Journal of Medicinal Plants Research Vol. 4(9), pp. 824-829, 4 May, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.118
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

Effect of fermentation temperature and time on the chemical composition of bush tea (*Athrixia phylicoides* DC.)

L. N. Hlahla*, F. N. Mudau and I. K. Mariga

[1]Department of Soil Science, Plant Production and Remote Sensing, University of Limpopo, Private Bag X1106, Sovenga 0727, Republic of South Africa.

Accepted 1 April, 2010

A study was conducted to determine the effect of fermentation temperature and time on chemical composition of bush tea (*Athrixia phylicoides* DC.). Bush tea was fermented in incubators at different temperatures and for different times for quality improvement. Treatments for fermentation temperature consisted of control (24°C) room temperature, 30, 34, 38 and 42°C where the tea leaves were fermented for 30 min. Treatments for fermentation time consisted of control (0), 60, 90 and 120 min at an incubator temperature of 22 - 26°C. A completely randomized design (CRD) was used with three replicates for both evaluations. The chemical analysis (polyphenols, tannins and antioxidants) were done using Waterman and Mole's (1994) method. The results of this study demonstrated that fermentation temperature significantly increases polyphenols at 30, 34 and 38°C whereas tannin content showed a great reduction at 38 and 42°C. Increasing fermentation time achieved a significant increase in both polyphenols (60 and 90 min) and tannin contents (90 and 120 min). However, changes in either fermentation temperature or time did not give any significant influence on antioxidant content of bush tea.

Key words: Polyphenols, tannins, antioxidants, flavour.

INTRODUCTION

Athrixia phylicoides (DC.), commonly known as bush tea, is an indigenous plant of South Africa. It belongs to the Asteraceae family. Bush tea is a popular beverage used as a herbal tea and as a medicinal plant by traditional African people (Roberts, 1990). Throughout history people gathered this plant from the mountainous regions of their homelands and used it for cleansing or purifying the blood, treating boils, acne, infected wounds and cuts, and for washing and as a lotion on boils or skin eruptions (Roberts, 1990). Herbal tea has high concentration of total polyphenols (Owour et al., 2000). Polyphenols are known to possess a wide range of beneficial biochemical and physiological properties (Hirasawa et al., 2002). The major polyphenol antioxidant reported in green tea is epigallocatechin-3-gallate (EGCG), which reduces the amount of free radicals and inflammatory prostaglandins in skin cells (Katiyara and Mukhtar, 1996). Bush tea leaves contain 5-hydroxy-6,7,3',4',5'-hexamethoxy flavon-3-ol which is a flavonoid, possibly responsible for bioactivity in plants as reported by Mashimbye et al. (2006). McGaw et al. (2007) reported that bush tea leaves do not contain caffeine or pyrrolizidine alkaloids, thus justifying its medical potential. Ivanova et al. (2005) reported that the roles of herbal tea in disease prevention and cure have been partly attributed to the antioxidant properties of phenolic compounds present in their extracts. Currently, there is widespread interest in the commercial development of plants with high levels of antioxidants as foods or beverages. Agronomic practices, such as the effects of mineral nutrition, have been reported to improve growth (Mudau et al., 2005), total polyphenols (Mudau et al., 2006), tannins (Mudau et al., 2007c), and total antioxidant contents (Mogotlane et al., 2007). Total polyphenols in tea leaves are the main potential indicators for medicinal potential due to their antioxidant

*Corresponding author. E-mail: l.hlahla@webmail.co.za. Tel: +2715 268 2203. Fax: +2715 268 2892.

activities (Hirasawa et al., 2002).

The major portions of total phenolic compounds in tea are catechins (flavanols and flavanol gallates) which can be oxidized to form theaflavins (TF) and thearubigins (TR) (Harbowy and Balentine, 1997; Lakenbrink et al., 2000). Tea phenolic compounds, known as tea polyphenols (Harbowy and Balentine, 1997), previously called tea tannins (Bokuchava and Skobeleva, 1980), are regarded as the quality parameters or indicators of tea (Deb and Ullah, 1968; Ding et al., 1992; Obanda and Owuor, 1992). In particular, TF were used to assess the market value (Owuor and Reeves, 1986), clonal variations (Deb and Ullah, 1968) and seasonal quality variations of black tea (Malec and Vigo, 1988). Thus, analysis of secondary compounds such as polyphenols, antioxidant content and tannins are effective methods for the determination of tea quality. Herbal tea quality is one of the critical factors determining the price of tea for export. It is currently measured by tea taster's scores from sensory evaluation, which is prompted to be subjective, depending upon the sensory tasting skills of the taster (Taylor et al., 1992). The sensory quality attributes are astringent taste, bitterness, sweetness, and aroma (Hu et al., 2001). Fermentation occurs when the tea polyphenols, such as catechins, are oxidized in the presence of enzymes, mainly peroxidase and polyphenol oxidase (Mahanta and Hazarika, 1985). They contribute to quality and colour of brewed tea. However, the data that describes the effect of fermentation time and temperature are not well established in bush tea. Therefore, the researcher tested different fermentation times and temperatures on the quality (chemical composition) of bush tea.

MATERIALS AND METHODS

Experimental site and plant collection

Bush tea samples were collected from the wild at Muhuyu village (Vhembe District, Limpopo province) [24°N 50'E, 31°S 17'E; 610 m.a.s.l. (meters above sea level); subtropical-type climate, that is summer rainfall, cold and dry winter] and the samples were dried in trays for 2 - 3 weeks under shaded conditions during August, 2008.

Experimental design and treatment details

The experimental design used for each of the two studies (To determine the effect of fermentation temperature on the quality of bush tea, and to determine the effect of fermentation time/period on the quality of bush tea.) was a completely randomized design (CRD) each replicated three times. The treatments for achieving the first objective comprised of different fermentation temperatures [Control (24°C) room temperature, 30, 34, 38 and 42°C] where the tea leaves were fermented for 30 min in an incubator; and the treatments for achieving the second objective consisted of different fermentation times/periods [control (0), 60, 90 and 120 min at 22 - 26°C in an incubator].

Extraction

The dried leaf samples were taken into an incubator and fermented at different temperatures and for different times. For fermentation temperature, 4 glass beakers, excluding the control, were placed into different incubators for 30 min where each sample was fermented at 30, 34, 38 and 42°C. For fermentation time, three glass beakers, excluding the control, were placed into an incubator where each was fermented for 60, 90, and 120 min at 22 - 26°C. Both experiments were replicated three times.

Sample extraction

A sample of two grams (2 g) was weighed into a centrifuge tube where 40 ml of methanol was added and vortexed every ten minutes for 2 h. After vortexing the centrifuge tubes were allowed to stand in order to achieve separation. After separation, the supernatant was removed into a new centrifuge tube and 20 ml of methanol was added to the residues which were vortexed every five minutes for 20 min and the supernatants were combined in one centrifuge tube where it was stored in a freezer set at -10°C until analyzed.

Polyphenol assay

Preparation of standards - a stock solution was prepared (0.1 g of tannic acid into a 100 ml methanol) and the stock solution (0, 2, 4, 6, 8, 10 ml) and the solvent which was methanol (10, 8, 6, 4, 2, 0 ml) were added to prepare a serial dilution. Folin reaction - approximately 10 ml of distilled water was added into each volumetric flask labeled 50 and 0.5 ml of the extracts or standards was added into the volumetric flask. Then 2.5 ml of Folin reagent was added into the volumetric flask and allowed to react for approximately 8 min. After the reaction, 7.5 ml of sodium carbonate was added into the volumetric flask and distilled water was added to the mark of the flask. This was then mixed well and allowed to react (room temperature) for two hours from the time of adding the Folin reagent. After the two hours reaction, a spectrophotometer was used to read the absorbance at 760 nm. A standard curve with concentration (x-axis) and absorbance (y-axis) was plotted where the R2 must be above (0.995).

Tannin assay

The procedures of tannin standards are similar to the one for polyphenols the only difference being that the stock solution in tannin is catechin (0.1 g into 100 ml methanol). Vanillin reagent - 1 g of vanillin was added into a 100 ml methanol and 8 ml of HCL (hydrochloric acid) was added into 92 ml of methanol. For the blank - 8 ml of HCL was added into 92 ml of methanol and the extracts and reagents were suspended in a thermostat-controlled water bath at 30°C for 20 min. Then 1 ml of the methanol extracts was added to 5 ml of vanillin reagent and a sample blank was prepared replacing the vanillin reagent. After the 20 min incubation the resultant colour was read on a spectophotometer at 500 nm. After the readings, the absorbance of the blank was subtracted from those of the samples and also a standard curve with concentration (x-axis) and absorbance (y-axis) was plotted (R2 should be at least 0.995).

Antioxidant assay

Mother Solution preparation - 24 mg of DPPH (2.2-diphenyl-1-picrylhydrazyl) was dissolved in 100 ml methanol and shook for 20 min ensuring that the DPPH was completely dissolved. Working

Table 1. Effect of different fermentation temperatures on the quality of bush tea.

Fermentation temperature (°C)	Polyphenols (mg/100 mg)	Tannins (mg/100 mg)	Antioxidants (µmol/g)
24 (control room temp)	3.4 c	0.9 a	8.3
30	5.0 a	0.8 a	8.3
34	4.1 b	0.7 a	8.3
38	4.1 b	0.3 b	7.9
42	3.7 bc	0.3 b	8.3
CV%	6.6	37.1	3.6

Means in a column followed by the same letter are not significantly different (P>0.05) ns - Non significant difference at 5% level.

solution preparation - 10 ml of the Working Solution was added to 50 ml methanol. The absorbance of this solution should be approximately 1.1 at 515 nm. If too low it should be adjusted with a few drops of the Mother Solution. Methanol was used to zero the spectrophotometer. Trolox Standard - Trolox solution was prepared by adding 2850 µl of Trolox in 100 ml methanol in order to obtain 1000 µM Trolox and a series of dilutions was prepared in methanol (0, 100, 200, 300, 400, 500, 600, 700, 800 µM Trolox from the 1000 µM solution). The 2850 µl of working solution was added to 150 µl of each of the Trolox series, and it was left to react in a shaker for 15 min and the absorbance was measured at 515 nm. A standard curve of change in absorbance (x-axis) versus Trolox concentration (y-axis) was prepared (R2 should be at least 0.995). Sample analysis - 2850 µl of the working solution was added to 150 µl of the sample extract in a vial with a tightly sealable cap, and let to react in a shaker for 6 h and the absorbance was measured at 515 nm.

Statistical analysis and data collection

Data collection (chemical analysis) was done by using Waterman and Mole (1994) method. All the analyses were done at Limpopo Agro-food Technology Station (LATS) at the University of Limpopo, South Africa. The collected data were subjected to analysis of variance (ANOVA) and the means were tested by confidence interval of 95% probability. Means were compared by least significant difference (LSD) test, with 5% level of significance. Data analyses were done using Statistix 8 (Statistix Institute Inc., New York, 1985 - 2003).

RESULTS AND DISCUSSION

Effect of different fermentation temperatures on the quality of bush tea

Total polyphenol, tannin and antioxidant content of bush tea fermented at different temperatures are given in Table 1.

Total polyphenols

Results in Table 1 show that fermentation temperature of 30°C significantly increased polyphenols (5.0/100 mg) followed by fermentation temperature of 34 and 38°C (4.1/100 mg). The lowest levels were obtained at 24 and 42°C. Therefore, this indicates that fermented bush tea at temperatures between 30 - 38°C significantly improved polyphenol contents than in tea fermented at 42°C and room temperature of 24°C.

These results suggest that when tea is fermented for 30 min, the temperature range 30 - 38°C produces the highest level of polyphenols. The high level of polyphenols in the bush tea will give advanced health benefits as it is associated with prevention of heart diseases. Moreover, the slight astringent and bitter taste associated with good teas will also be attained due to high polyphenol content present in bush tea. According to Toit and Joubert (1999), colour development in honey bush tea increased with increasing fermentation temperature while the water soluble solid and polyphenol contents decreased over the fermentation period. They also reported that fermentation at 70°C for 60 h and 90°C for 36 h produced the best flavoured tea. Similar results were reported by Weil (2002) that black tea leaves undergo a process of fermentation/oxidation that changes the colour and flavour and reduces the content of polyphenols. These results suggest that desirable colour and flavour in bush tea could be produced at 42°C as it has the least polyphenol content. However, the results from this study suggest that bush tea fermented at 34 - 38°C for 30 min may produce tea with impartial colour, flavour and polyphenols. Elizabeth and Ockert (1997) reported that the quality of rooibos tea improved with increasing fermentation temperature (30 - 42°C) whereas quality decreased with increasing drying temperature (40 - 70°C).

Tannins

Tannin content at 38 and 42°C were significantly reduced compared to 24, 30 and 34°C. Thus, bush tea leaves contain much tannin content when partially fermented at temperatures between 24 and 34°C. This signifies that increasing fermentation temperature will certainly decrease the tannin content of bush tea. As a result, to produce tea with bitter taste and astringent flavor (tannin

Table 2. Effect of different fermentation times/periods on the quality of bush tea.

Fermentation time (min)	Polyphenols (mg/100 mg)	Tannins (mg/100 mg)	Antioxidants (µmol/g)
0 (control)	3.4[b]	0.9[b]	8.3
60	4.4[a]	1.0[b]	8.4
90	4.4[a]	2.2[a]	8.4
120	3.7[b]	1.3[ab]	8.6
CV%	7.6	36.4	3.6

Means in a column followed by the same letter are not significantly different (P > 0.05). ns - Non significant difference at 5% level.

distinctiveness), fermentation temperatures below 34°C should be used in bush tea. Bush tea leaves fermented at room temperature (24°C) showed high tannin content as compared to other treatments and this will give positive health benefits effect as they eliminate bad bacteria in the mouth and impede development of dental cavities. These results suggest that when tea is fermented for 30 min, the temperature range between 24 - 34°C produces the highest level of tannin content. Furthermore, the low level of tannin in bush tea fermented at 38 and 42°C (0.3/100 m g) for 30 min will give advantage to people with digestive problems who have difficulty with tannin-rich beverages. According to Chakraverty (2003), fermentation temperatures in black tea vary between 24 and 27°C. Fermentation can be assessed by measuring the theaflavin and thearubign content, which are formed in the ratio of 1:10 under ideal conditions. Tannins decrease during this period, from 20% in green tea leaf to 10 - 12% in fermented tea (Chakraverty, 2003). However, there is no data that links fermentation temperature with theaflavin and thearubign contents in bush tea.

Antioxidants

Results in Table 1 show that there are no significant differences between treatments on antioxidant contents. These results signify that different fermentation temperatures do not have any significant impact on antioxidant content of bush tea. However, Erickson (2003) reported that unfermented rooibos, contains higher levels of antioxidants than traditional fermented rooibos. Furthermore, green tea leaves have high level of antioxidant content and healthier benefits than black tea with the least amount of antioxidant content. This antioxidant variation is due to the way in which the tea is processed.

Effect of different fermentation times/periods on the quality of bush tea

Total polyphenol, tannin and antioxidant content of bush tea fermented at different temperatures are given in Table 2.

Total polyphenols

Results in Table 2 show that fermentation time of 60 and 90 min significantly increased polyphenols (4.4/100 mg) in the bush tea leaves. There is a significant rise to a peak in total polyphenols values for tea fermented for 60 and 90 min before declining at 120 min. Polyphenol content at 0 and 120 min were similar (3.4 and 3.7/100 mg). These results suggest that high polyphenol contents can be produced at fermentation time between 60 and 90 min. There is no data that relates fermentation time to chemical composition in bush tea. However, Owuor and Obanda (2001) reported that fermentation duration of 90 min resulted in black tea with higher levels of theaflavins but lower thearubigins and colour than fermentation for 110 min. Honey bush tea fermented at 70°C for 24 h showed a significant increase in polyphenol content (129.2 g kg^{-1} ss) than honeybush tea fermented for 36 - 72 h (117.5 and 95.6 g kg^{-1} ss). These results suggest that increasing fermentation time in honeybush tea would lead to a decline in measured polyphenol concentration as complex colour and flavour compounds are formed. Furthermore, tea quality such as taste and astringent will be enhanced in bush tea as it showed an optimistic increase in polyphenol content when fermented between 60 and 90 min. Moreover, the presence of polyphenols in bush tea will have affirmative health benefits as it is said to reduce the incidence of skin, lung, stomach and liver cancer. The results from this study concur with the findings reported by Goldstein and Swain (1963), who reported that increasing fermentation time significantly reduced polyphenol concentration, brew colour and flavour in honeybush tea.

Tannins

Results in Table 2 show that fermentation of bush tea leaves for 90 min had peak tannin content as compared to 60 min and below, and fermentation for 120 min. These results suggest that desirable sensory attributes, such as taste and astringent will be produced with a fermentation time of 90 min if bush tea is fermented at 22

- 26°C as it produces high level of tannin content. Toit and Joubert (1998) reported that tannin content in honeybush tea when fermented at 70°C for 24 h (40.1 g kg-1 ss) showed a significant increase in tannin content than when fermented for 36 - 72 h (25.1 and 16.0 g kg-1 ss) respectively. Greaves (2009) reported that any fermentation process is responsible for the caffeine content of the tea. The longer it is fermented the more caffeine the tea will have. Chakraverty (2003) reported that time of fermentation in black tea varies between 45 min to 3 h, depending on the nature of the leaf, maceration technique and ambient temperature. According to Greaves (2009), green tea had the most health benefits when compared to other teas presumably due to longer fermentation process which resulted in increasing oxidation processes.

Antioxidants

No significant differences were observed on antioxidant contents in bush tea leaves due to fermentation time up to 120 min (Table 2). Unfermented bush tea and tea fermented for 120 min had 8.3 and 8.6/100 mg, respectively. This clearly indicates that fermentation time at any time interval had no significant influence on the antioxidant content of bush tea leaves. Greenwalt et al. (2000) reported that Kombucha tea is usually prepared at ambient temperature for up to 7 - 10 days but the role of fermentation time is not seriously considered. Greenwalt et al. (2000) also reported that Kombucha tea exhibits increase in antioxidant activities during fermentation. Thus, the extent of the activity depended upon culture period and starter origins, which in turn determine the forms of their metabolites. Green tea is processed differently from the way black tea is processed. Antioxidants in the tea leaves are nearly exhausted after black tea is processed whereas in green tea, almost all of its antioxidants are left in the leaves after processing. These suggest that fermented tea has the least amount of antioxidant content than unfermented tea leaves. However, this seems different in bush tea.

Conclusion

Different fermentation temperatures and times of bush tea exhibited significant influence on polyphenol and tannin contents, but had no influence on the antioxidant content of the tea. Further studies are required to determine the sensory quality parameters, such as taste and aroma.

ACKNOWLEDGEMENT

The authors are grateful to the National Research Foundation (NRF) for funding the study and to the Limpopo Agro-Food Technology Station for facilitating the study.

REFERENCES

Bokuchova MA, Skobeleva NI (1980). The biochemistry and technology of tea manufacture. Crit. Rev. Food Sci. Nutr. 12: 303-370.
Chakraverty A (2003). Handbook of Postharvest Technology. Cereals, Fruits, Vegetable, Tea and Spices. Second edition. Published by CRC Press, ISBN 0824705149, 9780824705145.
Deb SB, Ullah MR (1968). The role of theaflavins and thearubugins in the evaluation of black teas. Two and A Bud 15: 101-102.
Ding Z, Kuhr S, Engelhardt UK (1992). Influence of catechins and theaflavins on the astringent taste of black tea brews. Zeitschrift fur Lebensmitteluntersuchung und Forschung A. 195: 108-111.
Elizabeth J, Ockert T (1997). Effect of fermentation and drying conditions on the quality of rooibos tea. Int. J. Food Sci. Technol. 2: 127-134.
Erickson L (2003). Rooibos Tea: Research into Antioxidant and Antimutagenic Properties. J. Am. Bot. Counc. 59: 34-45. Goldstein JL, Swain T (1963). Changes in tannins in ripening fruits. Phytochem. 2: 371-381.
Greaves D (2009). Bodybuilding. All about green tea. Posted by Zulrushdi Bin Ismail.
Greenwalt CJ, Steinkraus KH, Ledford RA (2000). Kombucha, the fermented tea: microbiology, composition, and claimed health effects. J. Food Prot. 63: 976-981.
Harbowy ME, Balentine DA (1997). Tea chemistry. Critical Rev. Food Sci. Nutr. 16: 415-480.
Hirasawa M, Takada T, Makimura M, Otake O (2002). Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. Annual Revolut. 37: 433-438.
Hu Q, Pan G, Zhu J (2001). Effect of selenium on green tea preservation quality and amino acid composition of tea protein. J. Horticult. Sci. Biotechnol. 76: 344-346.
Ivanova D, Gerova D, Chervenkov T, Yankova T (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J. Ethnopharmacol. 96: 145-150.
Katiyara SK, Mukhtar H (1996). Tea in chemo-prevention of cancer: Epidemiologic and experimental studies. J. Oncol. 8: 221-238.
Lakenbrink C, Lapczzynski S, Maiwald B, Engelhardt UK (2000). Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. J. Agric. Food Chem. 48: 2848-2852.
Mahanta PK, Hazarika H (1985). Chlorophyll and degradation products in orthodox and CTC black teas and their influence on shade of colour and sensory quality in relation to thearubigins. J. Sci. Food Agric. 36: 1133-1139.
Malec LS, Vigo MS (1988). Seasonal variations in theaflavin, thearubign and caffeine contents of Argentinean black tea. J. Sci. Food Agric. 45: 185-190.
Mashimbye MJ, Mudau FN, Soundy P, Van Ree T (2006). A new flavanol from bush tea. South African J. Chem. 59: 1-2.
McGaw LJ, Steenkamp V, Eloff JN (2007). Evaluation of Athrixia bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. J. Earthnopharmacol. 110: 16-22.
Mogotlane ID, Mudau FN, Mashela PW, Soundy P (2007). Seasonal responses of total antioxidant content in cultivated bush tea leaves to fertilize rates. Med. Aromatic Plant Sci. Biotechnol. 1: 77-79.
Mudau FN, Soundy P, Du Toit ES (2005). Plant growth and development of bush tea as affected by nitrogen, phosphorus and potassium nutrition. Horticult. Sci. 40: 1898-1901.
Mudau FN, Soundy P, Du Toit ES (2007c). Nitrogen, phosphorus and potassium nutrition on total polyphenols content of bush tea in a shaded nursery environment. Horticult. Sci. 42: 334-338.
Mudau FN, Soundy P, Du Toit ES, Olivier J (2006). Variation in polyphenolic content of bush tea leaves with seasonal and nitrogen application. South African J. Bot. 71: 398-402.
Obanda M, Owuor PO (1992). Effects of wither plucking on the biochemical and chemical parameters of selected Kenya tea. Discov.

Innov 6: 190-197.
Owuor PO, Ng'etich KW, Obanda O (2000). Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard. J. Sci. Food Agric. 80: 439-446.
Owuor PO, Obanda M (2001). Tea: Influence of fermentation conditions and duration on the quality of black tea. Food Chem. pp. 319-327.
Owuor PO, Reeves SG (1986). Optimising fermentation time in black tea manufacture. Food Chem. 21: 195-203.
Roberts M (1990). Indigenous Healing Plants (first edition), Southern Book Publishers, Halfway Hose, South Africa.
Statistix 8 (Statistix Institute Inc., New York (1985-2003).
Taylor S, Baker D, Owuor P, Orchard J, Othieno C, Gay C (1992). A Model for predicting black tea quality from the carotenoid and chlorophyll composition of fresh green tea leaf. J. Sci. Food Agric. 58: 185-191.
Toit JD, Joubert E (1998). The effect of pretreatment on the fermentation of Honeybush tea. J. Sci. Food Agric. 76: 537-545.
Toit JD, Joubert E (1999). Optimization of the fermentation parameters of Honeybush tea. J. Food quality 22: 241-256.
Waterman P, Mole S (1994). Analysis of phenolic plant metabolites. Blackwell Scientific, London pp. 88-85.
Weil A (2002). Tea: Red, Green or Black? http://www.drweil.com/drw/u/id/QAA82788.