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ARTICLE

Effect of Pre-drying Treatments and Storage on Color and Phenolic Composition of Green Honeybush (*Cyclopia subternata*) Herbal Tea

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The effect of various pre-drying treatments and storage temperatures on the color (L^*, a^*, b^*, chroma, and hue angle) and phenolic composition of green *Cyclopia subternata* was investigated. Pre-drying treatments, which included comminution + drying (T2), steaming (ca. 90−93 °C/60 s) + comminution + drying (T3), and comminution + steaming + drying (T4), had a detrimental effect ($p < 0.05$) on the color parameters relative to the control (T1) (drying of intact leaves). All drying took place at 40 °C and 30% relative humidity (RH). Of the pre-drying treatments (T2, T3, and T4), the best retention of the green leaf color was observed for T3 ($p < 0.05$). T2 reduced the SS and TP contents of the leaves ($p < 0.05$) as well as the content of individual phenolic compounds, including mangiferin, isomangiferin, and eriocitrin. Scolymoside did not follow the same trend as the other compounds, with the intact leaves having the lowest content. It is postulated that oxidation of eriocitrin (eriodictyol-7-*O*-rutinoside) to scolymoside (luteolin-7-*O*-rutinoside) occurred with comminution and steaming. The phenolic composition of samples, subjected to T1 and T3 and a 6 month storage period at 0 and 30 °C, remained stable. Storage at 30 °C resulted in detrimental color changes, leading to the loss of the green leaf color irrespective of the pre-drying treatment.

KEYWORDS: Honeybush; herbal tea; *Cyclopia subternata*; steam treatment; CIELab; mangiferin; isomangiferin; eriocitrin; scolymoside

INTRODUCTION

Honeybush tea is prepared from a number of *Cyclopia* species (family Fabaceae), which are endemic to the Cape fynbos biome, found only in South Africa. The U.S.A., The Netherlands, and Germany represent some of the major markets. It is mostly consumed in its "fermented" (oxidized) form, but there is also a demand for the "unfermented" (green) product, both as herbal tea and source material for the preparation of extracts for the food, nutraceutical, and cosmetic industries (*1*). The presence of mangiferin (**Figure 1**), known for various pharmacological properties, including antioxidant activity (*2*), has also focused interest on *Cyclopia* as an important, sustainable source of mangiferin for the production of antioxidant extracts. Of several *Cyclopia* phenolic compounds tested in the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging assay, mangiferin displayed the highest activity (*3*). In the same test, eriocitrin (**Figure 1**) was less active than mangiferin but substantially more active than hesperidin. Eriocitrin, present in similar quantities to hesperidin in *Cyclopia subternata* (*3*), offered better protection against lipid peroxidation *in vitro* than hesperidin (*4*) and suppressed exercise-induced oxidative damage in rat liver (*5*). Both flavanone glycosides displayed cholesterol and triglyceride-lowering effects *in vivo* (*4*, *6*), findings that enhance the nutraceutical potential of *C. subternata*. Of the different *Cyclopia* species that are of commercial importance (*1*), *C. subternata* would be the species of choice for producing extracts with the highest eriocitrin content (*3*).

During the production of green *C. subternata*, slow greenish brown discoloration of the leaves occurs when the shoots are shredded, leading to a loss in perceived quality. The shredded plant material is in most cases sun- or oven-dried without any attempt to prevent possible degradation reactions. Because cell disruption and exposure to oxygen during shredding initiate phenolic oxidation (*7*), discoloration is assumed to indicate a change in the phenolic composition and a loss of bioactives, which will decrease the value of the final product. Chlorophyll degradation, leading to pheophorbide, which has an olive brown color (*8*), could also be expected to occur when the plant material is shredded. However, it is not known to what extent changes in color and phenolic composition take place in the interim period between shredding and drying. This lack of information was addressed in the present study. Leaves of *C. subternata* were subjected to different pre-drying treatments, of which their suitability was assessed in terms of the retention of the green color, extract yield (soluble solids), and phenolic composition.

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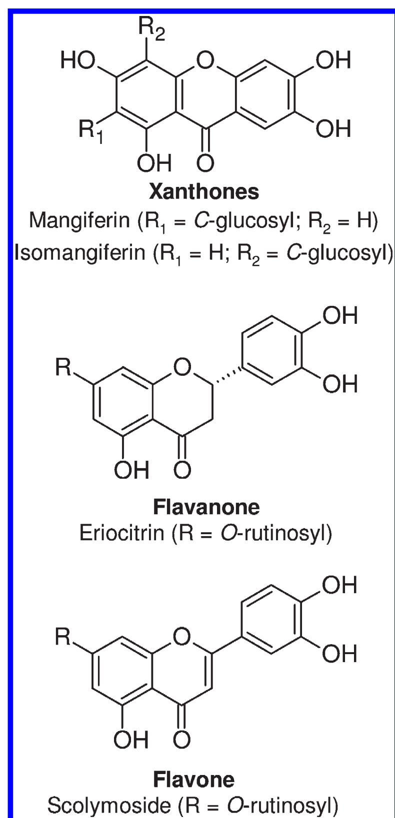

Figure 1. Structures of mangiferin, isomangiferin, eriocitrin, and scolymoside.

Incorrect storage conditions, such as high temperatures (30 °C), prevalent during summer could also be detrimental to the retention of color and phenolic compounds. To date, no long-term storage experiments have been carried out to determine the stability of honeybush. The stability of color and phenolic composition of the leaves of selected pre-drying treatments during storage at 0 and 30 °C were thus also evaluated.

MATERIALS AND METHODS

Chemicals. Eriocitrin, narirutin, hesperetin, naringenin, and luteolin were purchased from Extrasynthese (Genay, France). Mangiferin, gallic acid, hesperidin (97%), dimethyl sulfoxide (DMSO) (99.5%), acetonitrile R Chromasolv (Riedel-de Haën), and glacial acetic acid (99.8%) (Riedel-de Haën) were purchased from Sigma-Aldrich (Cape Town, South Africa), and sodium carbonate and Folin's reagent were purchased from Merck (Cape Town, South Africa). Carbon-filtered, reverse osmosis (RO)-treated, deionized water (Modulab Water Purification System, Continental Water Systems Corporation, San Antonio, TX) was further purified by means of a Milli-Q 185 Académic Plus water purification system (Millipore, Bedford, MA) for high-performance liquid chromatography (HPLC) solvent preparation.

Plant Material. *C. subternata* shoots were harvested in July 2005 at Helderfontein, the research farm of ARC Infruitec-Nietvoorbij (Stellenbosch), and at Kanetberg Flora (Barrydale district, Little Karoo), both in the Western Cape province of South Africa. Upon arrival at the research facilities, the plant material was immediately placed in cold storage (< 10 °C) until processed. Processing commenced within a day of harvest.

Effect of Pre-drying Treatments on Color and Composition. Fresh leaves from ca. 1.8 kg of shoots were manually stripped from the stems and mixed thoroughly to improve homogeneity of the sample. The batch of leaves was then divided into four subsamples of ca. 200 g each, which were subjected to the following treatments (**Figure 2**): T1 (control), drying of the intact leaves at 40 °C/30% relative humidity (RH) in a purpose-built dehydrator (*9*) for 4 h; T2, comminution of the leaves into small pieces in a Waring blender for 20 s (a handful of the leaves at a time), followed by drying; T3, steaming of the intact leaves for 60 s at 90−93 °C, followed by comminution and drying; and T4, comminution, followed by steaming and drying. During steaming in a cabinet-type steam blancher, the leaves were spread open in a thin layer on 30-mesh stainless-steel trays (370 × 310 mm) for good steam contact. The trays were placed in the preheated dehydrator immediately after steaming and dried. All samples were dried to less than 6% moisture content as determined gravimetrically.

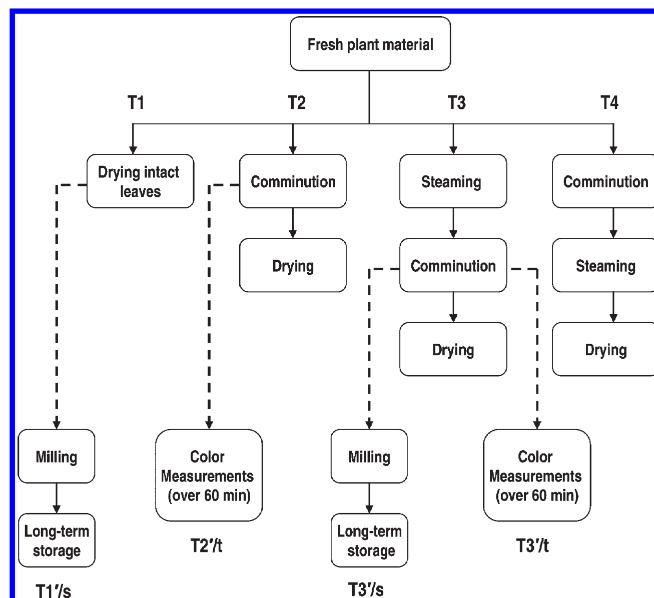

Figure 2. Outline of the different treatments. Solid lines indicate the major treatments. Dashed lines indicate secondary experiments (T′) to investigate the effect of long-term storage (T1′/s and T3′/s) and the change in color of freshly comminuted moist leaves over a 60 min period (T2′/t and T3′/t).

The experiment was repeated 6 times in blocks with six batches of plant material, with each batch representing plant material from different bushes. Color measurements were performed on the dry, comminuted leaves of treatments T2, T3, and T4. T1 (intact leaves) was excluded because of the difference in particle size. For comparison of all treatments, the samples were milled, passed through a 1 mm sieve (Retsch mill, Retsch GmbH, Haan, Germany), and subjected to color measurement and compositional analysis.

Effect of the Steam Treatment on the Color Change of Freshly Comminuted Leaves over Time. Leaves were comminuted as described, and color measurements commenced without delay over a period of 60 min (T2′/t). Measurements were taken every 2 min for the first 30 min, whereafter measurements were taken every 15 min. For the second treatment (T3′/t), ca. 200 g of leaves was first steamed for 60 s at 90−93 °C before comminution. Color measurements were carried out as for T2′/t. The experiment was repeated in triplicate on 2 consecutive days, giving six replicates in total. These samples were not subjected to compositional analyses.

Effects of the Storage Time and Temperature on Color and Composition. The leaves, stripped of ca. 1.8 kg shoots and mixed thoroughly, were divided into two subsamples. The leaves of the first subsample were dried intact at 40 °C/30% RH (T1′/s). The intact leaves of the second subsample were steamed for 60 s at 90−93 °C and then comminuted before drying (T3′/s). The experiment was repeated 3 times. The plant material was milled, and individual subsamples for each time point × storage temperature × treatment × replicate were prepared by sealing the samples in individual glass jars. The samples were stored in the dark in temperature-controlled rooms (0 and 30 °C), and at each time point, being 0, 1, 2, 3, and 6 months, samples were removed for color analysis. Directly after color measurement, the samples were stored in a freezer at −20 °C until compositional analyses of all samples could be performed simultaneously.

Compositional Analyses. The amount of soluble solids in the plant material was determined by extracting 5 g of milled plant material with

45 mL of acetonitrile/water (1:2, v/v) on a steam bath for 30 min. After filtration through Whatman No. 4 filter paper, duplicate aliquots (15 mL) were evaporated and the residual solids were determined gravimetrically. The total polyphenol (TP) content of extracts was determined in triplicate using a 20:1 reduced version (with a final volume of 5 mL) of the Folin−Ciocalteau method of Singleton and Rossi (*10*). Gallic acid was used for the preparation of the calibration curve.

Subsamples of plant material were remilled using a Retsch MM 301 ball mill (Retsch GmbH, Haan, Germany) before extraction for HPLC quantification of the major phenolic compounds. The milled plant material (20 mg) was extracted with 3 mL of acetonitrile/water (1:2, v/v) in vials, heated in a heating block at 100 °C for 20 min, followed by cooling and sonication for 10 min. A total of 1 mL of a 2% ascorbic acid solution was added to the extraction medium before filtering through disposable 0.45 μm Millipore Milex-HV hydrophilic polyvinylidene difluoride (PVDF) syringe filters (33 mm in diameter). HPLC analysis was performed using an Agilent 1200 series HPLC consisting of a quaternary pump, autosampler, inline degasser, column oven, and diode-array detector (Agilent Technologies, Inc., Santa Clara, CA), with Chemstation 3D LC software. Separation was performed on a Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm particle size, 80 Å pore size) from Agilent Technologies protected by a guard column with the same stationary phase. The solvents used for separation were 0.1% formic acid and acetonitrile at a flow rate of 1 mL/min using the solvent gradient reported previously (*11*). Mangiferin, isomangiferin, and scolymoside were quantified using the peak areas at 320 nm, while eriocitrin and the unidentified compounds, compounds **1** and **6** (**Figure 3**), were quantified using the peak areas at 288 nm. Standard dilution series of mangiferin (0.05−2.5 μg injected), eriocitrin (0.01−0.8 μg injected), and luteolin (0.01−0.4 μg injected) were used for external calibration. Isomangiferin and scolymoside were quantified in terms of mangiferin and luteolin, respectively, while compounds **1** and **6** were quantified in terms of eriocitrin equivalents.

Liquid chromatography−mass spectrometry (LC−MS) and LC−MS^2 analyses were performed on a Waters API QTOF Ultima apparatus with a Waters UPLC system (Waters, Milford, MA), operated in the negative elecrospray ionization mode. The identity of compounds was further confirmed by comparing the MS and MS^2 spectra to those of the available pure reference standards and the data previously reported (*11*). The effluent from the UPLC was split, and ca. 300 μL/min was introduced into the MS. The LC−MS analysis parameters were as follows: desolvation temperature, 370 °C; nitrogen flow rate, 370 L/h; source temperature, 100 °C; capillary voltage, 3700 V; and cone voltage, 35 V. For LC−MS^2 analysis the collision energy setting was 25.

Color Measurement. CIELab color measurements were performed with a Colorgard 2000/05 (BYK-Gardner, Geretsried, Germany) in reflectance mode, using the CIE 1931 standard colorimetric observer under illuminant C (geometry is 45° illumination and 0° viewing). Calibration with a quartz plate in place was performed with a black tile (Gardner number 05-1528) and a white tile (L^* = 94.53; a^* = −0.99; b^* = 0.92; Gardner number 05-1528). L^*, a^*, and b^* were measured directly, and chroma and hue angle (degree) were calculated as follows (*12*):

$$\text{chroma} = [(a*)^2 + (b*)^2]^{1/2} \quad (1)$$

$$\text{hue angle} = \tan^{-1}(b*/a*) \quad (2)$$

Negative values of the hue angle were converted to positive values by adding 180°, so that it could fall in the 90−180° quadrant ($+b^*$ = yellow; $-a^*$ = green). In the text, the hue angle will be referred to as hue. The quartz sample cup, $^3/_4$-filled, was covered with a black cover to exclude any external light, and measurements were performed from the bottom by illuminating a 10 mm diameter area. The color of the milled samples was measured once, while the color of the comminuted (not milled) samples was measured 5 times, taking into account the small area of illumination for color measurement. Before each consecutive measurement, the comminuted dried leaves were poured out and mixed and the sample cup was refilled to ensure that good average color values for a treatment could be obtained.

Color measurements of the moist, comminuted leaves of treatments T′2/t and T′3/t, performed over 60 min, required the sample cup to be removed from the sensor between measurements to prevent gradual heating of the sample and acceleration of degradation reactions. To ensure that the same sample area was illuminated again, the sample cup was positioned with a mark on the cup aligned with one on the sensor. All measurements of a treatment × replicate were completed before preparation and color measurement of the next sample commenced.

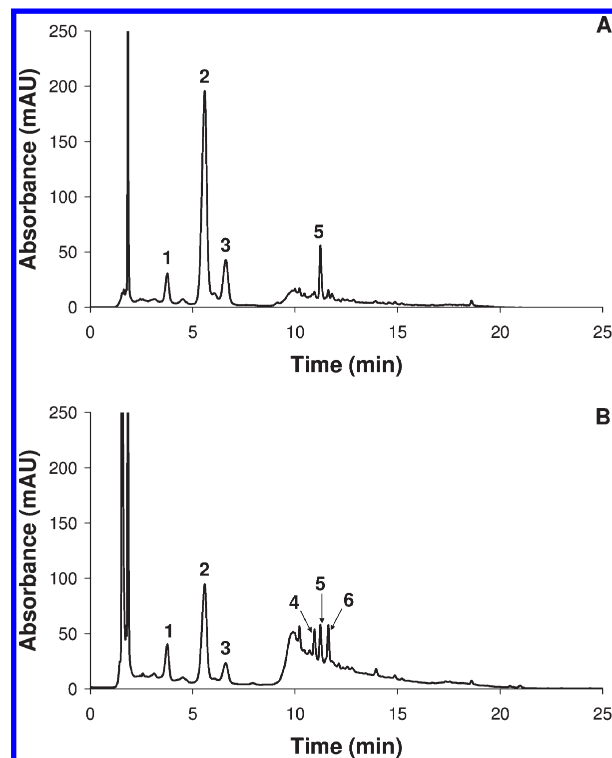

Figure 3. Typical HPLC chromatograms of extracts from dried, milled green *C. subternata* leaves (**1**, unidentified compound; **2**, mangiferin; **3**, isomangiferin; **4**, eriocitrin; **5**, scolymoside; **6**, unidentified flavanone glycoside) with detection at (A) 320 nm for the xanthones and flavone and (B) 288 nm for the flavanones.

Statistical Analyses. Analysis of variance (ANOVA) was used to determine whether the differences between treatment means were significant. In cases where significant ($p < 0.05$) differences were found, least significant difference (LSD) of the Student's t test ($p = 0.05$) was calculated to compare treatment means. Data were tested for normality using the Shapiro−Wilk procedure. Color and compositional data for the storage experiment were fitted to the models $y = a + b\ln(x + 1)$ and $y = a + bx$, respectively, to calculate their rate of change during storage, where x = time, y = parameter measured, i.e., mangiferin content, and b = rate of change. The SAS software package, version 9.1 (SAS Institute, Inc., Cary, NC), was used for all statistical analyses and curve fitting.

RESULTS

Figure 3 depicts typical HPLC chromatograms of an acetonitrile/water extract of green *C. subternata* at 320 and 288 nm. Large peaks representing mangiferin, isomangiferin, eriocitrin, scolymoside, compound **1**, and compound **6** were quantified to determine the effect of the pre-drying treatments and storage conditions on phenolic composition. Only small peaks were observed for hesperidin (0.06%), luteolin (0.03%), naringenin (<0.01%), hesperetin (<0.01%), and narirutin. The latter peak co-eluted with an unidentified compound and could thus not be accurately quantified.

Mangiferin, eriocitrin, narirutin, hesperidin, naringenin, hesperetin, and luteolin were tentatively identified by comparing their retention times and UV−vis spectra from HPLC analysis and their LC−MS and LC−MS^2 characteristics to those of authentic standards. Identification of isomangiferin, scolymoside, and compound **6** was based on corresponding data reported by De Beer and Joubert (*11*). Compound **1**, eluting

Table 1. Effect of Treatments T2, T3, and T4 on the Color Values of Dried, Comminuted Leaves of Green *C. subternata*

treatment[a]	L^{*b}	a^{*b}	b^{*b}	chroma[b]	hue[b]
T2	32.69 ± 1.48 a	1.31 ± 1.79 a	16.12 ± 1.32 b	16.27 ± 1.28 b	85.12 ± 6.35 c
T3	33.69 ± 1.38 a	−1.82 ± 0.94 c	17.27 ± 1.07 a	17.39 ± 1.04 a	96.09 ± 3.22 a
T4	33.37 ± 1.19 a	−0.23 ± 1.44 b	17.80 ± 1.00 a	17.86 ± 1.00 a	90.79 ± 4.61 b

[a] T2, leaves comminuted and dried; T3, leaves steamed, comminuted, and dried; T4, leaves comminuted, steamed, and dried. [b] Means in a column with different letters are significantly different ($p < 0.05$).

Table 2. Effect of Treatment on the Color Values and Composition of Dried, Milled Green *C. subternata* Leaves

parameter[a]	T1[b]	T2[b]	T3[b]	T4[b]
L^*	53.91 ± 1.88 a	47.86 ± 1.74 c	50.40 ± 1.73 b	49.27 ± 2.88 bc
a^*	−7.08 ± 3.69 b	−2.94 ± 2.37 a	−5.67 ± 1.24 b	−4.48 ± 1.45 ab
b^*	30.31 ± 1.43 a	27.40 ± 1.65 b	27.90 ± 1.11 b	27.71 ± 1.09 b
chroma	31.28 ± 2.08 a	27.63 ± 1.87 b	28.49 ± 1.25 b	28.10 ± 1.12 b
hue angle	102.83 ± 6.26 a	95.91 ± 4.53 b	101.44 ± 2.21 a	99.18 ± 2.88 ab
SS[c,d]	42.93 ± 1.95 a	40.09 ± 1.12 b	42.11 ± 1.38 a	41.80 ± 1.44 a
TP[c,d]	12.75 ± 0.66 a	11.72 ± 0.57 b	12.50 ± 0.50 a	12.25 ± 0.46 a
mangiferin[d]	1.22 ± 0.35 a	1.05 ± 0.25 b	1.11 ± 0.22 b	1.13 ± 0.30 ab
isomangiferin[d]	0.38 ± 0.05 a	0.34 ± 0.04 b	0.37 ± 0.05 a	0.37 ± 0.05 a
eriocitrin[d]	0.23 ± 0.06 a	0.19 ± 0.04 b	0.25 ± 0.04 a	0.22 ± 0.05 a
scolymoside[d]	0.48 ± 0.32 b	0.50 ± 0.31 ab	0.53 ± 0.28 a	0.49 ± 0.30 ab
compound **1**	0.25 ± 0.06 a	0.16 ± 0.04 b	0.24 ± 0.08 a	0.21 ± 0.04 a
compound **6**[c,d]	0.41 ± 0.01 a	0.25 ± 0.09 c	0.44 ± 0.11 a	0.35 ± 0.13 b

[a] Means in a row with different letters are significantly different ($p < 0.05$). [b] T1, leaves dried intact; T2, leaves comminuted and dried; T3, leaves steamed, comminuted, and dried; T4, leaves comminuted, steamed, and dried. [c] TP, total polyphenol content; SS, soluble solids content; compound **1**, unidentified; compound **6**, unidentified flavanone glycoside. [d] In grams per 100 g of dried plant material.

before mangiferin, could not be identified by type, e.g., flavanone, based on its UV−vis spectrum. Its LC−MS and LC−MS^2 characteristics were previously observed for an unidentified compound present in several *Cyclopia* species (*11*).

Effect of Pre-drying Treatments. As an initial evaluation of the pre-drying treatments, the color of the dry, comminuted leaves (not milled) was determined, with a^* ($+a^*$ indicating redness and $-a^*$ indicating greenness) and hue differing significantly between treatments (**Table 1**). The visual inspection indicated leaves subjected to T2 to be slightly brown−green, while T3 best retained the green leaf color, an observation supported by the relevant a^* and hue values. Higher b^* and chroma values were obtained for treatments T3 and T4 than T2 ($p < 0.05$), while L^* was not significantly affected by treatment.

Milling of the samples, required to include T1 (control), changed all color data parameters because of the effect of the particle size on reflectance (**Table 2**). Significantly higher values for L^*, b^*, and chroma ($p < 0.05$) were obtained for T1 compared to the other treatments. In the case of a^* and hue, only T1 was significantly different from T2. T2 was the most detrimental to composition (**Table 2**), with significant ($p < 0.05$) reductions in the TP and SS content of the leaves, and the least retention of individual phenolic compounds, i.e., isomangiferin, eriocitrin, and compounds **1** and **6**. The mangiferin content of leaves was equally reduced by T2, T3, and T4. For isomangiferin, eriocitrin, and compound **1**, T3 and T4 were as effective as T1 in retaining these compounds, whereas for compound **6**, T3 was as effective as T1. Interestingly, the highest scolymoside content was observed for leaves subjected to T3 and not T1.

Effect of the Steam Treatment on the Color Change of Freshly Comminuted Leaves over Time. In this experiment, the effect of steam treatment (T3′/t) versus no steam treatment (T2′/t = control) on the color change of the moist leaves during the first 60 min after comminution was followed. Rapid change in color was observed for all CIELab parameters of T2′/t, while L^*, b^*, and chroma (data not shown) of T3′/t remained relatively stable (**Figure 4**). Changes in a^* and hue were observed for T3′/t (**Figure 4**). Steam treatment lowered L^* initially, after which it remained stable. Comparing the color data of the leaves at 0 and 60 min showed that L^*, b^*, chroma, and hue of T2′/t were significantly ($p < 0.05$) lower after 60 min. The a^* value changed from negative (−6.43) to positive (1.66) ($p < 0.05$). Only the a^* and hue values of T3′/t changed significantly ($p < 0.05$) during the 60 min period.

Effect of the Storage Time and Temperature. For this experiment, samples were subjected to storage in sealed containers for 6 months. During this period, their moisture content remained stable at ca. 5.5%. Storage at 0 °C was effective in preventing both color (**Figure 5**) and compositional changes (data not shown). The control treatment (T1′/s) was included as a reference because it was expected to retain its color better than other treatments and, thus, to serve as benchmark. This was confirmed by data for the rate of change at 30 °C, with the color parameters of T3′/s (except L^*) changing faster than those of T1′/s (**Table 3**). **Figure 5** depicts the change in the color parameters L^*, a^*, b^*, and hue angle during storage. Chroma gave a similar trend to b^* (data not shown). The only significant ($p < 0.05$) compositional changes observed at 30 °C were an increase in the TP content of leaves subjected to both treatments and the SS content of T3′/s. Even at the higher storage temperature, the phenolic compounds remained stable, irrespective of the treatment (**Table 3**).

DISCUSSION

Consumers perceive the loss of food color as a loss of quality, because undesirable chemical changes are in many instances associated with color changes. This view is also shared by the regulatory body in South Africa dealing with the quality standards for the export of green honeybush, because emphasis is placed on leaf color as part of the standards for export. Not only is browning of green honeybush unacceptable, but it could negatively impact on traditional "fermented" honeybush, a dark-brown colored product, because consumers could perceive the product as a poor-quality "fermented" honeybush. It is thus of great importance that processors employ processing and

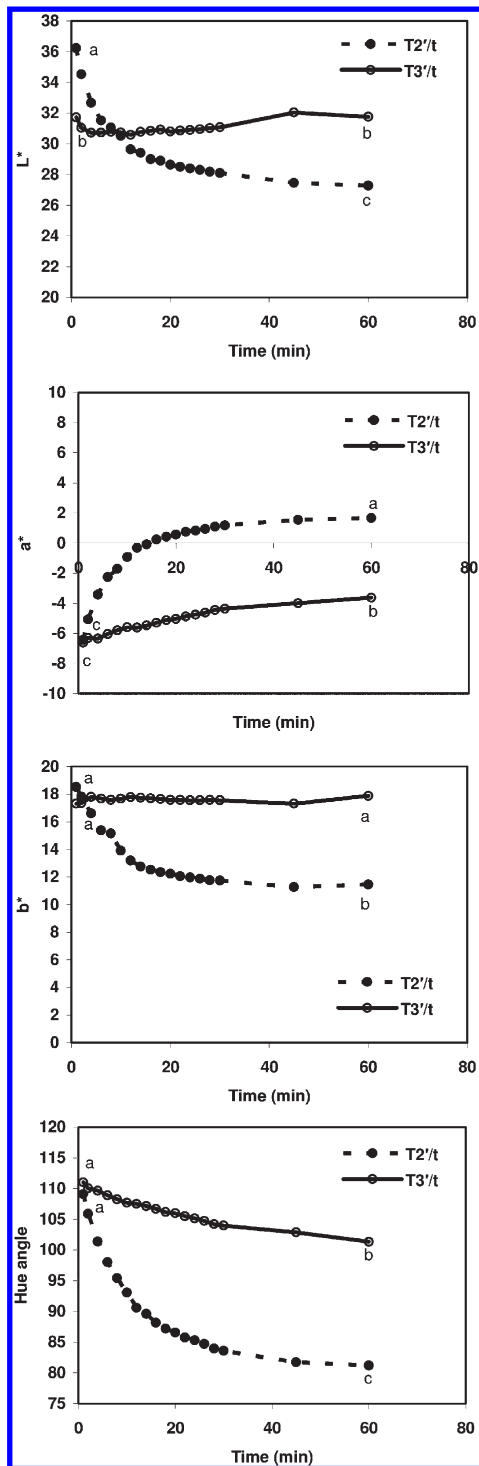

Figure 4. Change in color values of freshly comminuted *C. subternata* leaves subjected to treatments T2′/t and T3′/t (depicted in **Figure 2**). Data at 0 and 60 min were compared (ANOVA), and means with different letters are significantly different ($p < 0.05$).

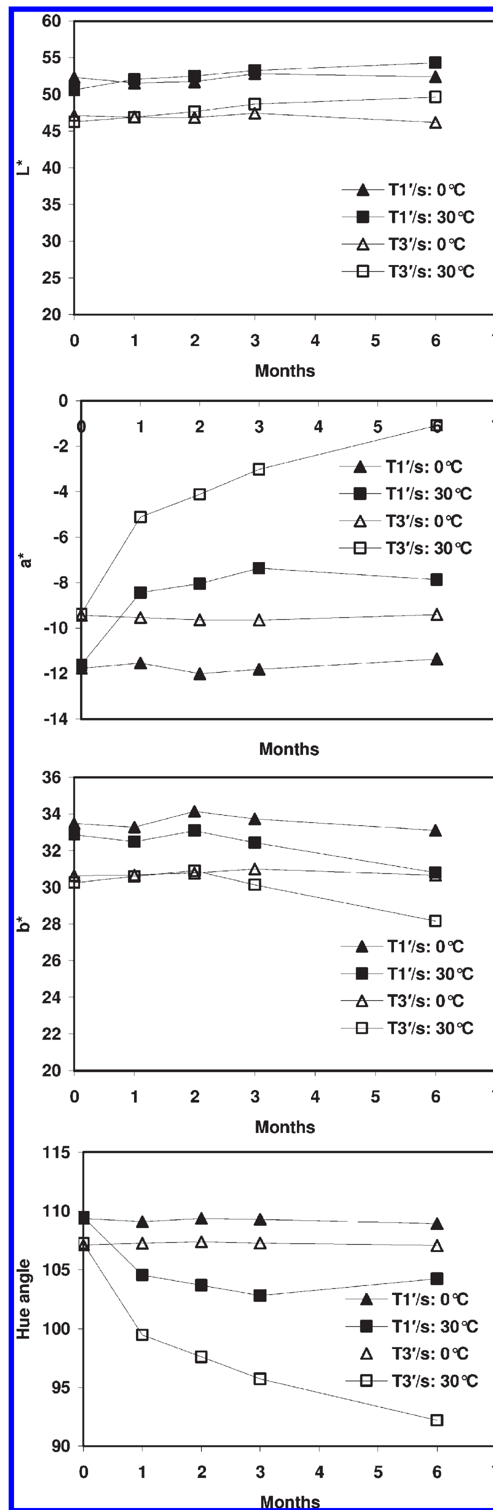

Figure 5. Effect of the storage time and temperature on the color values of dried, milled green *C. subternata* leaves subjected to treatments T1′/s and T3′/s (depicted in **Figure 2**).

storage conditions that mimimize color and compositional changes of green honeybush, irrespective its use as herbal tea or source material for the production of nutraceutical extracts. In the latter case, it is of utmost importance that processing conditions employed are optimum for maximum retention of bioactive compounds.

In this study, only leaves with very fine green stems attached were used for experimentation, solely because of practical considerations. The thick stems of shoots are yellow−brown to brown, which, if included in the sample, would have "masked" color changes. Under normal manufacturing conditions, the stem content of the final dried product is reduced by sieving. The use of mostly leaves, however, resulted in plant material with an atypical low hesperidin content. Plant material harvested according to normal practice, which consists of leaves and coarse stems, contains ca. 0.6% hesperidin.

Pre-drying Treatments. The pre-drying treatments were chosen to create situations that are either current practices (T2) or

Table 3. Rate of Change in Color Values and Composition of Dried, Milled Green *C. subternata* Leaves Stored at 30 °C

parameter[a]	T1′/s[b]	T3′/s[b]
L^*	1.888 ($p < 0.001$)	1.828 ($p < 0.001$)
a^*	1.963 ($p < 0.004$)	4.158 ($p < 0.001$)
b^*	−0.891 ($p = 0.024$)	−0.951 ($p = 0.055$)
chroma	−1.396 ($p = 0.002$)	−1.651 ($p = 0.002$)
hue angle	−2.819 ($p = 0.008$)	−7.531 ($p < 0.001$)
SS[c]	0.490 ($p = 0.487$)	−0.338 ($p = 0.010$)
TP[c]	0.635 ($p = 0.019$)	0.361 ($p < 0.001$)
mangiferin	0.004 ($p = 0.908$)	<0.000 ($p = 0.994$)
isomangiferin	0.002 ($p = 0.751$)	0.001 ($p = 0.805$)
eriocitrin	0.001 ($p = 0.723$)	0.005 ($p = 0.902$)
scolymoside	0.001 ($p = 0.926$)	<0.000 ($p = 0.956$)
compound **1**[c]	−0.001 ($p = 0.942$)	−0.003 ($p = 0.913$)
compound **6**[c]	0.004 ($p = 0.614$)	0.004 ($p = 0.793$)

[a] Rates for color change were calculated according to $y = a + b \ln(x + 1)$. Rate for compositional change (grams per 100 g of plant material) were calculated according to $y = a + bx$. [b] T1′/s, dried intact; T3′/s, steamed, comminuted, and dried. [c] TP, total polyphenol content; SS, soluble solids content; compound **1**, unidentified; compound **6**, unidentified flavanone glycoside.

provide the industry with a viable alternative (T4) during the production of green honeybush tea. Intact leaves (T1) were used as the control. Because the cellular structures were not damaged, contact between enzymes and substrates was prevented, so that optimum green leaf color and retention of bioactives could be obtained, without resorting to freeze-drying. In practice, when the fresh shoots are shredded to prepare particle sizes suitable for the tea retail market, rapid discoloration and the loss of green color occur, especially on warm days. Steaming of leaves before comminution (T3) is less feasible than steaming after comminution (T4), but the treatment was included to determine whether the sequence in which these two processing steps occur is critical.

Significant changes in phenolic composition, manifested by the decrease in TP content as well as the content of the individual phenolic compounds, except scolymoside, indicate that polyphenol oxidation occurred. Unnecessary delays in arresting enzyme activity after communition of the leaves will aggravate the losses. The change in color parameters after comminution (T2′/t) over 60 min showed that a delay was detrimental to color, with a^* changing from negative to positive after ca. 14 min.

Interestingly, scolymoside did not follow the same pattern as the other compounds. If the intact leaves (T1) reflect the "true" scolymoside content before processing, then it must be assumed that some scolymoside was formed during processing. Oxidation of eriocitrin, a flavanone, accompanied by the conversion of the C2−C3 bond to a double bond would lead to the formation of scolymoside, a flavone (**Figure 1**).

Treatment T2 was least effective in preventing compositional changes; similarly, it was also the least effective in retaining the green leaf color, as indicated by the positive a^* (and no visual green) and lowest hue angle (< 90°, thus locating T2 in the red/yellow quadrant of the color solid). Enzymatic degradation reactions, initiated by comminution, would be accelerated by the mild heat of the drying process. PPO can be active over a wide temperature range, i.e., 20−60 °C (*13*), while the optimum temperature of strawberry chlorophyllase is 40 °C (*14*). Evaporation during drying would keep the comminuted leaves at a lower temperature than that of the circulating air, until the almost dry state is reached. Enzyme activity would be possible during drying, especially during the initial stages, when the water activity (a_w) of the leaves is still high. Because the a_w of the leaves decreases during drying, enzyme activity would also decrease. LaJollo et al. (*15*) showed that chlorophyll was mostly converted to pheophytin at 37 °C and $a_w > 0.32$. At $a_w < 0.32$, the rate of pheophytin formation was low and other products were formed.

Steaming of intact leaves before comminution (T3) proved best for color retention based on the most negative a^* and the highest hue values, as well as visual observation. The hue angle was higher than 90°, locating the sample in the green/yellow quadrant of the color solid (*16*). The effect of T3, being the least detrimental to the green leaf color, was to be expected, because enzymes were inactivated before the leaves were comminuted. The extent to which PPO and chlorophyllase are inactivated depends upon the plant material. Treating sweet potato PPO at 90 °C for 1 min completely inactivated the enzyme (*17*), while PPO in *Camellia sinensis* leaves required 4 min of steam treatment (*18*). Chlorophyllase of strawberry has considerable resistance to heat treatment (*14*), whereas steaming of Tencha leaves for 18 s reduced its activity to about $^1/_{18}$th of that in fresh leaves (*19*).

Treatment T4 was less effective than T3 in retaining the green color, because comminution took place before steaming. Exposure of chlorophyll to acids as a result of cell disruprion combined with the heat of the steaming process could lead to substitution of magnesium in the porphyrin ring with two hydrogens and the formation of pheophytin (*20*). In leafy vegetables, this conversion to pheophytin is accompanied by an undesirable color change from bright green to olive brown (*21*). Enzymatic activity initiated by comminution would also cleave the phytol chain, leading to chlorophyllides and eventually pheophorbides (*20*).

The change in the honeybush leaf color with T4 was visually observed and confirmed by objective color measurement data, i.e., the substantially smaller $-a^*$ value (less green) and lower hue angle. This located T4 leaves just in the green/yellow quadrant of the color solid. T4, with further optimization, could be a viable treatment option for industry because equipment is available to rapidly steam comminuted plant material. T3, although retaining the green color better than T4, would not be feasible, because it would require steaming of whole shoots.

Storage. For the storage experiment, T3 was used to ensure that the starting material was of an adequate green color, so that any change in a^* and hue would mostly occur while the plant material was still green. T1 was once again included as the control. Storage temperatures were selected to include a control temperature (0 °C), at which no change was expected, and a high temperature (30 °C), which could be prevalent during normal summer conditions in areas where honeybush tea is produced. Treatment again affected the color parameters, consistent with the results of the first experiment, comparing the efficacy of treatments. As expected, no changes in leaf color and composition occurred when the plant material was stored at 0 °C. Storage at 30 °C was not detrimental to the phenolic composition of the leaves, irrespective of their pre-drying treatments, but color was affected, as indicated by the rate of change in the color parameters. A substantial difference in the rate of change of a^* and hue between T1′/s and T3′/s was observed, with the T3′/s samples having substantially higher rates (change in a^* and hue 2.1 and 2.7 times faster, respectively). These differences in the rate of change would suggest that treatments introduced qualitative changes in chlorophyll composition. Chlorophyllide, a green degradation product of chlorophyll, degrades faster than chlorophyll (*22*). Chlorophyllide *a* degraded 2 times faster than chlorophyll *a*, and chlorophyllide *b* degraded 4 times faster than chlorophyll *b* at high temperatures. It is thus possible that chlorophyllide was formed when the T3′/s samples were steamed. Their more rapid degradation would explain the difference between T1′/s and T3′/s.

The color parameters a^* and hue were shown to be the most relevant for quantifying color changes and could, in the future, be

used to assess the color of green honeybush. Previous studies on green vegetables and herbs showed good correlation between these instrumental parameters and their chlorophyll content (*9*, *23−25*). In terms of the processing of green *C. subternata*, the herbal tea processor can improve retention of color and phenolic compounds substantially by implementing a steam process directly after comminution and treating the tea without unnecessary delays. Because high summer temperatures would accelerate detrimental color changes, good thermal insulation in the storage area of the processed plant material is advisable.

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