

Effect of Seasonal Variations and Growth Conditions on Carbohydrate Partitioning in Different Organs and the Quality of Bush Tea

Mudau N. Fhatuwani¹

Department of Agriculture and Animal Health, University of South Africa, Private Bag X6, Florida 1710, South Africa

Makunga P. Nokwanda

Department of Botany and Zoology, Stellenbosch University, Private Bag XI, Matieland, Stellenbosch 7602, South Africa

Additional index words. flavonoids, harvest, seasonal, sugars, phenols, tea quality

Abstract. Bush tea (*Athrixia phylicoides* DC.) is a root perennial shrub used as indigenous tea and medicinal tea in South Africa. Thus, concurrent trials were conducted under different growing conditions as follows: in the glasshouse, field planted and wild, naturally grown, to investigate the effects of seasonal harvests and growing environments on carbohydrate reserves and quality parameters of bush tea. Of 50 plants, 25 single plants were allotted to each respective environment in a field and glasshouse conditions—and were arranged in a randomized complete block design. These were then harvested in summer, autumn, winter, and spring, respectively. For the wild bush tea trial, 25 single plants were randomly selected. Selected sugars and starch were quantified together with other quality parameters [total polyphenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and total antioxidant activities). The study revealed that the glucose content of bush tea plant organs was significantly higher during winter, followed by autumn, as compared with the other seasons. Similar fructose and sucrose trends were evident. However, the content of amylopectin was also significantly higher during summer, followed by autumn, compared with the other seasons. In winter, plants exhibited higher amylopectin content when compared with other seasons. No significant differences were found in the amylose content. Both wild and cultivated bush tea plants yielded the highest specific sugars in the study. The phytochemicals present in the leaves of field-grown bush tea and wild bush tea during winter were higher than in those grown in summer, autumn, and spring. No significant difference in tannin contents was observed, irrespective of seasons and growing conditions. Regardless of growing conditions, autumn yielded lower total antioxidant activities using both the DPPH and ferric reducing antioxidant power (FRAP) assays when compared with other seasons. To better resolve the metabolomic data, principal component analysis (PCA) was used and the first principal component showed a strong correlation within all parameters recorded over PC2. Future ecophysiological studies are recommended to establish region- and season-specific metabolomic biomarkers with canonical distinction on beverage, pharmacological, and organoleptic attributes of bush teas.

Bush tea (*A. phylicoides* DC.), which is indigenous to South Africa (Maudu et al., 2012), has been used as a beverage and as a medicinal herb that has curative metabolites with the ability to relieve the oxidative damage associated with modern lifestyle diseases. Traditionally, the herb is greatly treasured in folklore medicine for the treatment of diabetes, heart disease and hypertension, acne, boils, colds, cuts, headache,

infected wounds, loss of voice, and infected throat (Mudau et al., 2006; Nchabeleng et al., 2012; Roberts, 1990). In folklore, it is also recommended as a potent blood cleanser (Roberts, 1990), which is further substantiated by its high levels of total polyphenol content (Mudau et al., 2007a). As bush tea is a caffeine-free consumable, this is an advantage for those searching for an alternative tea that has many health benefits without risking the deleterious effects associated with the over consumption of caffeine or who may suffer from caffeine-related allergies (Lerotholi et al., 2017). Apart from its medicinal and culinary value, it has great potential as a horticultural species because of its attractive mauve flowers (Mbambenzi, 2005). Recently, Lerotholi et al. (2017) indicated that interest in this species as a commercialized tea (Malongane et al., 2017) is

growing but the lack of scientific information regarding the environmental regulation of the biochemicals produced by this species may be hampering its commercialization. Plants are found at various localities on the eastern regions of South Africa spanning from the Eastern Cape Province, KwaZulu-Natal to the northern province of Limpopo Province, and these areas are geographically and climatically different, leading to wide-ranging chemotypic variation of populations Lerotholi et al. (2017). Studies focused on metabolomic analysis of bush tea are, thus, urgently needed. Disparate environments and seasonal changes contribute to variable phytochemical accumulation in plants influencing both primary and secondary (specialized) metabolites (Nkomo et al., 2014).

Bush tea has a complex chemistry but some of the most prominent phytochemicals include the following: quercetin, 3-O-demethylgigicitrin, 5,6,7,8,3',4'-hexamethoxyflavone (Mudau et al., 2007a), 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavan-3-ol (Mashimbye et al., 2006), tannins (Mudau et al., 2007b), and antioxidants (Mogotlane et al., 2007). Although these secondary metabolites demonstrate their fundamental importance as quality control markers for profiling beverage quality and pharmacological attributes, there is limited understanding of their relationship with the dynamics of carbohydrate reserves in response to seasonal harvests of bush tea. The tea is currently harvested throughout the year without consideration of the influence of the time of harvest on phytochemical properties. Seasonal harvesting of shoot components is a critical cultural practice in tea production with the aim of influencing the sprouting of new shoots. Carbohydrate partitioning in the form of specific sugars, starch, and reserve carbohydrates is fundamental for perpetuating shoot growth in optimizing cumulative responses of biomass yields and quality attributes of tea crops. Mudau et al. (2016) demonstrated the influence seasonal pruning has on the variation of carbohydrate reserves and dry matter accumulation of bush tea under different growing conditions, without contrasting the responses with major tea quality parameters. The authors further reported that changes in carbohydrate reserves show a seasonal dependence, with the highest accumulation exhibited in winter, followed by summer (Mudau et al., 2016). Generally, in both *Camellia* species and herbal teas, to generate new shoots from older basal regions, consistent picking is the normal harvesting technique. Thus, early growth of new sprouts is associated with some degree of mobilization of previously accumulated reserves of nutrients, starch, sugars, and other soluble and insoluble energy reserves. However, according to our knowledge, data that describe the specific soluble sugars and starch that influence resprouting of bush tea with respect to season have not yet been quantified. As the type and amount of soluble sugars and starch accumulation are all implicated in the capacity for resprouting of bush tea and the latter is highly dependent on seasons, data

Received for publication 7 Feb. 2018. Accepted for publication 11 May 2018.

We would like to dedicate this paper to the late Matsobane Taboga Mathiba (University of South Africa) and acknowledge him for the analysis of the sugars and the statistical analyses of the study. ¹Corresponding author. E-mail: mudaufn@unisa.ac.za.

on these aspects are urgently needed. Therefore, the objective of this study was to investigate the influence of season-specific variation associated with sugars and starch reserves. This study also reports on growth and quality parameters of bush tea in response to growing seasons and environments.

Materials and Methods

Plant material and growth. A glasshouse trial was conducted at the Science Campus of the University of South Africa, which is situated in Florida (South Africa), under thermostatically regulated temperatures between 20 and 24 °C and 60% relative humidity. Average canopy responses in photosynthetic active radiation and chlorophyll content within the growing season ranged from 134.38 to 157.24 W·m⁻² and 30.96 and 44.65 μmol·m⁻², respectively. The plant materials were harvested from the Mudzidzidi village, where the tea grows wild, in the Limpopo Province of South Africa [24°50'S, 31°17'E; altitude 610 m above mean sea level]; with subtropical climate of summer rainfall, cold and dry winters. The area has mean annual rainfall between 600 and 800 mm. Field experiments were established at Morgenzon commercial farm (22°56'60S, 30°28'60E; altitude 709 m), with characteristic summer rainfall and dry winter conditions. Meteorological data were similar to that reported by Mudau et al. (2016). In all the trials, the stock plant materials used for developing cuttings were selected based on being true to name and type; free of disease and insects; and in a healthy physiological state as described by Mudau et al. (2016). Horticultural practices used to manage the trials were adopted from Mudau et al. (2007a, 2007b). The wild population of bush tea plants at the Mudzidzidi village was monitored throughout the 2013–14 growing season. For the wild bush tea, the transect monitored was demarcated into a 1500 m² area with 50 stratified random 100 m²-sample plots containing a minimum of 50 established plant bushes of similar plant height, size, and girth.

Treatment and experimental design. The treatments consisted of bush tea cultivated in four seasons being harvested in autumn, winter, spring, and summer during the 2013–14 growth period. The experiments started on 1 Mar. 2013 (beginning of autumn/fall) and ended on 28 Feb. 2014 (end of summer). In each season, 25 single plants were planted in a randomized, complete block design in the field. For the cultivated bush tea, 25 bush plants were used as replications leaving buffer trees. For the wild bush tea, 25 single plants were randomly tagged. Similarly, 25 single plants were selected for sample analysis.

Parameters recorded. At harvest, parameters recorded were soluble sugars (sucrose, glucose, and fructose), starch (amylopectin and amylose), total polyphenol content, TFC, total antioxidant activities (DPPH = 2,2-diphenyl-1-picrylhydrazyl and FRAP), and TTCs. The quality of bush tea from the glasshouse had already been reported, therefore,

the data set of the results will not be presented. Samples for carbohydrate analysis were partitioned into three plant organs being roots, stems, and leaves, and thereafter freeze-dried for analyses.

Sample preparation. The bush tea roots, stems and leaves, and twigs from all trials were harvested as per allotted season and growing conditions. The harvested leaves and twigs were freeze-dried for 2 weeks. After freeze-drying, they were blended separately, and the blended leaf and twigs materials (100 g) were soaked in 150 mL of 100% ethanol and shaken at room temperature for 24 h. The ethanol supernatant was filtered and then evaporated on a rotary evaporator under reduced pressure at 37 °C. The extract was stored in the cold room at 5 °C after which they were subjected for analysis.

Analysis of sugar. Twenty five percent of acetonitrile were of LiChrosolv[®] quality (Sigma-Aldrich, Johannesburg, South Africa); 75% of water was ultrapure. Glucose, fructose, and sucrose (Sigma-Aldrich) were used as pure standard sugar solutions of 1000 ppm galactooligosaccharides, xylooligosaccharides, fructooligosaccharides, and inulin standard solutions were prepared in acetone/water to give 1000-ppm stock solutions; followed by successive dilutions with acetone/water (2:1; v/v) to give solutions at different concentrations depending on the linear range of the calibration plots (Yemm and Willis, 1954). Samples were collected from fermentations every 2–3 h. These samples were centrifuged and filtered (cellulose acetate syringe filter, 0.22 μm; Sigma-Aldrich) to avoid time-dependent changes in analyte concentration resulting from continued metabolism. All samples were immediately chilled at 4 °C. Carbohydrates were analyzed in the supernatant based on the work of Koboyashi et al. (1985).

Chromatography. Standards (1–20 μL) and samples (1 μL) were applied to the plates using an automated sample applicator, Linomat V (Sigma-Aldrich) as 3-mm-wide bands 10 mm from the bottom of the plate and 6 mm apart with a delivery rate of 20 s·μL⁻¹. Five standards (in duplicate) were applied to the plate alternatively with 14 samples at different concentrations. The solubilized starch solutions were treated with a mixture of anthrone and sulfuric acid to hydrolyze the starch to glucose, producing a green color. Absorption was measured using a spectrophotometer at 620 nm wavelength; absorbances were regressed against readings from glucose standard solutions. A correlation factor of 0.9 was utilized in converting glucose equivalents to starch (McCready et al., 1950). Glucose content in the soluble sugar solution was determined by treatment with the anthrone/sulfuric acid mixture (Latt et al., 2000). Isocratic development was performed at 20 to 22 °C and 55% to 65% relative humidity in 20 × 10 cm Camag twin-trough chamber with 20 mL of mobile phase. Automated multiple development was performed by using Camag AMD-1 equipment with linear gradient in

15 steps of elution. The gradient was built by automatic mixing of four basic eluant solutions of acetonitrile/water, establishing a gradient composed of 32%, 30%, 26%, and 24% (v/v) of acetonitrile contents, respectively.

Glucose, fructose, and sucrose assays. Chromatography was performed on 20 × 10 cm Diol high performance thin-layer chromatography (HPTLC) plates (Sigma-Aldrich, Johannesburg, South Africa). The isocratic development was performed at 20 to 22 °C and 55% to 65% relative humidity in 20 × 10 cm Camag twin-trough chamber with 20 mL of mobile phase. Automated multiple development was performed by using Camag AMD-1 equipment with linear gradient in 20 steps of elution. The gradient was built by automatic mixing of four basic eluant solutions of acetonitrile:acetone (1:1; v/v) and water with quantities ranging from 25% to 15% (v/v).

Analyses of amylopectin and amylose. Isoamylase (59,000 units/mg protein, 1 mg·mL⁻¹) was purchased from Sigma-Aldrich. Before use, the enzyme solution was replaced with acetate buffer using a collodion bag by adding 10 mL (5 mL twice) of 0.1 M acetate buffer, pH 3.80, to 50 μL of the enzyme solution at 4 °C (McCready et al., 1950). The diluted buffer/enzyme solution (5 mL) was concentrated to 0.5 mL and diluted to 5 mL with acetate buffer. Distilled and deionized water was used. The water and dimethyl sulfoxide (DMSO) were filtered through a fritted disk funnel (pore size, 10–15 μm) and degassed at room temperature.

HPLC apparatus. A Model 510 pump equipped with a Model U6K universal liquid chromatograph injector and a differential refractometer R401 (Waters Corporation, Milford, MA) was used. The data were collected on an Apple II+ computer equipped with an ADALAB data acquisition/control card and CHROMATOCHART chromatography software for peak area integration. An E-Linear and an E-1000 μ-Bondagel gel permeation chromatography column were connected in series. The columns were protected with a guard column (Waters Corporation, 2.5 × 3.0 mm I.D.) packed with CO: PELL ODS (Whatman, Clifton, NJ). The columns were immersed in a 40 °C water bath after covering each column with a polyurethane film to prevent rust. The refractometer was also maintained at 40 °C with a circulating water bath.

Chromatographic conditions. Starch samples (40 mg of amylose/amylopectin mixtures or dextrans) were dissolved in 2 mL of DMSO in a boiling bath for 5 min, and the solution was centrifuged at 3000 g for 5 min. A 20-μL volume of the supernatant solution, removed from the top of the solution, was injected. All samples, including data collected for standard curves, were measured in triplicate. The columns were eluted with DMSO at a flow rate of 0.2 mL·min⁻¹ and the refractive index detector attenuation was set at eight.

Enzymatic debranching of amylopectin. A 100-μL volume of a 2% solution of amylopectin in DMSO, 100 μL of 0.1 M acetate

buffer (pH 3.80), 150 µL of water, and 50 µL of isoamylase solution were thoroughly mixed and allowed to stand for 17 h at 40 °C. A control of the same mixture, with 0.1 M acetate buffer replacing the isoamylase solution, was used with each debranching experiment. After the reaction, 80 µL of each mixture was analyzed by high-pressure size-exclusion chromatography as described.

Determination of TPC. The concentrations of total reactive phenolics present in leaf extracts were determined by using the Folin–Denis assay (Waterman and Mole, 1994) and slightly modified by Appel et al. (2001). The standard curves were developed using standards purified from hybrid poplar (clone OP-367) foliage. The Folin–Denis assay was conducted following the method proposed by Appel et al. (2001). Briefly, 7 mM ABTS ammonium was dissolved in potassium phosphate buffer (pH 7.4) and treated with 2.45 mM potassium persulfate. The mixture was allowed to stand at room temperature for 12–16 h to turn into dark blue. The solution was then diluted with potassium phosphate buffer. Until the absorbance reached 1.0 (0.02 at 734 nm), the absorbance was recorded with a spectrophotometer at 734 nm under room temperature after 6 min. Total polyphenolic content was expressed in µM of Trolox equivalent concentration per gram of tea samples, and the experiments were carried out in triplicate.

Determination of antioxidant activity. Radical scavenging activity of bush materials that were sampled from different growing environments and seasons were processed to determine antioxidant activity through the method described by Blois (1958) with modification described by Waterman and Mole (1994). The method assesses the capacity of 2,2-diphenyl-2-picrylhydrazyl (DPPH) stable radical to donate hydrogen ions or quench free radicals when reacting with a matrix of secondary metabolites constituting the sample material. Five mL of 0.1 µmol·L⁻¹ of methanol-dissolved DPPH solution was added into variable concentrations of methanol extracts of the plant materials. The mixtures were briefly vortexed and retained stationary at 27 °C for 20 min, to optimize reactions. Thereafter, the absorbance values were quantified through a spectrophotometer at 517 nm wavelength. Methanol and standards were used as negative and positive controls, respectively. Total antioxidant activity (TAA) was calculated as inhibition concentration of the plant material scavenging 50% of radical-donating source. Considering the natural diversity of anti and prooxidants, the method proposed by Pulido et al. (2001) was also used to assess antioxidant activity of the plant materials with respect to FRAP assay. Precision in the assays was qualified through the correlation of determination (*R*²) between absorbance and graduated sample concentrations.

Determination of TFC. The total flavonoids were measured using a modified calorimetric method described by Yoo et al. (2008). Briefly, 1 mL of the extracts

Table 1. Effects of seasons on the allocation of specific carbohydrates on different organs of bush tea.

Seasons	Avg concn (mg·g ⁻¹)										ANOVA							
	Root					Stem						Leaves						
	Glucose	Fructose	Sucrose	Amylose	Amylopectin	Amylose	Glucose	Fructose	Sucrose	Amylose	Amylopectin	Amylose	Glucose	Fructose	Sucrose	Amylopectin	Amylose	
Autumn	21.6 b ^B	10.7 a ^C	10.3 a ^C	25.1 c ^B	93.9 a ^A	18.8 b ^A	18.8 b ^A	9.5 b ^B	9.8 a ^B	19.7 b ^A	19.7 b ^A	18.7 b ^A	21.2 c ^C	12.1 b ^C	6.2 c ^C	44.8 a ^A	47.5 b ^A	
Winter	33.1 a ^B	11.2 a ^C	11.9 a ^C	70.1 a ^A	64.5 b ^A	25.0 a ^B	25.0 a ^B	10 b ^C	8.4 a ^C	40.6 a ^A	40.6 a ^A	19.8 b ^B	27.0 c ^B	10.5 b ^C	7.5 c ^C	42.0 a ^A	50.1 a ^A	
Spring	9.2 c ^C	7.8 b ^C	8.5 b ^C	40.3 b ^A	52.8 c ^A	16.0 b ^C	16.0 b ^C	8.4 b ^B	7.2 b ^B	21.2 b ^A	21.2 b ^A	20.1 b ^A	32.4 b ^B	10.9 b ^C	14.0 b ^C	41.2 a ^A	37.3 c ^A	
Summer	9.7 c ^C	9.6 b ^C	6.2 b ^C	29.5 c ^B	55.2 c ^A	22.0 a ^A	22.0 a ^A	11.6 a ^B	5.2 b ^B	23.5 b ^A	23.5 b ^A	21.5 b ^A	53.8 a ^A	22.3 a ^C	18.6 a ^C	43.6 a ^B	45.0 b ^B	
Probability	0.0001	0.0145	0.0001	0.0001	0.001	0.0001	0.0001	0.0125	0.0145	0.0001	0.0001	0.0001	0.0001	0.0245	0.001	0.5124	0.0001	
ANOVA																		
Sources of variation	df	SS	MS	F	P value													
S	3	14,601.2	7,962.3	6.17	0.0032													
O	8	488,199.9	49,467.1	37.12	0.0001													
S × O	24	425,407.12	42,561.2	—	—													
Error	72	19,743.12	2,341.1	—	—													
Total	107	96,76.46	—	—	—													

ANOVA = analysis of variance; SS = sum of squares; MS = means squares; F = test statistics; S = season; O = organ.

^aMeans with the small letter in a column are not significantly different using Duncan multiple range test at the 1% level.

^bMeans in capital letters in a row are not significantly different using Duncan multiple range test at the 1% level.

or standard solutions of catechin was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 mL of 10% (w/v) AlCl₃ was added and then, 6 min later, 2 mL of 1 M NaOH was also added to the mixture, followed by the addition of 2.1 mL distilled water. The absorbance was measured against the blank at 510 nm after 15 min. The standard curve was prepared by using different concentrations of catechin. The flavonoid content was expressed as mg catechin equivalents per 100 g of dry weight.

Determination of TTC. The method described by Cisowski et al. (1995) was used to determine the tannin content. The determination of tannin was carried out by a spectrophotometric method based on the formation of insoluble tannin salts with copper (II) cation. The tannins were extracted through boiling water followed by precipitation with copper II acetate and filtering after 12 h. The final precipitates were calculated from a proportion considering the quantity of copper taken from analysis and quantity of copper II oxide bound by tannin contents.

Statistical analysis. Data were subjected to analysis of variance using the general linear model of SAS version (version 9.2; SAS Institute, Cary, NC). Mean separations were performed using the Duncan multiple range test. Correlation and contribution of the parameters in projection of the whole data onto latent variables (PC1 and PC2) were represented through a general PCA biplot to contrast growth environments based on their chemical features. The application of this unsupervised statistical approach allows for extraction of information from large chemical data sets to observe patterns in a holistic fashion. PC1 represents the direction of the maximum variance. Cardinality of the assessed parameters was thus presented through biplots to demonstrate their contribution in the discrimination of seasonal harvest treatments.

Results

Seasonal response of specific carbohydrates in three bush tea organs

Roots. The carbohydrates of interest in this study (glucose, fructose, sucrose, amylopectin, and amylose) were significantly affected by seasons (Table 1). The glucose was significantly higher during winter (33.1 mg·g⁻¹) followed by autumn (21.6 mg·g⁻¹) as compared with other seasons. The difference between the highest sugar contents was 11.4 mg·g⁻¹. In addition to glucose, the two seasons (fall and winter) also exhibited the highest fructose concentrations (10.7 and 11.2 mg·g⁻¹, respectively). A similar trend observed for sucrose in that winter showed higher contents (11.9 mg·g⁻¹) followed by autumn 10.3 mg·g⁻¹) as compared with summer (6.2 mg·g⁻¹) and spring (8.5 mg·g⁻¹). Based on data presented in Table 1, the accumulation of glucose, fructose, and sucrose is more favored in the cooler to colder climates (autumn and winter) than in warmer

Table 2. Effects of growing conditions on the partitioning of specific carbohydrates on roots, stem and leaf.

GS	Leaves										Stem										
	Root					Stem					Root					Stem					
	Glucose	Fructose	Sucrose	Amylopectin	Amylose	Glucose	Fructose	Sucrose	Amylopectin	Amylose	Glucose	Fructose	Sucrose	Amylopectin	Amylose	Glucose	Fructose	Sucrose	Amylopectin	Amylose	
Glasshouse	10.0 c	5.37 c	4.9 b	22.1 c	15.9 c	11.6 b	6.4 c	17.9 c	12.1 c	18.9 c	17.9 b	16.4 b	2.66 c	22.13 c	11.8 c	17.9 b	16.4 b	2.66 c	22.13 c	11.8 c	
Field conditions	23.3 b	14.0 b	14.0 a	57.3 b	19.8 b	21.5 a	15.0 b	36.5 b	34.0 b	34.0 b	23.3 a	18.3 b	6.86 b	30.6 b	32.3 b	23.3 a	18.3 b	6.86 b	30.6 b	32.3 b	
Wild bush	55.1 a	36.57 a	14.9 a	80.0 a	26.9 a	22.6 a	37.5 a	60.2 a	53.3 a	53.3 a	25.2 a	37.6 a	14.3 a	49.3 a	43.8 a	25.2 a	37.6 a	14.3 a	49.3 a	43.8 a	
Probability	0.0001	0.0002	0.0001	0.0001	0.0423	0.001	0.0001	0.0001	0.0012	0.0015	0.0001	0.0145	0.0014	0.0001	0.001	0.0001	0.0145	0.0014	0.0001	0.001	
ANOVA																					
Sources of variation	df	SS	MS	F	P value																
GS	2	18,024	18,023	4,69	0.0001																
O	8	173,413	25,734	2,99	0.0206																
S × O	16	16,114.31	32,164	—	—																
Error	7	36,739	6,125	—	—																
Total	33	243,013	—	—	—																

ANOVA = analysis of variance; GS = growing sites; SS = sum of squares; MS = means squares; F = test statistics; S = season; O = organ. Means with the same letter are not significantly different using Duncan multiple range test at the 5% level.

to hot seasons (summer and spring). For the complex carbohydrate, amylopectin, the highest levels were detected in autumn and winter samples (93.9 and 64.5 mg·g⁻¹, respectively). A similar pattern was observed with the glucose, fructose and sucrose contents. Although the autumn and winter had the highest concentrations of amylopectin, spring and summer also showed moderate concentrations of this particular sugar (52.8 and 55.2 mg·g⁻¹, respectively). The other complex carbohydrate, amylose, was detected at significantly higher concentrations in winter and spring, respectively, with the lowest concentration observed in autumn. The results in Table 1 show that the accumulation of glucose, sucrose, fructose, and amylopectin in the roots of bush tea is more abundant in autumn and winter whereas that of amylose is correlated to increasing in winter and spring.

Stems. The stems had the highest glucose during winter (25.0 mg·g⁻¹) and summer (22.0 mg·g⁻¹) with the lower contents in autumn (18.8 mg·g⁻¹) and spring (16.0 mg·g⁻¹). The difference between the highest and the lowest glucose contents was 6.2 mg·g⁻¹. During the summer season, highest fructose (11.6 mg·g⁻¹) concentrations compared with other seasons were recorded. Both autumn and winter had highest sucrose concentrations (9.8 and 8.4 mg·g⁻¹, respectively) as compared with spring (7.2 mg·g⁻¹) and summer (5.2 mg·g⁻¹). Winter seasons exhibited higher amylopectin (40.6 mg·g⁻¹) compared with other seasons and no significant differences were discernible when the amylose content was examined in the stems.

Leaves. The leaf carbohydrates were significantly higher in summer (53.8 mg·g⁻¹) followed by spring (32.4 mg·g⁻¹) as compared with other seasons (Table 1). Fructose and sucrose also exhibited similar trends to that of glucose. No significant difference with regard to amylopectin was evident. The highest amylose content was obtained during winter (50.1 mg·g⁻¹). The difference between the highest and lowest was 12.8 mg·g⁻¹.

Seasonal allotted carbohydrates in all plant organs

Roots. In three seasons (autumn, spring, and summer), amylopectin exhibited higher (93.9 mg·g⁻¹) reserves as compared with other carbohydrates (Table 1).

Stem. The stem amylopectin exhibited higher reserves in all seasons. This was followed by amylose. Between the other three simple sugars (glucose, fructose, and sucrose), glucose accumulated as the highest reserve in all seasons viz., summer, autumn, winter, and spring, respectively.

Leaves. The amylose concentration was high in three seasons (autumn, winter, and spring) as compared with other sugars. In summer, glucose accumulation was predominant in the leaf tissue (Table 1).

Response of partitioning of carbohydrates on the roots in different growing sites

The growth location and environment had a significant effect on the partitioning of

carbohydrates in the organs of bush tea. The highest glucose content was obtained in the wild bush tea (55.1 mg·g⁻¹) followed by field-grown plants (Table 2). The difference between the highest and lowest glucose content was 45.1 mg·g⁻¹. A similar trend with that of glucose was recorded for fructose, amylopectin and amylose. Hence, root sucrose was significantly higher in both field (14.0 mg·g⁻¹) and wild bush tea (14.9 mg·g⁻¹) (Table 2) as compared with those plants that were left to grow in the glasshouse.

Response of partitioning of carbohydrates on the stem under growing conditions

Wild-grown bush tea had significantly increased glucose (22.6 mg·g⁻¹) as compared with glasshouse (11.6 mg·g⁻¹) and field-grown bush tea (21.5 mg·g⁻¹) (Table 2). The difference between the highest and lowest was 11.0 mg·g⁻¹. A similar trend as that of glucose was observed in fructose, sucrose, amylopectin, and amylose. Hence, the glasshouse-grown bush tea stem was constantly lower in carbohydrates throughout the growing seasons (Table 2).

Response of partitioning of carbohydrates on the leaves, in a different growing environment

Results in Table 2 also showed that a higher glucose content was exhibited in leaves of wild and field-grown bush tea, compared with those grown in the glasshouse. Similar consistent trends were also observed in fructose, sucrose, amylopectin, and amylose content being the lowest in leaves in glasshouse bush tea.

Phytochemicals in leaves of bush tea in different seasons

Results in Table 3 demonstrate that the phytochemicals present in the leaves of field-grown bush tea and wild bush tea during winter were higher than those grown in summer, autumn, and spring. Otherwise, autumn yielded the plants with the least phytochemical content when compared with the other three seasons. In general, a higher TPC (94.3 mg·g⁻¹) and TFC (97.67 mg·g⁻¹) were observed in winter, while the lowest TPC (57.67 mg·g⁻¹) and TFC (64.33 mg·g⁻¹) were observed in autumn. The difference between the highest and lowest TPC was 36.6 mg·g⁻¹, and the difference between the highest and lowest TFC was 33.34 mg·g⁻¹. The TTCs were 24.40, 23.07, 22.40, and 22.03 mg·g⁻¹ in winter, spring, summer, and autumn, respectively. No significant difference in tannin contents were observed, irrespective of seasons. In field-grown bush tea, the highest total polyphenols, and TFCs were obtained in winter compared with other seasons. Similarly, no significant differences in tannin contents were observed irrespective of growing conditions and seasons. Regardless of growing conditions, autumn yielded lower total antioxidant activities using both methods viz, DPPH and FRAP when compared with other seasons.

Table 3. Seasonal response of bush tea grown in different growing environment.

Seasons	Wild bush tea					Field-grown cultivate bush tea				
	TPC (µM of Trolox equivalent concn per gram)	TFC (mg CE per 100 g)	TTC (µmol·L ⁻¹)	DPPH (inhibition %)	FRAP (µmol equiv of Fe(II) per L)	TPC (µM of Trolox equivalent concn per gram)	TFC (mg CE per 100 g)	TTC (µmol·L ⁻¹)	DPPH (inhibition %)	FRAP (µmol equiv of Fe(II) per L)
Autumn	57.67 b ± 9.50	64.33 b ± 5.51	22.03 ^{ss} ± 0.64	160.70 b ± 7.19	164.42 b ± 6.69	57.67 b ± 9.50	58.33 b ± 15.89	22.01 ^{ss} ± 0.64	151.03 b ± 9.56	154.46 b ± 13.38
Winter	92.67 a ± 1.07	97.67 a ± 11.06	24.4 ± 1.73	198.47 a ± 1.57	353.70 a ± 106.38	90.33 a ± 5.84	97.67 a ± 11.06	24.40 ± 1.73	190.13 a ± 2.38	347.70 a ± 106.38
Spring	94.30 a ± 4.78	94.30 a ± 4.78	23.07 ± 0.58	194.97 a ± 4.57	356.93 a ± 6.03	88.30 a ± 8.02	94.63 a ± 5.19	23.07 ± 0.58	195.5 a ± 5.06	365.27 a ± 5.45
Summer	87.67 a ± 11.06	88.13 a ± 11.55	25.4 ± 1.00	191.83 a ± 5.97	365.47 a ± 43.71	88.33 a ± 12.01	88.13 a ± 11.55	24.73 ± 0.58	191.83 a ± 5.97	365.47 a ± 43.71
Probability value	0.0001	0.0001	0.1574	0.0001	0.0001	0.0001	0.0001	0.1684	0.0001	0.0001

TPC = total polyphenolic content; TFC = total flavonoid content; TTC = total tannin content; TAA = total antioxidant activity; FRAP = ferric reducing antioxidant power; CE = catechin equivalents. Means of the same letter in the column are significantly different using Duncan multiple range test at 1%.

PCA of all recorded parameters

Results in Table 4 demonstrated that in the variables measured, there was a strong correlation within all parameters recorded in PC1 with exceptions of weak correlation by TTC. The correlation drastically diminished in PC2, where the strongest correlation was 63%. Moreover, the results demonstrated that the first principal component had the highest contribution with TPC (10.36%), whereas the second component showed the highest contribution with FRAP assay and TAA (24.27%).

Discussion

There has been lack of literature, which clearly demonstrates the relationship between quality parameters and carbohydrate reserves in herbal teas, bush tea in particular. The relation is critical in the tea industry because periodic harvesting of shoots impose significant perturbation of reserve carbohydrates, which are important in plant growth and development to maximize yield as well as quality. Primary metabolites also influence the accumulation of secondary or specialized metabolites as they serve as precursors for the biosynthetic pathways that produce phenolics, flavonoids, and tannins that impart many pharmacological effects and the flavor profile of the tea (Bourgaud et al., 2001). Responses of plants to season and growth climates influence the biochemical makeup, leading to chemotypic variation. Because of their sessile nature, plants show considerable phenotypic plasticity and the metabolome responds appropriately to changing conditions linked to both abiotic and biotic factors. These changes are thought to impart metabolomic adjustments that allow for plants to be to cope with varying environments. The carbon balance is an important indicator of the overall physiological function of plants linked to carbon assimilation and photosynthesis and nonstructural carbohydrate accumulation may be associated with resting phases of the plants where photosynthesis is slowed down (Li et al., 2011). The idea of translocation of carbon metabolites from source to sink is a well-established phenomenon, and environmental conditions, thus, affect the carbohydrate pool and general sugar content in various plant organs. Conditions of water stress for instance affect the lower molecular weight sugars such as fructose (Li et al., 2011). Various life stages also result in variable accumulation of different sugars, and the movement of sugars from leaf tissue to nonsenescent tissues in plants is part of the overwintering process. Sugars affect the ψ_s and are thus important osmolytes during stress periods.

The present study reveals that the influence of growth environment and seasonal harvest on carbohydrate partitioning had a significant effect on quality of bush tea. Similar findings were also reported by Mudau et al. (2006, 2007a, 2007b) with regard to potted bush tea cultivated under 50% shade nets. A similar trend was consistent with the

Table 4. Correlation and contribution of recorded parameters in latent principal component analyses variables (PC1 and PC2), regardless of growing conditions.

Parameters recorded	Correlation		Contribution (%)	
	PC1	PC2	PC1	PC2
Root glucose	0.91	-0.085	3.398	0.432
Root fructose	0.744	-0.488	7.53	14.43
Root sucrose	0.586	-0.238	4.678	3.422
Root amylopectin	0.793	-0.04	8.555	0.096
Root amylose	0.750	-0.059	7.667	0.212
Stem glucose	0.815	-0.347	10.048	7.272
Stem fructose	0.847	-0.355	10.767	7.636
Stem sucrose	0.872	0.37	10.365	8.28
Stem amylopectin	0.862	0.341	10.115	7.022
Stem amylose	0.654	0.496	5.833	21.522
Leaves glucose	0.988	0.433	3.239	24.274
Leaves fructose	0.906	0.223	2.245	3.018
Leaves sucrose	0.744	-0.488	7.53	14.43
Leaves amylopectin	0.586	-0.238	4.678	3.422
Leaves amylose	0.862	0.341	10.115	7.022
Total polyphenolic content	0.872	0.37	10.365	8.28
Total flavonoid content	0.862	0.341	10.115	7.022
DPPH and total antioxidant activity	0.654	0.596	10.833	21.522
Ferric reducing antioxidant power and total antioxidant activity	0.888	0.633	10.239	24.274
Total tannin content	0.606	0.223	9.245	3.018
Eigen value	10.5	3.9	27.7	26.4
Variability (%)	35.5	15.4	14.9	12.5
Cumulative variability (%)	35.6	25.2	27.5	29.8

results reported by Mudau et al. (2016) who reported that seasonal pruning occurred as result of the variation of carbohydrate reserves and dry matter accumulation of bush tea under different growing conditions. Seasonal effects contributed to mobilization of starch reserves and export of sugars to overwintering root sink tissues, thereby, increasing total nonstructural carbohydrates in cultivated leafy spurge (Gesch et al., 2007). The current findings were in agreement with previous research reports in that the continual shoot growth has an association with the deposition of starch for development of root sink tissues as demonstrated with maple (Wargo et al., 1972; Wargo, 1979), poplar (Isebrands and Nelson 1982; Isebrands and Nelson 1983; Isebrands and Michael, 1986; Isebrands et al., 1988; Nelson and Dickson 1981), and mulberry (Yamashita, 1984, 1986). Thus, the association substantiates the role that the growth environment plays in the intricate balance between allometry of root and shoot growth of plants (Latt et al., 2000). As a result, the findings demonstrated a significant and distinct discrimination in field and wild growth environments, with the glasshouse serving as a benchmark growth environment.

The distinct differences may have been attributed to or inferred to the lack of homogeneity in plant age exhibited by the growing conditions in these trials. Wild-grown bush tea had limited physiological absurdness, although both field- and glasshouse-cultivated bush teas were clonal materials sourced from wild stock plants. Consequent to this constraint, environment-dependent effects on seasonal harvest treatments were comparatively assessed across all growth environments. In this regard, discrimination of winter seasonal harvest was influenced through partitioning of

soluble sugars, starch, and reserve carbohydrates, similar to discrimination of the wild growth environment. This was attributed by the seasonal changes of carbohydrate metabolism in plants. Sugar is generally accepted to play an important role in plants during cold acclimation and there is a growing body of evidence showing its contribution as a signaling molecule, which facilitates environmental adaptations in plants. In a recent study by Yue et al. (2015) in *Camelia sinensis* the putative role of sugars in a regulatory signaling network was studied using a genomic approach during cold acclimation. These authors illustrated starch hydrolysis, which leads to a higher accumulation of total sugars. Especially sucrose, fructose, and glucose are linked to higher expression of beta-amylases, invertase, raffinose synthase, and various sugar transporters in preparation for cold hardiness. This indicates a flexible homeostasis that is not only involving source-sink relationships, but also transport mechanisms and signaling mechanisms that are all part of season-to-season conditioning stimulated by changes in sugars. The higher accumulation of sugars in bush tea during the winter season shown in this study, thus, suggests similar mechanisms for cold tolerance being a possibility as the levels of sugars were not different during the other three seasons. Cryoprotection in plants is associated with changes in sugar metabolism during seasons, which are also affected by phytohormones such as abscisic acid and ethylene. Both of these phytohormones are involved in responses to environmental factors, and several studies have established their role in the accumulation of phenolics and terpenoids. Sucrose as a signaling molecule could, thus, crosstalk with ABA and ethylene signaling, leading to an overall change in the plant's antioxidant pool

(Bolouri-Moghaddam et al., 2010). Seasonal changes are regulated by various factors including free water availability in cells, which generally decreases during winter; all of these factors are known to contribute to changing phytochemical patterns in plants. In *C. sinensis*, genomic changes were shown to be tightly linked not only to carbohydrate metabolism, but also to glycan biosynthesis, amino acid metabolism, terpenoid biosynthesis pathways, polyketide production, and lipid metabolism among others (Wang et al., 2013).

In conclusion, the results of this study showed that there are seasonal differences with respect to the quantity of specific carbohydrates evaluated. The bush tea plant organs showed that the glucose content was significantly higher during winter followed by autumn, as compared with other seasons. Similar trends of sucrose were evident in the case of fructose. However, the amylopectin content was significantly higher during summer followed by autumn, compared with the other seasons. This effect may be associated with increasing starch levels. Winter also exhibited the highest amylopectin content compared with the other seasons. There was no significant difference with regard to amylose. Both wild and cultivated bush tea yielded the highest contents of specific, selected sugars in this study. The phytochemicals' acclimation to cooler seasons present in the leaves of field-grown bush tea and wild bush tea during winter was higher than those grown in summer, autumn, and spring. This may imply the importance of these chemicals for cold acclimation. No significant difference in tannin contents was observed irrespective of seasons. The first principal component showed a strong correlation within all parameters recorded over the second principal component. Future ecophysiological studies are recommended to establish region- and season-specific metabolomic biomarkers with canonical distinction on beverage, pharmacological and organoleptic attributes in herbal teas. Unfortunately, at this stage, no transcriptomic data are available for bush tea. It is, thus, of interest to obtain such information, especially seeing that the phytochemical profile of this plant was significantly altered in winter compared with other seasons. This would enable a better understanding of the genomic to metabolomic networks that are functioning in response to changing seasons.

Literature Cited

Appel, H.M., K.L. Govenor, M. D'Ascenzo, E. Siska, and J.C. Schultz. 2001. Limitations of Folin assays of foliar phenolics in ecological studies. *J. Chem. Ecol.* 27:761–778.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* 29:1199–1200.

Bourgaud, F., A. Gravot, S. Milesi, and E. Gontier. 2001. Production of plant secondary metabolites: A historical perspective. *Plant Sci.* 161:839–851.

Bolouri-Moghaddam, M., K.L. Roy, L. Xiang, F. Rolland, and D.E. Van. 2010. Sugar signalling

and antioxidant network connections in plant cells. *FEBS J.* 277:2022–2037.

Cisowski, W., M. Denbuiska, S. Gills, and Lacakiewicz. 1995. *Analiza fitochemica/phytochemical analysis*. Gidank Medical Academy Publishing.

Gesch, R.W., D. Palmquist, and J.V. Anderson. 2007. Seasonal photosynthesis and partitioning of non-structural carbohydrates in leafy spurge (*Euphorbia esula*). *Weed Sci.* 55:346–351.

Isebrands, J.G. and D.A. Michael. 1986. Effects of leaf morphology and orientation on solar radiation interception and photosynthesis in *Populus*, p. 359–381. In: T. Fujimori and D. Whitehead (eds.). *Crown and canopy structure in relation to productivity*. Forestry and Forest Products Institute, Ibaraki, Japan.

Isebrands, J.G. and N.D. Nelson. 1982. Crown architecture of short rotation intensively cultured *Populus II*. Branch morphology and distribution of leaves and specific leaf weight within the crown of *Populus* 'Tristis' as related to biomass production. *Can. J. For. Res.* 12:853–864.

Isebrands, J.G. and N.D. Nelson. 1983. Late-season photosynthesis and photosynthate distribution in an intensively-cultured *Populus nigra* × *P. laurifolia* clone. *Photosynthetica* 17:537–549.

Isebrands, J.G., R. Ceulemans, and B.M. Wiard. 1988. Genetic variation in photosynthetic traits among *Populus* clones in relation to yield. *Plant Physiol. Biochem.* 26:427–437.

Koboyashi, S., S.J. Schwartz, and D.R. Lineback. 1985. Rapid analysis of starch, amylose and amylopectin by high performance size-exclusion chromatography. *J. Chromatography* 319:205–214.

Latt, C.R., P.K.R. Nair, and B.T. Kang. 2000. Interactions among cutting frequency, reserve carbohydrates, and post-cutting biomass production in *Gliricidia sepium* and *Leucaena leucocephala*. *Agrofor. Syst.* 50:27–46.

Lerolithi, L., S.K. Chaudhary, S. Combrinck, and A. Viljoen. 2017. Bush tea (*Athrixia phylicoides*): A review of the traditional uses, bioactivity and phytochemistry. *S. Afr. J. Bot.* 110:4–17.

Li, T., S.L. Xu, J.A. Osés-Prieto, S. Putil, P. Xu, R.J. Wang, K.H. Li, D.A. Maltby, L.H. An, A.L. Burlingame, and Z.P. Deng. 2011. Proteomics analysis reveals post-translational mechanisms for cold-induced metabolic changes in *Arabidopsis*. *Mol. Plant* 31:361–374.

Malongane, F., L.Y. McGaw, and F.N. Mudau. 2017. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: A review. *J. Sci. Food Agr.* 97:4679–4689.

Maudu, M.E., F.N. Mudau, and I.K. Mariga. 2012. Quality profiles of cultivated and wild bush tea (*Athrixia phylicoides* D.C.) at various phenological stages. *Intl. J. Agr. Biol.* 16:1011–1014.

Mashimbye, M.J., F.N. Mudau, P. Soundy, and T. van Ree. 2006. A new flavonol from *Athrixia phylicoides* (bush tea). *S. Afr. J. Chem.* 59:1–2.

Mbambezeli, G. 2005. *Athrixia phylicoides* DC. 24 Mar. 2017. <<http://pza.sanbi.org/athrixia-phylicoides>>.

McCready, R.M., J. Guggolz, V. Silveira, and H.S. Owens. 1950. Determination of starch and amylose in vegetables. *Anal. Chem.* 22:1156–1158.

Mogotlane, I.D., F.N. Mudau, P.W. Mashela, and P. Soundy. 2007. Seasonal responses of total antioxidant contents in cultivated bush tea (*Athrixia*

phylicoides L.) leaves to fertilizer rates. *Med. Aromat. Plant Sci. Biotechnol.* 1:77–79.

Mudau, F.N., P. Soundy, and E.S. Du Toit. 2007a. Effects of nitrogen, phosphorus, and potassium nutrition on total polyphenol content of bush tea (*Athrixia phylicoides* L.) leaves in shaded nursery environment. *HortScience* 42:334–338.

Mudau, F.N., P. Soundy, and E.S. Du Toit. 2007b. Nitrogen, phosphorus, and potassium nutrition increases growth and total polyphenol concentrations of bush tea in a shaded nursery environment. *HortTechnology* 17:107–110.

Mudau, F.N., P. Soundy, E.S. Du Toit, and J. Olivier. 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. *S. Afr. J. Bot.* 72:398–402.

Mudau, F.N., A.R. Mudau, M. Nkomo, and W. Ngezimana. 2016. Variation in carbohydrate reserves and dry matter production of bush tea (*Athrixia phylicoides*) grown under different environmental conditions. *HortScience* 51:1–5.

Nchabeleng, L., F.N. Mudau, and I.K. Mariga. 2012. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. *J. Med. Plants Res.* 6:1662–1666.

Nelson, E.A. and R.E. Dickson. 1981. Accumulation of food reserves in cottonwood stems during dormancy induction. *Can. J. For. Res.* 11:145–154.

Nkomo, M.M., D.D. Katerere, H.H. Vismer, T.T. Cruz, S.S. Balayssac, M.M. Malet-Martino, and N.N. Makunga. 2014. *Fusarium* inhibition by wild populations of the medicinal plant *Salvia africana-lutea* L. linked to metabolomic profiling. *BMC Complement. Altern. Med.* 14(1):99.

Pulido, R., L. Bravo, and F. Sauro-Calixto. 2001. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agr. Food Chem.* 48:3396–3402.

Roberts, M. 1990. *Indigenous healing plants*. Southern Book Publishers (Pty) Ltd., Cape Town.

Wang, X.C., Q.Y. Zhao, C.L. Ma, Z.H. Zhang, H.L. Cao, Y.M. Kong, C. Yue, X.Y. Hao, L. Chen, J.Q. Ma, and J.Q. Jin. 2013. Global transcriptome profiles of *Camellia sinensis* during cold acclimation. *BMC Genomics* 22:14–15.

Wargo, P.M. 1979. Starch storage and radial growth in woody roots of sugar maple. *Can. J. For. Res.* 9:49–56.

Wargo, P.M., J. Parker, and D.R. Houston. 1972. Starch content in roots of defoliated sugar maple. *For. Sci.* 18:203–204.

Waterman, P.G. and S. Mole. 1994. *Analysis of plant metabolites*. Alden Press Limited, Oxford.

Yamashita, T. 1984. Effect of top pruning on stem carbohydrate reserves and foliar ¹⁴C₂ assimilation in mulberry trees during autumn. *Plant Cell Physiol.* 25:677–681.

Yamashita, T. 1986. Mobilization of carbohydrates, amino acids and adenine nucleotides in hardwood stems during regrowth after partial shoot harvest in mulberry trees (*Morus alba* L.). *Ann. Bot.* 57:237–244.

Yemm, E.W. and A.J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* 57:508–514.

Yoo, K.M., C.H. Lee, H. Lee, B. Moon, and C.Y. Lee. 2008. Relative antioxidant and cytoprotective activities of common herbs. *Food Chem.* 106:929–936.