EFFECT OF FERMENTATION TEMPERATURE AND DURATION ON CHEMICAL COMPOSITION OF BUSH TEA (*Athrixia phylicoides* DC.)

by

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Agriculture (Horticulture) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

_____ _____

Initials & Surname (Title) **Date**

Student Number**: _____**

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ABSTRACT

A study was conducted to determine the effect of fermentation temperature and duration on chemical composition of bush tea (*Athrixia phylicoides* DC.). Bush tea was fermented in incubators at different temperatures and for different lengths of time for quality improvement. Treatments for fermentation temperature consisted of control (24°C; room temperature), 30°C, 34°C, 38°C and 42°C where the tea leaves were fermented for 30 minutes. Treatments for fermentation time consisted of control (0), 60, 90, and 120 minutes 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 (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 minutes) and tannin contents (90 and 120 minutes). However, changes in either fermentation temperature or time did not give any significant influence on antioxidant content of bush tea.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

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 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, Ng'etich and Obanda, 2000). Polyphenols are known to possess a wide range of beneficial biochemical and physiological properties (Hirasawa, Takada, Makimura and Otake, 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, Mudau, Soundy and Van Ree (2006). McGaw, Steenkamp and Eloff (2007) reported that bush tea leaves do not contain caffeine or pyrrolizidine alkaloids, thus justifying its medical potential. Ivanova, Gerova, Chervenkov and Yankova (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, Soundy and Du Toit, 2005), total polyphenols (Mudau, Soundy, Du Toit and Olivier, 2006), tannins (Mudau, Soundy and Du Toit, 2007c), and total antioxidant contents (Mogotlane, Mudau, Mashela, and Soundy, 2007). Total polyphenols in tea leaves are the main

potential indicators for medicinal potential due to their antioxidant 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, Lapczynski, Maiwald and Engelhardt, 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, Kuhr and Engelhardt, 1992; and 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, Baker, Owuor, Orchard, Othieno and Gay, 1992). The sensory quality attributes are astringent taste, bitterness, sweetness, and aroma (Hu, 2001a).

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.

1.2 Problem statement

Previous studies showed that bush tea has a significant commercial potential. Currently, there is no information regarding the effect of fermentation temperature and time on the quality of bush tea. Therefore, the researcher proposes testing different fermentation temperatures and times on the quality (chemical composition) of bush tea.

1.3 Motivation of the study

Bush tea is a popular beverage used as a herbal tea and as a medicinal plant by traditional African people. It is also used as an aphrodisiac by Vhavenda people, and the Zulu people use a decoction of the root as a cough remedy and a purgative. Bush tea contains no caffeine thus suggesting that bush tea is a healthy drink. The current study will contribute towards development of processing techniques for standard bush tea products.

1.4 Aim and objectives of the study

1.4.1 Aim

To investigate the effect of fermentation temperature and time on the quality (chemical composition) of bush tea.

1.4.2 Objectives

To determine the effect of fermentation temperature on the quality of bush tea.

To determine the effect of fermentation time/period on the quality of bush tea.

1.5 Hypotheses

Fermentation temperature does not have an effect on the quality of bush tea.

Fermentation time/period does not have an effect on the quality of bush tea.

CHAPTER 2

LITERATURE REVIEW

2.1 Fermentation

Fermentation is the process during which the polyphenols in the tea leaf are oxidized in the presence of enzymes and subsequently condensed to form coloured compounds contributing to the quality attributes of tea (Mahanta and Hazarika, 1985). It starts immediately after cell rupture (Kumar, 1999). A series of chemical reactions take place during this process due to the severe damage to the leaf cells. Heat (Owour and McDowell, 1994), light (Cha, 1995) and pH (Mahanta and Hazarika, 1985) affect the degradation of carotenoids (Mahanta, 1988).

According to Boruah (1992), some non-volatile compounds such as theaflavins (TF) and thearubigins (TR) are produced in the fermentation stage. These compounds together impart liquor and taste to tea (Owour and McDowell, 1994). In addition to the formation of TF and TR, some other chemical changes also take place in the leaf tissues during the fermentation process (Boruah, 1992). Proteins get degraded, the chlorophyll is transformed into pheophytins and some volatile compounds are generated due to transformation of certain aroma precursors present in the tea leaf (Mahanta and Hazarika, 1985). There are currently two distinct methods of fermentation used in teas; heap and oven fermentation (Du Toit, 1996). However, there are no standard methods for bush tea fermentation.

2.1.1 Factors that affect fermentation

Tea quality: The effect of fermentation is one of the most fundamental processes that determine factors for tea quality (Taylor *et al.,* 1992). Furthermore, quality is one of the critical factors determining the price in any market (Taylor *et al.,* 1992). It is currently measured or valued in terms of price realization or tea taster's scores from sensory evaluation (Taylor *et al.,* 1992). Generally, in the tea industry quality has also been reported to be influenced by active chemical compounds, but there are arguments that tea drinkers do not drink chemicals. Therefore, sensory quality parameters such as taste and aroma have been considered as major quality

indicators in the tea industry (Fernando and Roberts, 1984; Taylor *et al.,* 1992). There are several factors such as cultivars (Owour *et al.* 2000), environmental conditions (Chiu, 1989), cultural practices (Taylor *et al.,* 1992) and seasonal variations (Sud and Baru, 2000) that affect tea quality.

Temperature: The colour of honeybush tea improves with increasing fermentation temperature whereas polyphenols decreased with increasing drying temperature (Toit and Joubert, 1999). According to John (1980), teas produced at higher temperatures contain more of the thearubigin pigments, including the polymeric fraction known as the non-dialysable material. In the case of black tea, low fermentation temperatures improve black tea quality hence long fermentation duration and high temperature favour the production of more intense coloured black teas with high thearubigin levels (John, 1980).

Time: After cutting, tea is subjected to a so-called fermentation. This process is not actually a fermentation, which is an anaerobic process, but rather an enzymatic oxidization of the polyphenols in the tea leaves (Owour and McDowell, 1994), yielding theaflavins and thearubigins (Robertson, 1983). When the tea leaves are dry, fermentation stops, allowing some control of the process by manipulation of the drying rate or adding water after drying. The compounds responsible for tea quality, such as theaflavins (TF) and thearubigins (TR) increase with fermentation time (Hampton, 1992). Caffeine concentration remained unchanged (Hampton, 1992). Polyphenols declined more quickly during the initial stages, followed by a steadily declining trend. Fermentation time had little impact on the gallic acid concentration (Lakshminarayanan and Ramaswamy, 1978). Among the catechins, epigallocatechin oxidized fastest, followed by epigallocatechin gallate and epicatechin gallate (Katiyara and Mukhtar, 1996).

Processing: It consists of four steps: withering, rolling, fermentation and drying. Withering of tea leaves is necessary to physically condition the fresh tea leaves making them amenable to subsequent processing (Lin, Juan, Chen, Liang, and Lin 1996). In addition, loss of moisture and a number of important biochemical changes take place during withering. Leaves are subjected to rolling in which the cell structures are disrupted and leaves are macerated (Gutman and Ryu, 1996). During this stage, enzymes such as polyphenol oxidase present in tea leaves are brought into intimate contact with substrate (catechins). The chemical and biochemical reactions initiated during rolling are allowed to continue and completed during the next stage of tea processing, referred to as fermentation (Subramanian, Venkatesh, Ganguli, and Sinkerm, 1999). It was previously believed that the changes occurring during this stage are caused by microorganisms. It is now well known that the principal reaction is oxidation of catechins by the enzyme polyphenol oxidase which results in the formation of two types of dimeric and polymeric products (Robertson, 1983), theaflavins and thearubigins. The fermentation step is followed by drying or firing which is necessary for cessation of enzyme activity and reducing moisture content of the fermented products (Bhatia, 1964).

2.2 Chemical composition

The main difference in the manufacture of tea is heat inactivation of enzymes in the flush (Lopez and Barcelo, 2001). In the case of green tea, steaming of leaves is the first step, by which polyphenol oxidase and other enzymes are inactivated and thus retaining its green colour (Katiyara and Mukhtar, 1996). The steamed leaves are then rolled and subsequently dried to get the final product.

Flavonoids, a group of phenolic compounds occurring abundantly in vegetables, fruits, and green plants, attract special attention as they show high antioxidant property (Bu-Abbas, Copeland, Clifford, Walker and Loannides, 1997). The antioxidants are known to prevent cellular damage caused by reactive oxygen species (Sergediene, Jonsson, Szymusiak, Tyrakowska, Rietjens, and Cenas, 1999). Catechins are highly potent flavonoids present in tea and serve perhaps as the best dietary source of natural

antioxidants (Sarkar and Bhaduri, 2001). Approximately 30% of the tea solids in a typical infusion are composed of flavonoids, whereas less than 5% of the water soluble solid extract of tea is formed by flavonols like quercetin, kaempferol, myricetin and their glycosides (Subramanian *et al,* 1999). Of the catechins, epigallocatechin gallate (EGCG) is present maximally (more than 10% of dry weight) in green tea (Hour, Liang, Chu, and Lin, 1999).

The flavonoid content of plants, vegetables and fruits varies with plant variety and environmental conditions (Lampe, 1999). Light is required for the synthesis of flavonoids and they are generally found in the outer portion of plants (Cha, 1995), vegetables and fruits, like skin of the fruit, or in the leaves as in the case of tea. From a chemical point of view (Sergediene *et al.,* 1999), the accepted definition of flavonoids is dibenz pyrans and pyrones and their derivatives, which are any compound with a C6-C3-C6 ring structure, including those which are oxidized and those attached to sugar molecules (glycosides), as long as the derivatives retain the ring structure (Fig. 1).

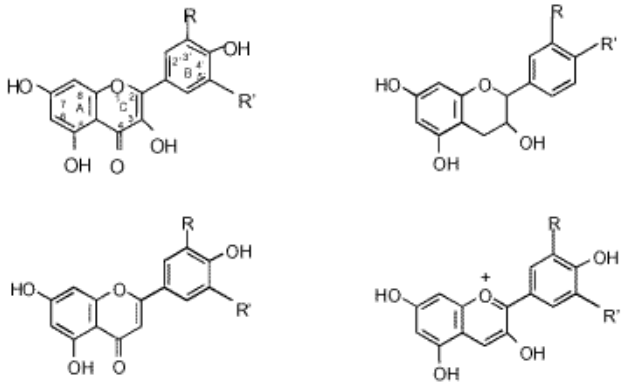
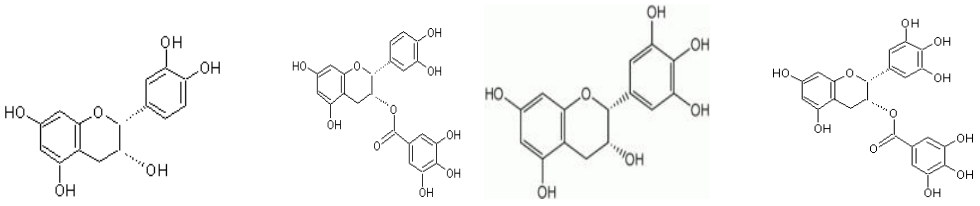


Figure 1: Flavonoids (C6-C3-C6). Basic structure and system used for carbon numbering of the flavonoid nucleus. Structural variations within the rings subdivide the flavonoids into several families (Sergediene *et al.,* 1999)

Bush tea contains many nutrients, but the primary nutritious constituents are the polyphenols. The chief polyphenols are flavonoids such as catechin and proanthocyanidins, with the four major polyphenols (Fig. 2) being epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG).

Polyphenols are everywhere, some give plants their colour, while others give plants their taste. The strong astringent flavour of tea is attributed to its polyphenol content (Hirasawa *et al.,* 2002). In fact, these special flavonoids have been credited with having more potency than vitamins C and E.

Polyphenols appear to thwart cancer by at least three methods: they can shut off the formation of cancer cells (Fujiki, Suganuma, Okabe, Sueoka, Suga, Imai, Nakachi, and Kimura, 1999), turn up the body's natural detoxification defences (Vendemiale, Grattagliano, and Altomare, 1999), and suppress cancer advancement (Halder, and Bhaduri, 1998). Most of the green tea catechins, during the manufacture of black tea, are oxidized and converted into orange or brown products known as theaflavins (TF) and thearubigins (TR) (Harbowy and Balentine, 1997 and Lakenbrink *et al.,* 2000). These compounds retain the basic C6-C3-C6 structure and are thus still classified as flavonoids.



(EC) (ECG) (EGC) (EGCG)

Figure 2: Structures of polyphenols (Wang, Khan and Bickers, 1989)

2.3 Nutritional value

Bush tea has a high level of polyphenolic antioxidants. Furthermore, the absence of caffeine is a desirable feature of a health beverage, as is the presence of antioxidants which may have beneficial health effects (McGaw *et al.*, 2007). This gives it a distinct advantage over regular green and black teas made from *Camellia sinensis*. Furthermore, it also possesses a low level of tannins, thus bypassing the bitter, astringent taste experienced with many other teas (Hu, Pan, and Zhu, 2001a). The low tannin content of bush tea is an advantage for people with

digestive problems who have difficulty with tannin-rich beverages (Bokuchava and Skobeleva, 1980). Tannins bind iron and reduce the absorption of non-heme iron (Bokuchava and Skobeleva, 1980). This can be significant for those with marginal iron intake. Some teas, like black and peppermint, may inhibit iron uptake by as much as 80 to 90 percent.

CHAPTER 3

EFFECT OF FERMENTATION TEMPERATURE ON THE QUALITY OF BUSH TEA

3.1 Introduction

Most varieties of tea come from the leaves of a single plant, *Camellia sinensis*. However, it is the way the leaves are processed that determines the outcome of the final product. In rooibos tea, quality improved with increasing fermentation temperature whereas quality decreased with increasing drying temperature (Elizabeth and Ockert, 1997). They also reported that colour development increased with increasing fermentation temperature while polyphenol content significantly decreased. Long fermentation duration and high temperature favoured production of more intense coloured black teas with high thearubigin level and lower theaflavin (Owuor and Obanda, 2001). Owuor and Obanda (1998) were of the opinion that long fermentation decreased brightness and flavour index and increased volatile flavour compound levels in black tea. The objective of this study was to determine the effect of fermentation temperature on the quality of bush tea.

3.2 Materials and methods

3.2.1. 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, i.e. summer rainfall, cold and dry winter] and the samples were dried in trays for 2-3 weeks under shaded conditions.

3.2.2 Experimental design and treatment details

The experimental design used to determine the effect of fermentation temperature on the quality of bush tea was a completely randomized design (CRD) replicated three times. The treatments comprised of different fermentation temperatures [Control (24°C; room temperature), 30°C, 34°C, 38°C and 42°C] where the tea leaves were fermented for 30 minutes in an incubator.

3.2.3 Extraction

The dried leaf samples were taken into an incubator and fermented at different temperatures. Four glass beakers excluding the control were placed into different incubators where each sample was fermented at 30°C, 34°C, 38°C and 42°C for 30 minutes. The experiment was 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 two hours. 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 twenty minutes and the supernatants were combined in one centrifuge tube where they were 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 ml 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 eight minutes. 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 of 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 R^2 must be above 0.995.

Tannin assay: The procedures of tannin standards are similar to the ones 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 minutes. 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 20 minute 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 (R^2 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 minutes ensuring that the DPPH was completely dissolved. Working 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 minutes and the absorbance was measured at 515 nm. A standard curve of change in absorbance (x-axis) versus trolox concentration (y-axis) was prepared (R^2 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 six hours and the absorbance was measured at 515 nm.

3.2.4 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) at, with 5% level of significance. Data analysis were done using Statistix 8 (Statistix Institute Inc., New York. 1985-2003).

3.3 Results and Discussion

3.3.1 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^{o}C significantly increased polyphenols (5.0 mg/100 mg) followed by fermentation temperature of 34 and 38^{o}C (4.1 mg/100 mg). The lowest levels were obtained at 24^{o}C and 42^{o}C. Therefore, this indicates that fermented bush tea at temperatures between 30-38^{o}C significantly improved polyphenol contents than in tea fermented at 42^{o}C and room temperature of 24^{o}C. These results suggest that when tea is fermented for 30 minutes, the temperature range of 30-38^{o}C produces the highest level of polyphenols.

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

Fermentation temperature (^{o}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
LSD 0.05	0.5	0.4	ns
CV%	6.6	37.1	3.6

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

The high level of polyphenols in 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 honeybush 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^{o}C for 60 hours and 90^{o}C for 36 hours 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 to 38°C for 30 minutes 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^{o}C) whereas quality decreased with increasing drying temperature (40-70^{o}C).

Tannins: Tannin content at 38 and 42^{o}C were significantly reduced compared to 24, 30 and 34^{o}C. Thus, bush tea leaves contain much tannin content when partially fermented at temperatures between 24 and 34^{o}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 distinctiveness), fermentation temperatures below 34^{o}C should be used in bush tea. Bush tea leaves fermented at room temperature (24 ^{o}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 minutes, the temperature range between 24-34^{o}C produces the highest level of tannin content. Furthermore, the low level of tannin in bush tea fermented at 38 and 42^{o}C (0.3 mg/100 g) for 30 minutes 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^{o}C and 27^{o}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 content 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.

CHAPTER 4

EFFECT OF FERMENTATION TIME/PERIOD ON THE QUALITY OF BUSH TEA

4.1 Introduction

Tea is the most widely consumed and cheapest non-alcoholic drink next to water. Catechins are the major biochemical constituents present in tea leaves and they get oxidized to form theaflavins (TF) and thearubigins (TR) during fermentation (Hampton, 1992). Catechins and their oxidation products are mainly responsible for the taste and astringent character of black tea. Fermentation is one of the important processes in black tea manufacture. During fermentation, the simple substrates (catechins) are acted upon by the oxidative enzymes, polyphenol oxidase and peroxidase to form theaflavins and thearubigins (Lakshminarayanan and Ramaswamy, 1978). The time, temperature, pH, relative humidity and oxygen availability during fermentation are the crucial factors responsible for the formation of high levels of desired products (Cloughley, 1980; Cloughley and Ellis, 1980; Obanda, Owuor, and Mangoka, 2001; Rajeev, Rajappan, and Balasubramanian, 2000). Of these, the time of fermentation is important, since both increase and decrease in fermentation time can lead to poor quality tea. The objective of this study was to determine the effect of fermentation time/period on the quality of bush tea.

4.2 Materials and methods

4.2.1. 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, i.e. summer rainfall, cold and dry winter] and the samples were placed in trays for 2-3 weeks under shaded conditions.

4.2.2 Experimental design and treatment details

The experimental design used to determine the effect of fermentation time/period on the quality of bush tea was a completely randomized design (CRD) replicated three times.

The treatments consisted of different fermentation times/periods [control (0), 60, 90, and 120 minutes at 22-26°C in an incubator].

4.2.3 Extraction

The dried leaf samples were taken into an incubator and fermented at different times/periods. Three glass beakers excluding the control were placed into an incubator where each was fermented for 60, 90, and 120 minutes at 22-26°C. The experiment was 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 two hours. 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 twenty minutes and the supernatants were combined in one centrifuge tube where they were 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 ml 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 eight minutes. 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 R^2 must be above 0.995.

Tannin assay: The procedures of tannin standards are similar to the ones 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 minutes. 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 minute 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 (R^2 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 minutes ensuring that the DPPH was completely dissolved. Working 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 minutes and the absorbance was measured at 515 nm. A standard curve of change in absorbance (x-axis) versus trolox concentration (y-axis) was prepared (R^2 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 six hours and the absorbance was measured at 515 nm.

4.2.4 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, at 5% level of significance. Data analysis were done using Statistix 8 (Statistix Institute Inc., New York. 1985-2003).

4.3 Results and discussion

4.3.1 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 minutes significantly increased polyphenols (4.4 mg/100 mg) in bush tea leaves. There was a significant rise to a peak in total polyphenols values for tea fermented for 60 and 90 minutes before declining at 120 minutes. Polyphenol content at 0 and 120 minutes were similar (3.4 and 3.7 mg/100 mg). These results suggest that high polyphenol contents can be produced at fermentation time between 60 and 90 minutes. There are no data that relates fermentation time to chemical composition in bush tea. However, Owuor and Obanda (2001) reported that fermentation duration of 90 minutes resulted in black tea with higher levels of theaflavins but lower thearubigins and colour than fermentation for 110 minutes. Honeybush tea fermented at 70^{o}C for 24 hours showed a significant increase in polyphenol content (129.2 g kg^{-1}) than honeybush tea fermented for 36-72 hours (117.5 and 95.6 g kg^{-1}). 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 minutes. 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.

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

Fermentation time (minutes)	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
LSD 0.05	0.6	0.9	Ns
CV%	7.6	36.4	3.6

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

The results from this study concur with the findings 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 minutes had peak tannin content as compared to 60 minutes and below, and fermentation for 120 minutes. These results suggest that desirable sensory attributes, such as taste and astringency will be produced with a fermentation time of 90 minutes if bush tea is fermented at 22-26°C as it produces high levels of tannins. Toit and Joubert (1998) reported that tannin content in honeybush tea when fermented at 70°C for 24 hours (40.1 g kg^{-1}) showed a significant increase in tannin content than when fermented for 36-72 hours (25.1 and 16.0 g kg^{-1}). 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 minutes to 3 hours, 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 minutes (Table 2). Unfermented bush tea and tea fermented for 120 minutes had 8.3 and 8.6 mg/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, Steinkraus and Ledford (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.

CHAPTER 5
SUMMARY AND CONCLUSSIONS

The study was conducted to determine the effect of fermentation temperature and duration on chemical composition of bush tea (*Athrixia phylicoides* DC.). Bush tea was fermented in incubators at different temperatures and for different periods of time for quality improvement. Bush tea samples were collected from the wild at Muhuyu village (Vhembe District, Limpopo Province). Treatments for fermentation temperature consisted of control (24°C; room temperature), 30°C, 34°C, 38°C and 42°C where the tea leaves were fermented for 30 minutes. Treatments for fermentation time consisted of control (0), 60, 90, and 120 minutes at an incubator temperature of 22-26°C. A completely randomized design (CRD) was used with three replicates for both evaluations. 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, at 5% level of significance. Data analyses were done using Statistix 8 (Statistix Institute Inc., New York. 1985-2003).

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 duration achieved a significant increase in both polyphenols (60 and 90 minutes) and tannins (90 and 120 minutes). However, changes in either fermentation temperature or period did not have any significant influence on antioxidant content of bush tea. In conclusion, the interactive effect of temperature and time during fermentation on quality of bush tea may be

fundamental for future investigation. Further studies are also required to determine the sensory quality parameters such as taste and aroma since they are the most dominant parameters for quality determination during fermentation in the tea industry.

LITERATURE CITED

BOKUCHAVA, M.A., and N.I. SKOBELEVA. 1980. The biochemistry and technology of tea manufacture. *Critical Review in Food Science and Nutrition* 12:303-370.

BORUAH, P.K. 1992. Electronic Instrumentation for Tea Industry. Instrumentation The United States Industry Coalition, Gauhati University, Volume 1, No 1.

BHATIA, I.S. 1964. Biochemical investigations in relation to tea manufacture. *Two and A Bud* 7:8-15.

BU-ABBAS, A., COPELAND, E., CLIFFORD, M.N., WALKER, R., and C. IOANNIDES.1997. Fractionation of green tea extracts: Correlation of anti mutagenic effect with flavanol content. *Journal of the Science of Food and Agriculture* 75:453-462.

CHA, J. 1995. The Classic of Tea: Origins and Rituals, Carpenter, Francis Ross; New York, United States of America. Ecco Press.

CHAKRAVERTY, A. 2003. Handbook of Postharvest Technology. Cereals, Fruits, Vegetable, Tea and Spices. Second edition. Published by CRC Press, ISBN 0824705149, 9780824705145.

CHIU, W.T.F. 1989. Factors affecting the production and quality of partially fermented tea in Taiwan. *Taiwan Tea Experimental Station* 2:99-102.

CLOUGHLEY, J. B. 1980. The effect of fermentation temperature on the quality parameters and price evaluation of Central African black teas. *Journal of the Science of Food and Agriculture* 31:911-919.

CLOUGHLEY, J. B., and R. T. ELLIS. 1980. Effect of pH modification during fermentation on the quality parameters of Central African black teas. *Journal of the Science of Food and Agriculture* 31:924-934.

DEB, S.B., and M.R. ULLAH. 1968. The role of theaflavins and thearubugins in the evaluation of black teas. *Two and A Bud* 15:101-102.

DING, Z., KUHR, S., and U.K. ENGELHARDT. 1992. Influence of catechins and theaflavins on the astringent taste of black tea brews. *Zeitschrift fur Lebensmitteluntersuchung und Forschung A* 195:108-111.

Du TOIT, J. 1996. Development of standardized processing method for honeybush tea. *Master of Science in Food Science.* Department of Food Science, Faculty of Agriculture, University of Stellenbosch, South Africa.

ELIZABETH, J., and T. OCKERT. 1997. Effect of fermentation and drying conditions on the quality of rooibos tea. *International Journal of Food Science and Technology* 2:127-134.

ERICKSON, L. 2003. Rooibos tea: Research into antioxidant and antimutagenic properties*. The Journal of the American Botanical Council* 59:34-45.

FERNANDO, V., and G.R. ROBERTS. 1984. The effect of process parameters on sea seasonal development of flavour in black tea. *Journal of the Science of Food and Agriculture* 35:71-76.

FUJIKI, H., SUGANUMA, M., OKABE, S., SUEOKA, E., SUGA, K., IMAI, K., NAKACHI, K., and S. KIMURA. 1999. Mechanistic findings of green tea as cancer preventive for humans. *Processing Social Experimental of Biological Medicine* 220:225-228.

GOLDSTEIN, J.L., and T. SWAIN. 1963. Changes in tannins in ripening fruits. *Phytochemistry* 2:371-381.

GREAVES, D. 2009. Bodybuilding. All about green tea. Updated April 19, 2009. http://www.bodybuilding.com/fun/greentea.htm.

GREENWALT, C.J., STEINKRAUS, K.H., and R.A. LEDFORD. 2000. Kombucha, the fermented tea: Microbiology, composition, and claimed health effects. *Journal of Food Protection* 63:976-981.

GUTMAN, R.L., and B.H. RYU. 1996. Rediscovering tea. An exploration of the scientific literature. *Herbal Gram* 37:33-44.

HALDER, J., and A. BHADURI. 1998. Protective role of black tea against oxidative damage of human red blood cells. *Biochemestry Biophysical Research Community* 244:903-907.

HAMPTON, M.G. 1992. Tea: Cultivation to consumption*.* Chapman and Hall: London.

HARBOWY, M.E., and D.A. BALENTINE. 1997. Tea chemistry. *Critical Reviews in Food Science and Nutrition* 16:415-480.

HIRASAWA, M., TAKADA, T., MAKIMURA, M., and O. OTAKE. 2002. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. *Annual Revolution* 37:433-438.

HOUR, T.C., LIANG, Y.C., CHU, I.S., and J.K. LIN. 1999. Inhibition of eleven mutagens by various tea extracts, (-) epigallocatechin.3-gallate, gallic acid and caffeine. *Food Chemistry Toxicology* 37:569-579.

HU, Q., PAN, G., and J. ZHU. 2001a. Effect of selenium on green tea preservation quality and amino acid composition of tea protein. *Journal of Horticultural Science and Biotechnology* 76:344-346.

IVANOVA, D., GEROVA, D., CHERVENKOV, T., and T. YANKOVA. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology* 96:145-150.

JOHN, B. 1980. The effect of temperature on enzyme activity during the fermentation phase of black tea manufacture. *Journal of the Science of Food and Agriculture* 9:920-923.

KATIYARA, S.K., and H. MUKHTAR. 1996. Tea in chemo-prevention of cancer: Epidemiologic and experimental studies. *Journal of Oncology* 8:221-238.

KUMAR, P.K. 1999. Electronic instrumentation and control systems for critical manufacturing stages in tea industry. *Global Advances in Tea Science* 1:825-836.

LAKENBRINK, C., LAPCZYNSKI, S., MAIWALD, B., and U.K. ENGELHARDT. 2000. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *Journal of Agricultural and Food Chemistry* 48:2848-2852.

LAKSHMINARAYANAN, K., and S. RAMASWAMY. 1978. Towards optimization of fermentation during manufacture. *United Planters' Association of South India Tea Scientific Department Bulletin* 35:34-47.

LAMPE, J.W. 1999. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *American Journal of Nutrition* 70:47-55.

LIN, Y.L., JUAN, I.M., CHEN, Y.L., and Y.C. LIANG. 1996. Composition of polyphenols in fresh tea leaves and association of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *Journal of Agricultural Food Chemistry* 44:1387-1394.

LOPEZ, S., and A.R. BARCELO. 2001. Reversed phase and size exclusion chromatography as useful tools in the resolution of peroxidase-mediate (+) - catechin oxidation products. *Journal of Chromatograph* 919:267-273.

MAHANTA, P.K. 1988. Biochemical basis of colour and flavour of black tea. Proceedings of 30th Tocklai Conference: Assam, India.

MAHANTA, P.K., and M. HAZARIKA. 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. *Journal of the Science of Food and Agriculture* 36:1133-1139.

MALEC, L.S., and M.S. VIGO. 1988. Seasonal variations in theaflavin, thearubign and caffeine contents of Argentinean black tea. *Journal of the Science of Food and Agriculture* 45:185-190.

MASHIMBYE, M.J., MUDAU, F.N., SOUNDY, P., and T. VAN REE. 2006. A new flavanol from bush tea. *South African Journal of Chemistry* 59:1-2.

McGAW, L.J., STEENKAMP, V., and J.N. ELOFF. 2007. Evaluation of Athrixia bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. *Journal of Earthnopharmacology* 110:16-22.

MOGOTLANE, I.D., MUDAU, F.N., MASHELA, P.W., and P. SOUNDY. 2007. Seasonal responses of total antioxidant content in cultivated bush tea leaves to fertilize rates. *Medicinal and Aromatic Plant Sciences and Biotechnology* 1:77-79.

MUDAU, F.N., SOUNDY, P., and E.S. Du TOIT. 2007c. Nitrogen, phosphorus and potassium nutrition on total polyphenols content of bush tea in a shaded nursery environment. *HortScience* 42:334-338.

MUDAU, F.N., SOUNDY, P., Du TOIT, E.S., and J. OLIVIER. 2006. Variation in polyphenolic content of bush tea leaves with seasonal and nitrogen application. *South African Journal of Botany* 71:398-402.

MUDAU, F.N., SOUNDY, P., and E.S. Du TOIT. 2005. Plant growth and development of bush tea as affected by nitrogen, phosphorus and potassium nutrition. *HortScience* 40:1898-1901.

OBANDA, M., and P.O. OWUOR. 1992. Effects of wither plucking on the biochemical and chemical parameters of selected Kenya tea. *Discovery and Innovation* 6:190-197.

OBANDA, M., OWUOR, P. O., and R. MANGOKA. 2001. Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chemistry* 75:395-404.

OWUOR, P.O., and I. McDOWELL. 1994. Changes in theaflavin composition and astringency during black tea fermentation. *Food Chemistry* 51:251-254.

OWUOR, P. O., and M. OBANDA. 1998. The changes in black tea quality due to variations of plucking standard and fermentation time. *Food Chemistry* 61:435-441.

OWUOR, P.O., and M. OBANDA. 2001. Tea: Influence of fermentation conditions and duration on the quality of black tea. *Food Chemistry* 72:319-327.

OWOUR, P.O., NG'ETICH, K.W., and O. OBANDA. 2000. Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard. *Journal of the Science of Food and Agriculture* 80:439-446.

OWUOR, P.O., and S.G. REEVES. 1986. Optimising fermentation time in black tea manufacture. *Food Chemistry* 21:195-203.

RAJEEV, M., RAJAPPAN, S. T. and S. BALASUBRAMANIAN. 2000. Effect of oxygen addition during fermentation on CTC black tea quality: Plantation crops research and development in the new millennium. Hyderabad, India.

ROBERTS, M. 1990. Indigenous Healing Plants (first edition), Southern Book Publishers: Halfway House, South Africa.

ROBERTSON, A. 1983. Effects of physical and chemical condition on the *in vitro* oxidation of tea catechins. *Photochemistry* 22:89-96.

SARKAR, A., and A. BHADURI. 2001. Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: Comparison with its individual catechin constituents and green tea. *Biochemestry Biophysical Research Community* 284:173-178.

SERGEDIENE, E., JONSSON, K., SZYMUSIAK, H., TYRAKOWSKA, B., RIETJENS, Y.M.C.M., and N. CENAS. 1999. Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure-activity relationship. *Federation of Biochemical Societies Letters* 462:392-396.

STATISTIX INSTITUTE Inc. 1985-2003. Statistix Analytical Software. New version Statistix 8, New York.

SUBRAMANIAN, N., VENKATESH, P., GANGULI, S., and V.P. SINKER. 1999. Role of polyphenol oxidase and peroxidase in generation of black tea theaflavins. *Journal of Agricultural Food Chemistry* 47:2571-2578.

SUD, R.G., and A. BARU. 2000. Seasonal variation in the flavins, total colour and brightness of Kangra orthodox tea [*Camellia sinensis (L.)* O Kuntze] in Himachal pradesh. *Journal of the Science of Food and Agriculture* 80:1291-1299.

TAYLOR, S., BAKER, D., OWUOR, P., ORCHARD, J., OTHIENO, C., and C. GAY. 1992. A model for predicting black tea quality from the carotenoid and chlorophyll composition of fresh green tea leaf. *Journal of the Science of Food and Agriculture* 58:185-191.

TOIT, J.D., and E. JOUBERT.1998. The effect of pretreatment on the fermentation of honeybush tea. *Journal of the Science of Food and Agriculture* 76:537-545.

TOIT, J.D., and E. JOUBERT.1999. Optimization of the fermentation parameters of honeybush tea. *Journal of Food Quality* 22:241-256.

VENDEMIALE, G., GRATTAGLIANO, E., and E. ALTOMARE. 1999. An update on the role of free radicals and antioxidant defense in human disease. *International Journal of Laboratory Research* 29:49-55.

WATERMAN, P., and S. MOLE. 1994. Analysis of phenolic plant metabolites. Blackwell Scientific: London.

WANG, Z.Y., KHAN, W.A., and D.R. BICKERS. 1989. Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis* 10:411-415.

WEIL, A. 2002. Tea: Red, Green or Black? Updated March 21, 2005. http://www.drweil.com/drw/u/id/QAA82788.

APPENDICES

Appendix A.1 Effect of different fermentation temperature on the quality of bush tea

A.1.1 Analysis of variance (ANOVA) for polyphenols

SOURCE	DF	SS	MS	F	P
BETWEEN	4	4.67611	1.16903	16.4	0.0002
WITHIN	10	0.71287	0.07129		
TOTAL	14	5.38897			

A.1.2 LSD (T) comparison of means of polyphenols by treatment

TREATMENTS	MEAN	HOMOGENEOUS GROUP
30	5.0400	a
38	4.1400	B
34	4.0633	B
42	3.7000	Bc
24: control	3.3800	C

CRITICAL T VALUE	2.228	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	0.4857		
STANDARD ERROR FOR COMPARISON	0.2180		

A.1.3 Analysis of variance (ANOVA) for tannins

SOURCE	DF	SS	MS	F	P
BETWEEN	4	0.99989	0.24997	5.30	0.0149
WITHIN	10	0.47147	0.04715		
TOTAL	14	1.47136			

A.1.4 LSD (T) comparison of means of tannins by treatment

TREATMENTS	MEAN	HOMOGENEOUS GROUP
24: control	0.8867	A
30	0.7667	A
34	0.7233	A
42	0.2800	B
38	0.2733	B

CRITICAL T VALUE	2.228	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	0.3950		
STANDARD ERROR FOR COMPARISON	0.1773		

A.1.5 Analysis of variance (ANOVA) for antioxidants

SOURCE	DF	SS	MS	F	P
BETWEEN	4	0.28117	0.07029	0.82	0.5400
WITHIN	10	0.85520	0.08552		
TOTAL	14	1.13637			

A.1.6 LSD (T) comparison of means of antioxidant by treatment

TREATMENTS	MEAN	HOMOGENEOUS GROUP
34	8.3100	A
30	8.2900	A
24: control	8.2767	A
42	8.2533	A
38	7.9433	A

CRITICAL T VALUE	2.228	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	0.5320		
STANDARD ERROR FOR COMPARISON	0.2388		

APPENDIX A.2 Effect of different fermentation time/period on the quality of bush tea

A.2.1 Analysis of variance (ANOVA) for polyphenols

SOURCE	DF	SS	MS	F	P
BETWEEN	3	2.54170	0.84723	9.18	0.0057
WITHIN	8	0.73840	0.09230		
TOTAL	11	3.28010			

A.2.2 LSD (T) comparison of means of polyphenols by treatment

TREATMENTS	MEAN	HOMOGENEOUS GROUP
60	4.4333	A
90	4.4133	A
120	3.6733	B
0: control	3.3800	B

CRITICAL T VALUE	2.306	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	0.5720		
STANDARD ERROR FOR COMPARISON	0.2481		

A.2.3 Analysis of variance (ANOVA) for tannins

SOURCE	DF	SS	MS	F	P
BETWEEN	3	2.99000	0.99667	4.29	0.0443
WITHIN	8	1.86047	0.23256		
TOTAL	11	4.85047			

A.2.4 LSD (T) comparison of means of tannins by treatment

TREATMENTS	MEAN	HOMOGENEOUS GROUP
90	2.1467	A
120	1.2933	Ab
60	0.9667	B
0: control	0.8867	B

CRITICAL T VALUE	2.306	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	0.9080		
STANDARD ERROR FOR COMPARISON	0.3937		

A.2.5 Analysis of variance (ANOVA) for antioxidants

SOURCE	DF	SS	MS	F	P
BETWEEN	3	0.12549	0.04183	0.46	0.7162
WITHIN	8	0.72340	0.09042		
TOTAL	11	0.84889			

A.2.6 LSD (T) comparison of means of antioxidant by treatment

TREATMENTS	MEAN	HOMOGENEOUS GROUP
120	8.5633	A
60	8.4100	A
90	8.3867	A
0: control	8.2767	A

CRITICAL T VALUE	2.306	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	0.5662		
STANDARD ERROR FOR COMPARISON	0.2455		