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Rooibos tea: equilibrium and extraction kinetics of aspalathin

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Abstract

A mobile phase for the extraction of aspalathin was developed and used to study the kinetics of extraction of the compound from Rooibos tea. The rate of infusion of aspalathin into water at 80 °C was measured for Rooibos tea, using a leaf of size 1.00–1.40 mm. The first order rate constant ($k_{obs} = 23.4 \times 10^{-4}$ s^{-1}) was determined from the rate of increase of aspalathin concentration in a tea infusion with time and the result interpreted using a steady state model. The results were compared with those of rose-hip, black and green teas. The first order rate constant for extraction of aspalathin was found to be comparable with that of L-ascorbic acid in rose-hip tea. However, the value was about five times smaller than that of flavanols from Japanese green tea and 3–10 times smaller than the extraction of caffeine from black Assam Bukial and green Chun Mee teas. The pH of the liquor at equilibrium was found to be 4.6. This value is comparable to that of both black and rose-hip tea but 20% smaller than that of green Chun Mee tea.

Keywords: Rooibos; Aspalathin; Extraction kinetics

1. Introduction

The leaves and fine stems of the leguminous shrub *Aspalathus Linearis*, known as Rooibos, are used to produce Rooibos tea, a beverage that is becoming increasingly popular due to its unique taste, versatility and, most importantly, its reputation as a health drink. This reputation stems from claims of antioxidant activity and its therapeutic and physiological advantage (Rooibos Ltd., 1995). Normal consumption involves brewing the leaves and then consuming the liquor hot or cold. It is often used as an ingredient in various recipes.

Much of the attention that Rooibos tea has received in the world of research is due to its antioxidant properties. These have been found to be comparable with that of green, Oolong and black tea (von Gadow, Joubert, & Hansmann, 1997b). The antioxidant activity of the tea is associated with a number of its constituents, which have the ene-diol functionality, often in an electron rich B-ring system (see Fig. 1). It has also been established that Rooibos tea contains substances that mimic superoxide dismutase (SOD) in its antioxidant activity (Yoshikawa et al., 1990; Ito, Shinohara, & Kator, 1991). One of the compounds that show antioxidant properties is aspalathin. *Aspalathus linearis* is the only known natural source of this compound (Koeppen & Roux, 1965b), making the composition of the plant unique.

Research has been done to compare the antioxidant activity of aspalathin with other phenolics within the tea, and known antioxidants, such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), alpha tocopherol (von Gadow, Joubert, & Hansmann, 1997a). Aspalathin showed one of the highest degrees of DPPH (alpha, alpha-diphenyl-beta-picrylhydrazyl) radical scavenging. It has also been postulated by Ferreira, Joubert, Mavais, and Steenkamp (1995) that dihydrochalcones, such as aspalathin, contribute to the naturally sweet taste of Rooibos tea.

Research on the brewing of Rooibos tea has been, and still is, concerned with the kind of constituents extracted during the infusion process and their benefits to human health. A literature survey showed that no published information is available on the physico-chemical aspect of the dissolution process of the various species from the leaf. This type of information has proved useful in the manufacturing of instant black tea. The aim of this paper is to provide new experimental

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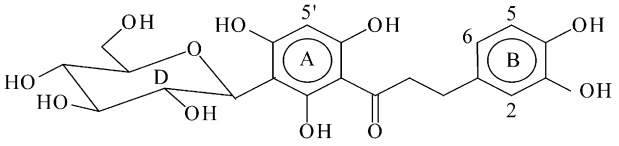

Fig. 1. 2′,3,4,4′,6′-pentahydroxy-3′-C-β-D-glucopyranosylhydrochalcone (aspalathin).

results by applying a simple two-phase model in determining kinetic and equilibrium data that are of relevance to the extraction of aspalathin in water at 80 °C.

2. Material and methods

Rooibos tea leaves used in this investigation were obtained from the Rooibos tea Board in Clanwilliam, South Africa. These leaves were first sieved into different fraction sizes, using a set of stainless steel and brass coated Endecott sieves and a mechanical shaker. The fraction range selected for kinetic studies was 1.40–1.00 mm. The flask used in the experiments (brewing flask) was a plastic conical flask (500 ml) containing 400 ml of MilliQ water and a teflon coated magnetic stirrer. The bottom of the flask was heated and moulded into a gentle concave shape so as to house the magnetic stirrer at the centre for efficient stirring of the liquor. It was sealed with a lid that had two holes drilled into it for the fitting of a thermometer and a plastic sampling tube. The end of the tube that was inside the flask consisted of a small cylindrical plastic sheath (inner diameter 5 mm) inside which a Gilson filter (Anachem) was fitted. This prevented the withdrawal of tea leaves, which would have blocked the plastic tube. The filter also removed any tiny particles that would have affected the HPLC. Nylon gut was tied through the open end of the plastic sheath to prevent the filter from dislodging. This is because, before sampling, air in the syringe was always used to flush the tube and filter. The filter was replaced after each experimental run. The brewing flask was weighed without the thermometer before it was submerged in the bath and allowed to equilibrate to the set temperature.

The bath containing the brewing flask is illustrated in Fig. 2.

Once the liquid in the brewing flask had reached 80 °C, the lid was temporarily removed and a wide bore glass funnel modification of the device used by Spiro and Siddique (1981) was used to add a standard amount of tea leaf (4.0 g) to the flask. The lid was replaced after the addition to prevent evaporation. An underwater magnetic stirrer with a variable speed control was used to stir the mixture. This process ensured that the concentration of the extracted aspalathin and the temperature of the solution were uniform within the flask.

Tea samples (1 ml) were withdrawn from the flask using a 1 ml plastic syringe (Promex). Each sample was then transferred into a glass polytop. The 1 ml samples were then diluted with 0.666 ml of methanol (BDH, HPLC Grade). Methanol was used because the HPLC peaks were better resolved as compared to when water was used as the diluting solvent. The experiments were performed in quadruplicate and monitored on the HPLC.

Initially, a total of 14 samples were withdrawn from the flask, the sampling times being 0.0 (blank), 0.5, 1.0,

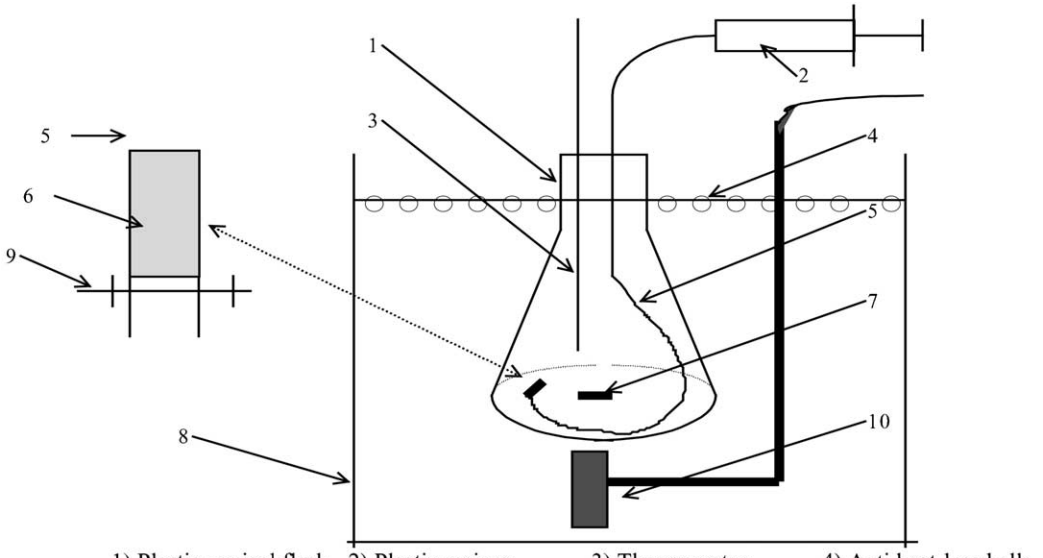

Fig. 2. The brewing flask and water bath components.

1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 15.0, 30.0, 45.0, 60.0 and 90.0 min. Once it was established that the component was exhibiting the trend shown in Fig. 3 and the results were reproducible, the sampling times between 5.0 and 60.0 min were excluded. At the end of the run, the brewing flask was again weighed with its contents. The difference in weight was taken as ΔV, the volume lost through sampling and evaporation. This was used for volume correction purposes (Spiro & Jago, 1982). The pH of the solution was also monitored at the end of each experiment, using an Orion Research microprocessor pH/millivoltmeter 811 while the solution was being stirred. The value obtained is compared with other tea values in Table 3.

High pressure liquid chromatography (HPLC) was used to monitor the concentration of aspalathin. The instrument employed was a Thermo Separation Products HPLC, equipped with a Nucleosil 100-5 C_{18} (Macherey-Nagel) reversed phase column of length 250 mm and inner diameter of 4 mm. This was calibrated with known concentrations of aspalathin (courtesy of Prof. D. Ferreira, University of the Orange Free State, Bloemfontein, South Africa). The UV-spectrum of aspalathin in Roiboos tea sample was identified by comparing the sample and standard peak. The UV-spectra showed the wavelength of maximum absorbance (λ_{max}) to be 290 nm. This value agreed very well with data available in the literature (Harborne, 1984; Koeppen & Roux, 1965a; Markham, 1982). The optimum wavelength to which the detector, Spectra System UV3000 (Scanning) was set in the current investigation was therefore 290 nm. Integration at this wavelength ensured a flat baseline and absence of interference.

The mobile phase system that was developed and used in the current work was a gradient elution as shown in Table 1, and illustrated diagrammatically in Fig. 4. Solvent A was methanol (Merck; HiperSolv for HPLC) and solvent B was 5% (v/v) formic acid (Merck, 98–100%), in water (MilliQ). All solvents, except the formic acid which attacked the filters causing contamination, were filtered using 0.45 m filters (Micropore) prior to use. The tea samples were injected straight into the HPLC for analysis.

3. Results and discussion

The corrected concentrations were used to plot Fig. 3, which represents the changing of aspalathin concentration with respect to time. These plots clearly show that the concentration rises rapidly, initially, and then asymptotically approaches a limiting equilibrium concentration (C_∞), seen at the profile plateau occurring between 60 and 90 min. The shape of these curves closely resembles those already published for similar infusions (Jaganyi, Vanmare, & Clark, 1999).

It is mentioned in the literature that aspalathin possibly undergoes oxidation to (2*R*)- and (2*S*)-2,3-dihydro-iso-orientin (Marais, Marais, Steenkamp, Malan, & Ferreira, 1997) and/or gets converted to an as-yet unidentified brown substance in the presence of oxygen and sunlight (Koeppen & Roux, 1966). This is not evident from the curve shown in Fig. 3. Therefore, in these cir-

Table 1
Mobile phase composition in terms of percentages (v/v) of the respective solvents

Time/min	% Methanol	% (5% formic acid in water)
0	15.0	85.0
4	15.0	85.0
8	30.0	70.0
15	40.0	60.0
18	50.0	50.0
20	50.0	50.0
23	15.0	85.0
28	15.0	85.0

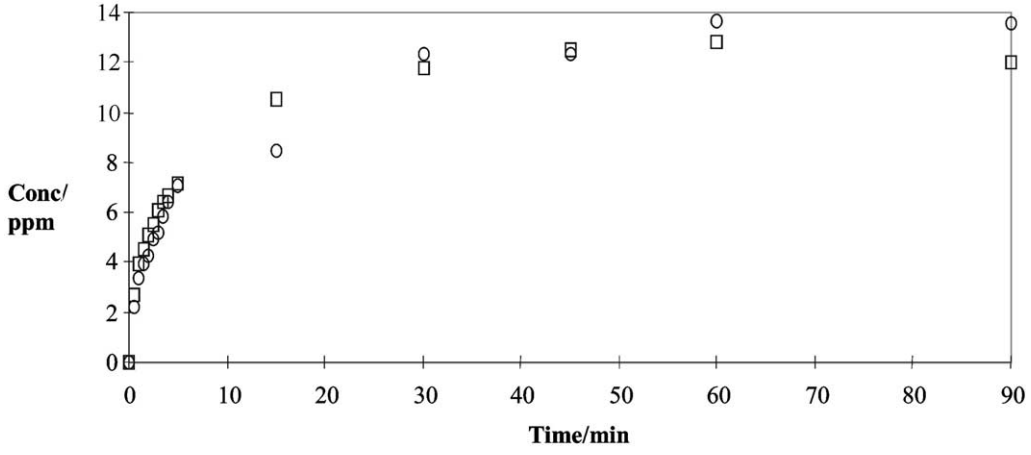

Fig. 3. Plot of concentration versus time for aspalathin showing the typical trend of rapid initial concentration increase, and leading up to the equilibrium concentration (C_∞). The different symbols used represent two of the four analyses performed.

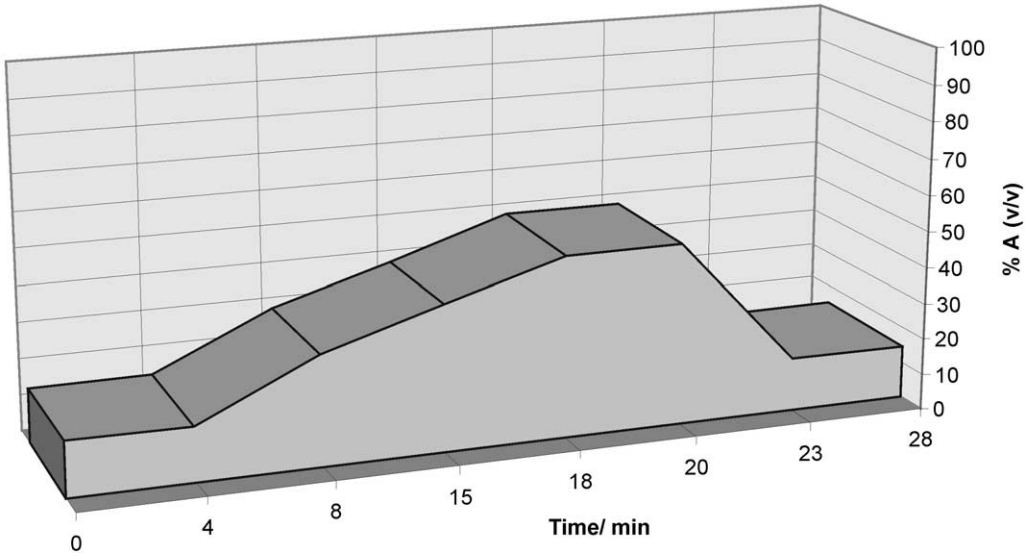

Fig. 4. Pictorial representation of the gradient elution system for the mobile phase, where A represents methanol and B represents 5% (v/v) formic acid in water.

cumstances, the 90 min C_∞ value was used in the calculation of the observed rate constant (k_{obs}).

The corrected values of C_∞ and C, the concentration at time t were fitted into Eq. (1),

$$\ln\left(\frac{C_\infty}{C_\infty - C}\right) = k_{obs}t \quad (1)$$

and the k_{obs} values obtained by plotting the ln function against time. The resulting mean k_{obs} and the intercept values obtained from the plot are shown in Table 2, and included is the equilibrium concentration. The first order graph was plotted and can be seen in Fig. 5.

The theory, through Eq. (1), suggests that the line should pass through the origin. This was not the case as seen in the first order plot presented in Fig. 5, as has been reported in many other situations (Jaganyi & Mdletshe, 2000; Price & Spiro, 1985; Price & Spitzer, 1994; Spiro & Lam, 1995) where a semi-empirical intercept (a) was found in each instance. Price and Spiro (1985) suggested that the intercept is the result of a complex infusion process. These workers postulate that the intercept is affected by the loss of solubles, the leaf structure and its uptake of water at the beginning of the infusion process. Price and Spitzer (1994) suggest that the intercepts serve as indicators of the quality of the data and deviations from the model employed. Note that an intercept of $a = 0.5$ implies that about 25% of the soluble component is present in the solution at $t = 0$. An intercept corrected rate constant k_{eqn} can be calculated in two steps. First, once a and k_{obs} have been obtained, the half life ($t_{1/2}$) can be calculated (Jaganyi & Mdletshe, 2000) from

$$t_{1/2} = (\ln 2 - a)/k_{obs} \quad (2)$$

Second, substituting the value of $t_{1/2}$ back into Eq. (1) and setting $C = 0.5C_\infty$ will yield the value of k_{eqn}. The half life $t_{1/2}$ and k_{eqn} values given in Table 2 were cal-

Table 2
The mean (from quadruplicate analysis) C_∞, a, k_{obs}, $t_{1/2}$ and k_{eqn} values of aspalathin at 80 °C

Aspalathin	Mean values
C_∞/ppm	13.0±0.07
intercept	0.20±0.05
$k_{obs}/10^{-4}$ s^{-1}	22.0±0.4
$t_{1/2}$ / s	224
$k_{eqn}/10^{-4}$ s^{-1}	30.9

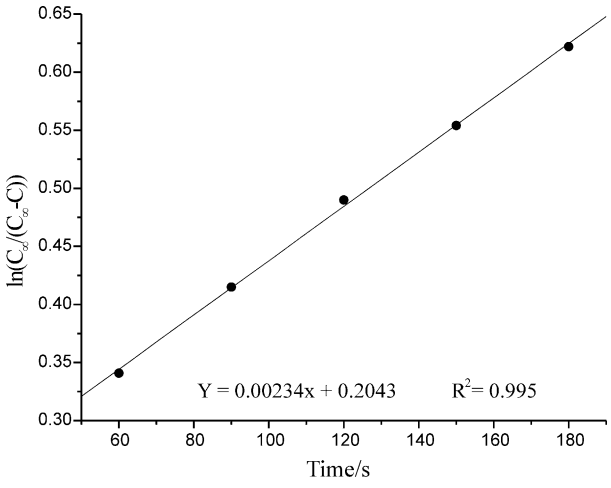

Fig. 5. First order plot for the infusion of aspalathin from Rooibos tea at 80 °C.

Table 3
Comparison of Rooibos tea rate constants with literature values at 80 °C

	Tea type k_{obs} /(10^{-4} s^{-1})				
	Rooibos tea	Japanese Green	Rose-hip tea	Black Assam	Green Chun Mee
Range	22[a]	116–164[b]	23–33[c]	197[d]	245[d]
pH[e]	4.6	–	4.1	4.8	5.6

[a] This work (aspalathin).
[b] Price and Spitzer (1994) (epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate).
[c] Spiro and Chen (1993) (range representing values from German, Chilean and French teas).
[d] Spiro and Lam (1995) (caffeine).
[e] Measured at equilibrium.

culated according to a method devised by Price and Spitzer (1994) and, although they represent essentially the same data both are given to aid assessing the impact of the non-zero intercept.

Most rate constants given in the literature are based on investigations at 80 °C, and thus it is expedient to compare these values with the rate constant obtained in this work. Literature reported rate constants, using different sieving fractions, were converted to reflect those that would have been obtained using a 1.40–1.00 mm sieving fraction (Spiro & Chong, 1997). This was achieved knowing that $k_{obs} \propto 1/d^2$ as shown by Spiro and Jago (1982). The value of d was taken to be the mean of the upper and lower sieving fraction limits. The value for aspalathin and those reported in the literature are compared in Table 3.

It appears that only the data from the rose-hip tea investigations compares favourably with data obtained in the present work. The range of infusion rate constants for L-ascorbic acid in German, Chilean and French rose-hip teas (Spiro & Chen, 1993) compares well with that of aspalathin in Rooibos tea. However, the rate constants for the flavanols from Japanese green tea, as investigated by Price and Spitzer (1994), are very different, with values over 5 times as large as that found for the Rooibos tea flavanol. Similarly the rate constants for caffeine, in black Assam Bukial and green Chun Mee teas, range from 3 to 10 times that of aspalathin in Rooibos tea. This difference can be attributed to the fact that the teas are different and undergo very different manufacturing processes (Jaganyi & Price, 1999). In the green and black tea cases, the leaf is subjected to a tearing process, which opens the leaf and makes the infusion path for water in and species out of the leaf matrix much easier. Also, the difference in leaf structure between the plant species could account for the difference in rate constants. The pH value obtained for Rooibos tea liquor is comparable with those found for the other teas, except green Chun Mee tea, whose pH value was approximately 20% higher.

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