Analytical Characterization of the Fermentation Process of Rooibos Tea (Aspalathus linearis)

Introduction

Rooibos Tea (aka as Massai Tea), a caffeine free drink originating in South Africa, is becoming an increasingly popular in recent years because of the health promoting properties ascribed to it. Studies confirm its anti-mutagenic and anti-carcinogenic as well as its anti-inflammatory properties. Rooibos Tea is a member of the family of legumes, grows to up to 2 m high bushes with needle leaves of 2 to 6 cm length and was first described in 1972. Rooibos prefers a Mediterranean climate, and occurs sole in a small region in South Africa in the Clanwilliam District, and is only being cultivated and bred since the beginning of the 20th century.

Rooibos is harvested from the second year during the summer. The shoot-tips are chopped up into approximately 0,5 cm lengths, spread on stone floor and are mixed with water to initiate fermentation. Depending on the weather the green tea ferments for between 8 – 24 hrs at approximately 35 ºC until Rooibos has achieved its typical reddish-brown colour [1]. During the fermentation the flavour changes from unpleasantly resinous to sweet, apple or caramel-like. Subsequently the tea is sun dried until a water content of < 10% is achieved (approximately 24 hrs), where it is important to keep the drying period as short as possible so as to prevent over fermentation. Over 120 aroma relevant compounds have so far been identified in Rooibos by means of GC-MS, where the aroma profile is very dependent on the preparation process.

Thereafter Rooibos is customarily passed through 3 mm sieves, during which the waste (coarse material and stems) as well as the dust fraction (fine dust) are separated out. The quality assay is conducted by members of the Rooibos Tea Board, who check the fermented product as to purity, cut length, aroma, colour imparting strength and residual moisture, subsequently grading it in up to eight different qualities.

Constituents and Analysis

Rooibos Tea has a comparatively high mineral content and up to 15,7 mg / 100 gm Ascorbic acid [2]. The health promoting properties are mainly derived from the presence of numerous flavonols such as Quercitin, Quercitrin, Luteolin, Orientin, Rutin and Vitexin [3], as well as the dihydrochalcones Aspalathin and Nothofagin which occur only in Rooibos (Fig. 1)

Fig. 1: R=OH – Aspalathin; R=H - Nothofagin

After the characterization of the flavonol profile by thin film chromatography, a HPLC method was later developed, which served to quantify Nothofagin and Aspalathin during the fermentation process [4]. As a requirement exists to be able to complete such analysis in as short a time as possible, the following will cover the development of a Near Infra Red Spectroscopy (NIRS) method [5], based on the results of a newly improved, with respect to separation and accuracy, HPLC process. Furthermore, the possibilities and limitations of differentiating between different fermentation products based on the recorded spectral data.

Material and Methodology

120 samples of fermented and unfermented Teas from different cultivation regions in South Africa (Nieudwoudville, Gifberg, Clanwilliam, Citrusdal, Eendekuil) were analyzed.

HPLC

After grinding the sample 500 mg of the material are extracted at 100 ºC (15 min) in water, then cooled and mixed with 1 % Ascorbic acid. The HPLC analysis of the centrifuged, filtered solution were then done in a 3.5 µ ZORBAX SB-C18 column (3,0 x 15 mm) with gradient elution (1 % HCOOH / CH3CN) within 30 minutes.

NIRS

The sample was measured in a dispersive NIR apparatus NIR Systems 5000, supplied by Foss, in reflection mode in the range 1100 to 2500 nm. Based on spectral data a major component analysis (PCA) was done and followed by a quantitative analysis using a modified PLS algorithm (Tecatur ISI Software) after a successful scatter correction (WMSC) was completed. The confidence of the calibration was established with the aid of the cross validation parameter R^2 (Correlation between predicted and reference values) as well as SECV (satanderd error of correlation).

Results

HPLC

With regard to the flavonol constituents in respect of separation, time required to complete an analysis and reproducibility the HPLC-DAD determination could be significantly optimized compared to previously published methods. The addition of ascorbic acid enables the reliable determination of the oxidation susceptible Aspalathin, the content of which reduces to as little as 1.5 %, while Nothogfagin reduces to 10 % of the original concentration in the green tea (Table 1). The other secondary constituents remain large unchanged during the fermentation process.

Unfermented	**Aspal.**	**Notho.**	**Fermented**	**Aspal.**	**Notho.**
Clanwilliam (A)	4,52	0,34	Choice Grade	0,14	0,10
Clanwilliam (M)	5,44	0,56	Super Grade	0,21	0,12
Gifberg (A)	6,25	0,66	Sun dried	0,11	0,09
Clanwilliam (J)	9,34	1,03	Tunnel dried	0,16	0,11
Waste (leaves)	4,45	0,68	Stems, coarse leaves	0,11	0,02
Waste (stems)	7,89	1,25	Unsieved	0,11	0,03
Process field (leaves)	11,9	1,33	Dust	0,12	0,03

Table 1 : Comparison of various unfermented and fermented tea qualities

(J-Mainly young plants, A-mainly old plants, M-mixture of old and young plants)

NIRS

On the basis of the spectral data it is possible, with the use of PCA , to obtain clearly defined grouping for fermented and unfermented Rooibos tea. As expected the semi-fermented samples are found in the range between the two main groupings, while the dust fraction and the fungal infection samples obtained during further processing are recognizable as separate clusters (Fig. 2).

The Aspalathin content of the unfermented tea (Content 3,9 – 9%) yielded a calibration with an average experimental bias of 0,65 % with a coefficient of correlation (R^2) between NIRS prediction and HPLC reference value of 0,76. The relatively large error is a result of the low band intensity of the CH- valency oscillation (v_{CH}) in the infra red range.

Despite the relatively high method bias (error) the described NIRS method is well suited to provide sufficiently accurate data for the breeding and cultivation of Rooibos and in conjunction with PCA to determine additional quality characteristics of various fermentation products.

Fig. 2: PCA of different Tea samples
(*) – unfermented; (+) – fermented; (o) – Waste / Dust

Fig. 3: NIRS – Callibration for Aspalathin in unfermented Rooibos Tea