calibration set).

Validation plots of a) the predicted mangiferin values versus the measured (HPLC) mangiferin values and b) the predicted hesperidin values versus the measured (HPLC) hesperidin values for the calibration models for green *C. genistoides* plant material.

