EFFECTS OF BREWING TEMPERATURE AND DURATION ON QUALITY OF BLACK TEA (*Camellia sinensis*) AND EQUAL (50:50) COMBINATION OF BUSH TEA (*Athrixia phylicoides* DC.) AND BLACK TEA

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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

Black tea (*Camellia sinensis)* and black tea combined with bush tea *(Athrixia phylicoides*) were analyzed for their polyphenol content, antioxidant activity and tannin content. Solvent extraction methods were used for extracting polyphenols, antioxidant activity and tannin content. Total phenols were determined using the Folin-Ciocalteu method, antioxidant activity using Trolox Equivalent Antioxidant Capacity (TEAC) assay and tannin content using vanillin-HCl method. Polyphenol content, antioxidant activity and tannin content were calculated using regression equation Y=MX+C, where C=intercept, Y= Absorbance of the sample and M= Gradient**.**

Results of black tea in this study showed that total polyphenols, antioxidant activity and tannin content decreased with decrease in temperature and time and this was attributed to the release of polysaccharides at 90°C. At 90°C for 3 minutes 7.68mg/100g of total polyphenol, 3.85µmol/g of antioxidants and 2.81mg/100g of tannin were obtained and this amount decreased to 5.50mg/100mg for total polyphenols, 1.31µmol/g for antioxidant activity and 0.72mg/100mg for tannin content at 30°C for 10 minutes.

Combining the two tea types (50:50) resulted in a significant ($p<0.005$) decrease in total polyphenols, antioxidant and tannin content. Results of this study showed that at high temperature of 90°C for 3 minutes 2.64mg/100g for total polyphenol, 2.48µmol/g for antioxidants and 1.8mg/100g for tannin were obtained and these amounts decreased to 1.39mg/100mg for total polyphenols, 0.35µmol/g for antioxidant activity and 0.64mg/100mg for tannin content at 30°C for 10 minutes. Thus high brewing temperature causes increase in total polyphenols, antioxidant activity and tannin content of the combined teas. This study showed that black tea and combined black tea and bush tea solutions had large TEAC radical scavenging activity which can be related to total polyphenols and catechins. The antioxidant activity of the tea solution increased with increase in extraction

temperature and duration of soaking. It is recommended that 3-min soaking temperature of 90 °C of black tea and combined bush tea and black tea can be used due to the highest total polyphenols and antioxidant activity obtained.

CHAPTER 1

INTRODUCTION

It is of great interest to the general public, medicinal and nutritional experts, and health and food science researchers to know the antioxidant capacity and constituents in the food we consume (Bonoli, Verardo, Marconi and Caboni, 2004). Health advantages of diets rich in antioxidants include lowering the risk of cardiovascular diseases, certain cancers and natural degeneration of the body associated with the aging process (Miyachi, 1995). Bush tea (*Athrixia phylicoides* DC.) and black tea (*Camellia sinensis*) are beverages rich in antioxidants, which make them excellent sources for increased health benefits (Turkmen, Velioglu, Sari and Polat, 2007; Mudau, Hitsa, Araya, Du Toit, Soundy, and Olivier, 2007). Black tea is one of the most commonly consumed beverages in the world for its desirable aroma, taste and putative positive physiological functions**.** It is the most widely used tea in making iced tea and English tea. New taste and flavors have been created by blending black tea with chrysanthemums, plums, ginger, hibiscus or lemon (Gupta, Saha and Giri, 2002).

Bush tea is an indigenous tea of South Africa predominantly used for many years for treating boils, cleansing or purifying blood, bad acne, infected wounds and cuts, skin eruption, and for bathing (Roberts, 1990). There are 14 species in the genus *Athrixia*, nine of which are found in South Africa (Leistner, 2000). Bush tea contains 5-hydroxy-6,7,8,3',4,'5,' hexamethoxy flavonoid (Mashimbye, Mudau, Soundy and Van Ree, 2007) with no caffeine content or pyrrolizidine (McGaw, Steekamp and Eloff, 2007). The stems of bush tea are also tied up in bundles for brooms and traded on a small scale in Limpopo Province (Van Wyk and Gerike, 2000).

The usage of *Athrixia* tea has declined over time with the availability of commercially produced teas but the plant is considered to have economic

potential as herbal infusion (Mudau, Soundy and du Toit, 2007). According to research done, 68 of the 92 people in rural places in Wolkberg region of the Limpopo Province still consume the tea, only 8% used it for medicinal properties (McGaw *et al.,* 2007). In urban surveys conducted in Soweto, Mamelodi and Marabastad (Gauteng Province) 83 out of 150 people who use the plant indicated that they would buy it if it were available for purchase in stores (McGaw *et al.*, 2007). Black tea is the most important source of polyphenols, since it accounts for almost 80% of the world's tea production and contain polyphenols in high concentration (Lakenbrink, Lapczynski, Maiwald, and Engelhardt, 2000). Black tea polyphenols are produced by complex oxidation during fermentation, epigallocatechingallate (EGCG) is converted to theaflavin and thearubigin during fermentation. These polyphenols have been shown to possess a wide range of biological and pharmaceutical benefits including cancer prevention, such as intestinal and breast cancer (Celestino and Augustin, 2000).

Black tea is generally stronger in flavour and contains more caffeine than the less oxidized teas. The major chemicals found in black tea are flavonoids, a natural source of antioxidants, which are found in many naturally derived foods. Antioxidants rid the body of molecules called free radicals, which are side products of damage done to the body by pollution and the natural aging process. Free radicals in the body's cells are very unstable and tend to react negatively with other molecules like DNA causing malfunctions at cellular level (Celestino *et al*., 2000). The destruction of these molecules paves the way for diseases like heart diseases and cancer. In the case of heart diseases, antioxidants in tea may prevent death from heart attack by helping blood vessels relax, thereby allowing blood to flow through more easily, potentially lowering blood pressure and reducing stress of the heart (Celestino *et al*., 2000).

Due to the complexity of the composition of foods, separating each antioxidant compound and studying it individually is costly and inefficient. Determinations of polyphenols and antioxidants have challenged scientists for years. The challenge

that lies before scientists is that the number of methods and variation of methods for measuring antioxidant capacity are numerous (Prior, Wu and Schaick, 2005), thus making it difficult to have consistent conclusive results. There are various ways consumers brew their tea. The main difference is in brewing time and temperature. Studying the influence of brewing time on polyphenols and antioxidants content of tea can contribute to the information on how to utilize the product effectively. Data that describe the synergistic influence of black and bush tea has not yet been established.

1.1 The objectives of this study were to:

1. Investigate phenolic compounds and antioxidant activity of black tea under various brewing temperatures and duration.

2. Determine the effects of soaking conditions on polyphenol content, antioxidant activity and tannin content of equal (50:50) combination of black tea and bush tea.

1.2 Hypotheses

1. Brewing temperatures and duration of time do not influence phenolic compounds and antioxidant activity in black tea.

2. Soaking conditions have no effect on polyphenol content and antioxidant activity of equal (50:50) combination of black tea and bush tea.

CHAPTER 2

LITERATURE REVIEW

2.1 Antioxidants, phenolic compounds and free radicals

Phenols are presumed to be responsible for the beneficial effects derived from the consumption of fruits, vegetables and teas (Han, Shen and Hongxiang, 2007). Phenolic compounds have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reaction and chelate metals (Han *et al*., 2007). Moreover, consumption of phenolics has been correlated with reduced risk of cardiovascular diseases and certain cancer. Tea is rich in flavonoids and other polyphenols that have been shown to posses a wide range of biological and pharmaceutical benefits (Buschman, 1998).

According to Mavundza, Tshikalange, Meyer and Mudau (2007), bush tea ethanol extract has a strong antioxidant activity *in vitro*; inhibition of DPPH was shown to be 81.6% when lowest concentration was used to test antioxidant activity. Bush tea also has inhibitory effects against micro-organisms such as *Staphylococcus aureus*, *Bacillus cereus*, *Enterecoccus* and *Esherichia coli* and *Mycobacterium smegmatis.* Bush tea has strong inhibitory effects against gram positive bacteria than gram negative bacteria.

Numerous antioxidants are plant based and play a fundamental role in protecting plants that are open to elements such as sunlight and severe oxygen stress (Han *et al*., 2007). It has been suggested that antioxidants may amend cellular oxidative status and prevent biologically significant molecules such as DNA, proteins and membrane lipid from oxidative damage and as a result lessen the risk of several chronic disease including cancer and cardiovascular disease (Zhou and Liangli, 2004). A sufficient ingestion of natural antioxidants in food is

therefore of great consequences for the defense of macromolecules against oxidative damage (Halliwell and Gutterdge, 1999; Wallace, 1997). According to Stratil, Klejdus and Kuban (2006), cells most frequently damaged by oxidative stress are unsaturated fatty acids in lipids, cholesterol, different functional polypeptide, proteins and nucleic acids. Mechanism of antioxidant consists of transition metal chelating, reducing peroxide, and stimulation of *in vivo* antioxidative enzyme activities (Zhou *et al*., 2004). In living systems, antioxidants may elevate the level of endogenous defense. The action of antioxidant in food and biological system is reliant on the system, composition, interfacial phenomena, and partitioning properties of the antioxidants between lipids and aqueous phase (Diaz-Reinoso, Moure, Dominguez and Parajo, 2006).

Bulger and Helton (1998) defined oxidative stress as the state in which the level of toxic reactive oxygen intermediate (ROI) overcomes the endogenous antioxidant defense of its host. This results in surplus of free radicals, which can react with cellular lipids, proteins, and nucleic acids leading to damage and eventual organ dysfunction. Lipids are the most exposed biomolecules to a free radical attack. Free radicals play a vital role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by neutrophil granulocytes (Bulger *et al*., 1998). Free radicals have also been implicated in certain cell signaling processes. The peroxyl radical is the most common radical in human biology, but the hydroxyl radical singlet oxygen, superoxide radical and reactive nitrogen species are all present in biological system (Wu, Beecher, Holden, Haytowitz, Gebhardt and Prior, 2004). They result from molecular oxygen under reducing conditions because of their reactivity, and this same free radical can have a role in unwanted side reactions causing cell damage. A variety of aging symptoms such as atherosclerosis are credited for free radical-induced oxidation of numerous chemicals. Free radicals are essential for life, the body has a number of mechanisms to reduce free radical induced damage and restore damage that does occur, such as the enzymes, superoxide

dismutase, catalases glutathione peroxidase and glutathione reductase (Wu *et al.,* 2004).

According to the Mitochondrial Free Radical Theory of Aging (1999), free radicals are a class of molecules with a very simple definition. The nature of atomic structure and of the covalent chemical bond (the feature that gives an atom its valence) are fixed by the rule that electrons occupy orbital's of atoms, such that an orbital can contain zero, one or two electrons and the electrons carry less energy when they are one of a pair in an orbital than when they are unpaired. A molecule is only a free radical if it posses one or more unpaired electrons (Figure 1).

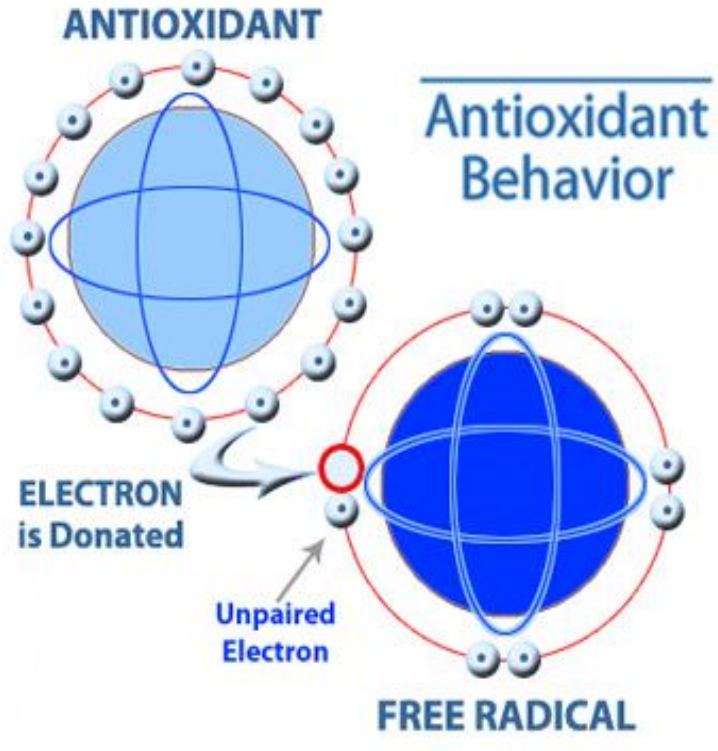

Figure 1**:** Free Radical Molecule (www.biomatrixone.com)

2.2 Green tea and black tea health benefits

Antioxidants: Green tea polyphenols have attracted much attention because of their relatively high antioxidant activity (Han *et al*., 2007). Antioxidants are compounds that interact with harmful molecules in the body and may enhance the body's defense mechanisms against diseases like cancer and coronary heart disease (Han *et al*., 2007). The reactive oxygen species (ROSs) including oxygen ions, free radicals and peroxide are the main causes of much pathology. Their reactivity is due to the presence of unpaired valence shell electrons. Reactive oxygen species are formed as a natural by-product of the normal metabolism of oxygen and have important roles in cell signaling (Cotelle, Bernier, Catteau, Pommery, Wallet and Gaydou, 1996). However, during times of environmental stress, reactive oxygen species levels can increase dramatically, which can result in significant damage to cell structures (Han *et al*., 2007). The reactive oxygen species scavenging activity of the flavonoids is imputable to the hydroxyl groups in the molecules (Cotelle *et al*., 1996). It has been observed that 2',3',4' hydroxyl substitution on the B ring plays a crucial role in radical scavenger activity in the DPPH radical assay and in the inhibitory effect on peroxidation of tissue lipids (Cotelle *et al*., 1996).

Protective effect on immune cell functions: Polyphenols appear to have a protective effect on immune cell functions. Alvarez, Alvarado, De la fuente, Jimenez, Puerto and Schlumberger (2006) showed that leukocyte functions were improved in prematurely aging mice after five weeks of diet supplementation with polyphenol-rich cereals.

Anti-diabetic effects: Johnston, Sharp, Clifford and Morgan (2005) demonstrated that glucose uptake into cells under sodium-dependent conditions was inhibited by flavonoid glycosides and non-glycosylated polyphenols in polarised caco-2 intestinal cells. Under sodium-free conditions, aglycones and non-glycosylated polyphenols inhibited glucose uptake whereas glycosides and phenolic acids were ineffective (Johnston *et al*., 2005). These results suggest that glycones

inhibit facilitated glucose uptake whereas glycosides inhibit the active transport of glucose. The non-glycosylated dietary polyphenols appeared to exert their effects via steric hindrance, while EGCG, ECG and epigallocatechin were effective against both transporters (Johnston *et al*., 2005). Kobayashi, Suzuki, Hideo, Arai, Yukihiko, Suzuki, Miyamoto and Shimizu (2000), have shown that the green tea polyphenols EGCG and ECG also inhibited glucose transport, possibly by sodium-dependent glucose transporter 1 (SGLT1) inhibition in the rabbit small intestine.

Anti-mutagenic/anti-carcinogenic properties: Polyphenols could modulate diverse biochemical processes involved in carcinogenesis inhibition of cellular proliferation and angiogenesis, blockage of tumor cell cycle progression, and induction of programmed cell (Han *et al*., 2007). Consumption of food/beverages rich in polyphenols can contribute to the reduction of cancer through many mechanisms.

2.3 Health benefits reputed for bush tea

Bush tea (*Athrixia phylicoides DC*.) is an indigenous plant of South Africa. It has been used for many years by traditional people for various treatments of diseases. A decoction of leaves and twigs is widely used as “tea’’ by local communities. Root decoctions of this species are also taken as purgatives and cough remedies (Watt and Breyer-Brandwijk, 1962). Plant infusions are used by the Zulus as blood purifiers and to treat sores and boils (Mudau *et al*., 2007). The Vhavenda people drink extracts made from leaves and roots as anthelminthics (Mabogo, 1990) and for aphrodisiac properties (Van *et al*., 2000). It is chewed for sore throats and coughs by the Sotho and the Xhosa peoples. The health benefits of consuming bush tea could be related to its polyphenol content. The major phenolics in the bush tea are hydroxycinnamic acids, recently a unique methoyxylated flavonol (5-hydroxy-6,7,8,3',4,5'-hexamethoxy-flavonol) with no caffeine content or pyrrolizidine was isolated from bush tea (Mashimbye *et al*., 2006). Bush tea phenolic compounds and tannin shows seasonal variation with

fertilizer application. According to Mavundza *et al.,* (2007), bush tea contains the highest concentration of tannins in autumn and in summer.

Tannin content in tea leaves is the main potential indicator of medicinal potential due to their anti oxidant activities (Hirasawa, Takada, Makimura and otake, 2002). Tannins help to prevent cancers and heart problems by lowering the tendency of blood platelets to stick together (Stensveld, Tversdal and Solvoll, 1992). McGaw *et al*., (2007) found no detectable levels of pyrrolizidine alkaloids, an important group of plant toxins with serious health risk to humans and animals, in two separate *Athrixia phylicoides* samples analyzed. They also found that aqueous extracts of *Athrixia phylicoides* were not toxic to brine shrimp and renal cell line. In another study done by Chellan, De Beer, Muller, Joubert and Louw (2008), it was demonstrated that aqueous extract of *Athrixia phylicoides* is non-toxic to mammalian cells when consumed in high doses. Surveys have shown that the consumption of bush tea is wide spread, and commercialization of the extract holds economic and developmental potential (McGaw *et al*., 2007).

2.4 Effect of high water temperature on tea quality

Historically, it has been the tradition of Chinese tea connoisseurs to place special emphasis on infusion temperature. Too high water temperature has the undesirable effect of ‘overcooking’ the ‘tea green’ in confinement, resulting in a yellowish and cloudy infusion which is much bitter in taste. Its substantial vitamin content could easily be destroyed. In China, oolong tea is usually prepared by soaking the tea in hot water (> 80°C) using a covered ceramic pot (Gong and Gu, 2001). This is followed by stirring and steeping procedure using appropriate time and temperature that are critical to extract catechins and theaflavins from teas (Astill, Birch, Dacombe, Humphrey and Martin, 2001; Khokhar and Magnusdotirr, 2002).

Polyphenols: Although tea offers much more benefits, tea preparation and processing can decrease polyphenols if proper steps are not followed, while enzymatic oxidation can result in degradation of important polyphenols and high water temperature can reduce polyphenol content (Polydera, Stoforos and Taoukis, 2005). Su, Duan, Jiang, Duan and Chen (2007) reported that higher brewing temperature for longer time (100°C for 10 minutes) cause decreases in phenolic content of oolong tea.

Antioxidants: Antioxidants are a group of chemicals capable of extending the shelf life of food that contain lipid (Madhavi, Deshpande and Salunkhe, 1996). It is known that antioxidants inhibit lipid peroxidation by their radical scavenging activity (Bloknina, Virolainene, Fargersted, 2003). They retard oxidation of lipids by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers (Shahidi and Wanasundara, 1992). Su *et al.* (2007) reported that higher temperature and longer time (100°C for 10 minutes) brewing time causes reduction in antioxidant activity of oolong tea.

Individual polyphenols: The major components of green and black tea polyphenols are: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), theaflavin and thearubigin (Han *et al*., 2007). In a study done by Su, Duan, Jiang, Shi and Kakudi (2006), they noted that the contents of EGCG and EGC significantly decreased at higher temperature and longer steeping duration.

2.5 Methods for the determination of total phenols, tannins and antioxidant activity

2.5.1 Determination of total phenols

The Folin-Ciocalteu method is a rapid widely used method for the determination of total phenolic content (Waterman and Mole, 1994). The method is simple and provides reasonably good and reliable estimates of concentration of total

reducing phenolic groups. The method is based on the reducing power of the phenolic hydroxyl groups (Hahn, Rooney and Earp, 1984), which react with Folin-Ciocalteu phenol reagent (an oxidizing agent comprised of heteropolyphosphotungstate-molybdate) under basic conditions to form chromogens that can be detected spectrophotometrically at 760 nm.

2.5.2 Determination of tannins

The widely used assay for tannins is the vanillin-HCl method (Prince, Van Scoyoc and Butler, 1978) and is specific for condensed tannins. This method was used to analyze tannin since the tea was extracted using methanol and is specific for catechins and proanthocyanididns. The method is based on the ability of the condensed tannin units to react with the vanillin reagent in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. The vanillin reagent reacts with flavanols with a single bond between C-2 and C-3 of the C ring (Earp, Akingbala, Ring and Rooney, 1981), and also with the free meta-oriented OH groups on the B ring, and since condensed tannins are condensation products of flavan-3-ols and flavan-3,4-diols, they give a positive reaction with vanillin (Gupta and Haslam, 1980). A blank subtraction is done to correct for non-tannin compounds that give a positive vanillin reaction.

2.5.3 Determination of antioxidant activity

Much attention however is being focused on determination of antioxidant capacity of compounds using the TEAC assay (Van den Berg, Haenen, Van den Berg and Bast, 2000). The method is preferred for its simplicity and speed of analysis. It can be used over a wide pH range and can be used to study effects of pH on antioxidant mechanism (Lemaska, Szymusiak, Tyrakowska, Zielinski, Soffer and Rietjens, 2001). Moreover, the ABTS radical is soluble in both aqueous and organic solvents and is not affected by ionic strength, thus it can be used in multimedia to determine both hydrophilic and lipophillic antioxidant capacities of extracts and body fluids.

The assay measures the relative ability of an antioxidant to scavenge the 2,2'-azinobis (3-ethyl-benzothiazoline-6 sulfonic acid) radical cation ($ABTS^{\bullet+}$) generated in aqueous phase, as compared with Trolox (water soluble vitamin E analogue) standard. Reacting a strong oxidizing agent such as potassium permanganate or potassium persulfate with the ABTS salt generates the $ABTS^{\bullet+}$. The extent of the decolorization of the blue-green $ABTS^{\bullet+}$ radical cation by hydrogen donating antioxidant is measured spectrophotometrically at 734 nm. The assay is applicable for assessing antioxidant capacity of single compounds, food components, and food extracts as well as biological systems (Van den Berg *et al*., 2000), and is applicable to both aqueous and lipophilic systems (Arnao, Cano & Acosta, 2001).

CHAPTER 3

DETERMINATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF BLACK TEA BREWED AT DIFFERENT TEMPERATURES AND DURATION OF TIME

3.1 Introduction

Potential health benefits of tea, together with its popularity as a beverage, have prompted numerous investigation on the chemical constituents of tea and their biological properties (Gupta *et al.,* 2002), such as antimutagenic (Halder, Pramanick, Mukhopadhyoy and Giri, 2005), anticarcinogenic and antioxidant (Han, 1997; Sarkar and Bhaduri, 2001), antiallergic (Maeda, Ngai, Suzuki, Ema, Kanda and Mitsuda, 2005) and, antibacterial activities.

Tea is rich in flavonoids that can be divided into anthocyanins and anthoxanthins. Anthocyanins are glycosylated derivatives of anthoxanthins most commonly found in tea and fruits. Anthoxanthins include flavones, flavans, flavonols and isoflavones. Depending on manufacturing process teas can be classified into three major groups: non-fermented green tea, semi-fermented oolong tea and fermented black tea (Zuo, Chen and Deng, 2002). Black tea is made from the same leaves as those of green tea whereas black tea goes under full oxidation (Gupta *et al*., 2002). The brew colour and sensory properties of black tea are generated during the manufacturing process, during which the colourless (gallo) catechins present in fresh tea leaves are oxidized both enzymatically and non-enzymatically to give two major groups of pigments: theaflavins and thearubigins (Gupta *et al*., 2002). Eventually in black tea 75% of the substrate flavan-3-ol may be converted to thearubigins, whereas 10% accounted for the formation of theaflavins and approximately 15% of flavanols would remain unchanged (Celestino *et al*., 2000). Thearubigins may account for 15-20% of the dry weight in black tea leaves and infusions (Celestino *et al*., 2000).

Although there have been some reports on polyphenols and antioxidant activities on different black teas from other regions, little or no information is reported on total polyphenols and antioxidant activities at various brewing temperatures and times. The total polyphenol amounts determined from the same tea and their corresponding antioxidant activities may vary widely depending on extraction conditions, such as time used during extraction and temperature. According to the research done by Turkmen *et al*., (2007), aqueous extract of black tea extracted for long time using aqueous acetone contained high levels of polyphenols and antioxidants activity whereas lower levels of polyhphenols and antioxidant were obtained when using absolute acetone as an extracting solvent for a short time. Furthermore, black tea also possessed strong antibacterial activity against *Staphylococcus aureus*. Su *et al*. (2007) reported a decrease in polyphenol and antioxidant activities at higher temperatures (100°C) and longer times (10 minutes). Therefore, the aim of this study was to determine the total polyphenol content and antioxidant activity of black tea at various brewing temperatures and times.

3.2 Materials and methods

Chemicals and materials: Black tea was obtained from Mukumbani Tea Estate (22°53'60S 30°25'0E 724 m.s.a.l). The tea was processed using standard commercial practices. Folin-Ciocalteau, methanol, sodium carbonate (anhydrous), potassium persulfate, 2,2'azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (Steinheim, Germany). Tannic acid was obtained from Saarchem-Holpro Analytical (Pty) Ltd (Johannesburg, South Africa) while vanillin and sodium dihydrogen orthophosphate (anhydrous) were obtained from Associative Chemicals Enterprise (Pty) Ltd (Johannesburg, South Africa). Deionized water was used in all analysis, and all other chemical and solvents were of analytical grade.

Sample preparations: Black tea was brewed using water bath (Labotec, Johannesburg, South Africa) at different temperatures and duration. An amount of 6 g of black tea was measured and poured into 1000 ml beaker and 600 ml of distilled water was added. Tea was then brewed at 30°C for 3 minutes, 30°C for 10 minutes, 60°C for 3 minutes, 60°C for 10 minutes, 90°C for 3 minutes and 90°C for 10 minutes. Brewed tea was then dried in an oven at 80°C until thoroughly dried. Dried tea was packaged and stored for one week at -1°C in a refrigerator.

Determination of total polyphenol content: Methanol was used as the extraction solvent for the determination of total phenols. Duplicate of 2 g of black tea were extracted using 30 ml of the solvent as follows:

An amount of 10 ml of methanol was added to 2 g of sample in centrifuge tubes and the samples were vortex mixed every 10 minutes for 2 hours to improve extraction efficiency. The samples were then centrifuged at 3 500 rpm for 10 minutes (25°C) using centrifuged tubes and decanted. Each sample residue was rinsed twice with 10 ml of solvent, vortex mixed for 5 minutes, centrifuged as above, and decanted. The two supernatants were combined and used for analysis. The Folin Ciocalteau method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total phenols in the black tea extract. This method was based on the reducing power of phenolic hydroxyl groups (Hahn *et al*., 1984) which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, methanol extract (0.5 ml) was added to a 50 ml volumetric flask containing distilled water and mixed. Folin Ciocalteau phenol reagent (2.5 ml) was then added and mixed, followed by 7.5 ml sodium carbonate solution (20 g/100 ml) within one to eight minutes after addition of the Folin Ciocalteau phenol reagent. The contents were mixed and the flask made up to volume with distilled water, stoppered and thoroughly mixed. Absorbance of the reactants was read after 2 hours at 760 nm using UV-visible genesys 20 Spectrophotometer. Catechin was used as standard

to prepare a standard curve and results were expressed as mg equivalents/100 mg of samples on a dry weight basis.

Determination of Tannins: The Vanillin HCL method of Prince *et al*. (1978) was used for the determination of tannins. This method is based on the ability of flavoids to react with vanillin in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. The extracts and reagents were maintained at 30°C in a thermostat-controlled water bath before mixing the reactants. The methanolic extract (1 ml) was added to 5 ml vanillin reagent (4% HCl in methanol and 0.5 ml vanillin in methanol) and mixed. Sample blanks were done with 4% HCl in methanol replacing vanillin reagent. The reactants were maintained at 30°C and absorbance read at 500 nm after 20 minutes. Absorbance reading of the blanks was subtracted from those of the samples. Catechin was used as a standard and results were expressed as mg catechin equivalents/100 mg sample on a dry weight basis.

Determination of antioxidant activity: Antioxidant activity of the extracts was determined using Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika and Rooney (2004). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the $ABTS^+$ radical cation chromogen in relation to that of Trolox, the water soluble vitamin E analogue which is used as an antioxidant standard. The $ABTS^+$ was produced by mixing equal volume of 8 mM ABTS with 3 mM potassium persulfate prepared in distilled water and allowed to react in the dark for at least 12 hours at room temperature before use. The $ABTS^+$ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH_2PO_4, 0.2 M $NaHPO_4$ and 150 mM NaCl in 1 litre of distilled water, with pH adjustment using NaOH where necessary. This solution was made fresh for each analysis. The $ABTS^+$ solution (2900 µl) was added to the methanol extracts of tea (100 µl) of Trolox in a test tube and mixed. Absorbances reading were done at 734 nm and were taken after 30 minutes (for the samples) and 15 minutes (for the standard)

of the initial mixing of the samples and standard respectively. The results were expressed as μM Trolox equivalents/g of sample on a dry weight basis.

3.3 Statistical Analysis

The experiment was replicated three times and all measurements were done in duplicate. Data were subjected to analysis of variance (ANOVA), computed using SAS (2003) General Linear Model procedure, and the Least Significant Difference test was used to identify differences among the means.

3.4 Results and Discussion

Yield of methanol extract from black tea: Tea powder, extraction yield and concentration of black tea are shown in Table 1.1. The amount of tea powder extracted increased from 1.88 g to 2.40 g with increasing temperature and time. At 30°C for 3 minutes 1.88 g was obtained which was not significantly different from 1.97 g and 1.98 g of tea boiled for 10 minutes and tea boiled for 3 minutes at 60°C. At 60°C for 10 minutes 2.20 g was obtained which is not significantly different from 2.31 g of tea boiled at 90°C for 10 minutes. Tea powder was highest at 90°C for 3 minutes.

Extraction yield ranged from 31.41% and 32.91% at 30°C for 3 minutes and 10 minutes, but the two values were not significantly different from each other. It increased to 32.93% and 36.66% at 60°C for 3 and 10 minutes which were significantly different from each other. It further increased to 37.91% at 90°C for 3 minutes which was significantly different from 40.66% obtained at 90°C for 10 minutes. The latter was significantly higher than the rest.

Brewing tea at 90°C for 10 minutes also achieved the highest tea concentration of 14.64 mg/ml while brewing at 30°C for 3 minutes achieved lowest concentration of 11.31 mg/ml (Table 1.1). At 30°C for 3 minutes 11.31 mg/ml was obtained which was significantly different from 11.85 mg/ml obtained for tea

boiled for 10 minutes. At 60°C for 3 minutes it increased to 11.88 mg/ml and 13.20 mg/ml which were significantly different from each other. The increase in tea powder can be attributed to high molecular compounds found in black tea. Ni, Xie, Song and Yu (2003) reported that soaking tea at higher temperature increased the extraction yield of tea polysaccharides and other soluble solids.

Table 1.1 Yield of methanol extract from black tea at different soaking conditions

Tea solution	Treatments	Powder (g)	Yield extract (%)	Concentration (mg/ml)
1	30°C 3 min	1.88^{c}	31.41^{d}	11.31^{f}
2	10 min	1.97^{c}	32.91^{d}	11.85^{e}
3	60°C 3 min	1.98^{c}	32.93^{d}	11.88^{d}
4	10 min	2.20^{b}	36.66^{c}	13.20^{c}
5	90 °C 3 min	2.31^{ab}	37.91^{b}	13.65^{b}
6	10 min	2.40^{a}	40.66^{a}	14.65^{a}
Standard error		0.042	0.274	0.005

Means within each column followed by different letters are significantly different ($P<0.005$).

Effects of soaking conditions on total polyphenol content of black tea: Total polyphenols decreased with decreasing temperature and at each temperature, declined with an increase in time (Table 1.2). Brewing black tea at 90°C for 3 minutes achieved 7.68 mg/100 g total polyphenol and it decreased to 5.50 mg/100 g at 30°C for 10 minutes. The results indicated that at different temperatures and duration there were different amounts of tea polyphenols obtained, and tea boiled at 90°C for 3 minutes had significantly ($p<0.05$) higher amount of polyphenols than tea boiled for 10 minutes at the same temperature. Polyphenols continued to decrease with temperature and duration of time. At 60°C for 3 minutes 6.81 mg/100 g of polyphenol was achieved which were significantly different from 6.60 mg/100 g obtained when tea was boiled for 10

minutes at the same temperature. The amount of polyphenols further decreased to 5.60 mg/100 g which was obtained from tea boiled at 30°C for 10 minutes and this amount was significantly different from 5.50 mg/100 g which is the lowest amount of polyphenol obtained in tea boiled for 10 minutes at same temperature. The increased temperature assisted to hydrolyze bonds of phenolic compounds and free them to become extractable phenolic compounds. Folin Ciocalteau method is based on reducing power of hydroxyl group (Hahn *et al*., 1984). Therefore, the greater the level of phenolic hydroxyl groups the higher the level of total phenols assayed using Folin Ciocalteau method. Similar findings where reported by Su *et al.* (2007), when oolong tea was soaked at different temperatures and durations. These results suggest that brewing tea at 90°C for 3 minutes produced the best tea high in polyphenols which are the main potential indicator of tea quality.

Table 1.2 Extraction yields of total polyphenols, antioxidants activity and tannin content

Treatments		Total polyphenols (mg/100g)	Antioxidant activity (µmol/g)	Tannin (mg/100mg)
1	90°C 3 min	7.68[a]	3.85[a]	2.81[a]
2	10 min	7.45[b]	3.54[b]	2.79[a]
3	60°C 3 min	6.81[c]	2.48[c]	1.45[b]
4	10 min	6.60[d]	2.42[c]	1.32[c]
5	30°C 3 min	5.60[e]	1.32[d]	0.81[d]
6	10 min	5.50[f]	1.31[d]	0.72[e]
Standard error		0.014	0.022	0.015

Means within each column followed by different letters are significantly different ($P<0.005$)

Effects of soaking conditions on total antioxidant activity of black tea: Results in Table 1.2 show that antioxidant activity decreased with decrease in temperature and duration. Tea boiled at 90°C for 3 minutes had significantly ($P<0.05$) stronger TEAC radical scavenging activity of 3.85 µmol/g than 3.54 µmol/g obtained from tea boiled for 10 minutes at the same temperature. Tea boiled at 60°C for 3 and 10 minutes contained 2.48 µmol/g and 2.42 µmol/g antioxidant activity which were not significantly different from one another. The lowest TEAC antioxidant activity was obtained from tea boiled at 30°C for 3 (1.32 µmol/g) and 10 (1.31µmol/g) minutes which were not significantly different from each other. These results again suggest brewing tea at 90°C for 3 minutes gives optimum total antioxidant activity but that, the use of lukewarm water to brew black tea must be discouraged.

Effects of soaking conditions on total tannin content of black tea: Tannin content decreased with decreases in temperature and duration and at 90°C for 3 minutes highest amount of tannin 2.81 mg/100 g was obtained which was not significantly different from 2.79 mg/100 g obtained from tea boiled for 10 minutes at the same temperature. Tannin content decreased to 1.45 mg/ml at 60°C for 3 minutes and this amount was significantly different from 1.32 mg/ml obtained for tea boiled for 10 minutes at the same temperature. The lowest amount of tannin of (0.72 mg/100 g) which was obtained at tea boiled at 30°C for 10 minutes and was significantly different from 0.81 mg/ml obtained at tea boiled for 3 minutes. Strong TEAC radical scavenging activity and high polyphenol content in black tea was associated with presence of tannin at 90°C for 3 minutes.

In conclusion, black tea boiled at 90°C for 3 minutes contained higher levels of polyphenols and antioxidants. However, tannin content was not significantly affected at 90°C for either 3 minutes or 10 minutes. Thus brewing tea at high temperature increases polyphenols, antioxidant activity and tannin content in black tea. This study showed that black tea solutions had large amount of polyphenol and strong TEAC radical scavenging activity. The antioxidant activity

of the tea solution increased with an increase in extraction temperature and duration. It is recommended that 3-min brewing temperature of black tea at 90 °C can be used due to the highest total polyphenols and antioxidant activity. This tea will offer more health benefits to consumers as compared to other teas brewed at lower temperatures.

CHAPTER 4

EFFECTS OF SOAKING CONDITIONS ON POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF EQUAL (50:50) COMBINATION OF BUSH TEA AND BLACK TEA

4.1 Introduction

Phenolic compounds from plant sources have become a subject of interest for researchers due to their antioxidant properties. Phenols have many favorable effects on human health. They may reduce the incidence of cancer and heart disease by inhibiting oxidation of low density lipoprotein, boost the immune system, detoxify contaminants and pollutants, and reduce inflammation (Bonilla, Mayen, Merida and Medina, 1999).

The standard processing techniques used in China and South West Asia for oolong tea is to soak tea in hot water (>90 °C) using a covered ceramic pot (Gong *et al*., 2001) carefully followed by stirring and steeping conditions for a few minutes (Su *et al*., 2006; Su *et al*., 2007) in order to avoid extraction of catechin or theaflavins from tea extracts. Too high water temperature has the undesirable effect of 'overcooking' the 'tea in confinement, resulting in a yellowish and cloudy infusion which is much bitter in taste. Its substantial vitamin content could easily be destroyed. Zhu, Hackman, Ensuansa, Holt and Keen (2002) reported that 100 g of extract of dry oolong tea using boiling water (1L) had greater antioxidant activity and free radical scavenging capacity than 0.02% butylated hydroxyanisole (BHA).

Benzie and Szeto (1999) tested 25 different tea extracts including five oolong teas, and found that one cup (about 20 mL) of tea extract (12 % w/w tea in water) had the same antioxidant activity potential as 150 mg of ascorbic acid. Su *et al*. (2006) reported that infusion conditions had major effect on antioxidant potentials

and sensory assessment of oolong tea. Su *et al,* (2007) reported that higher temperature for longer time decreased polyphenol compounds. Blending creates a well balanced flavour and also ensures the variation in quality. Determination of total polyphenol content and measurement of total antioxidant activity are necessary for better understanding of the health benefits of tea. Therefore the objectives of this study were to evaluate the effect of temperature and duration on polyphenol content and antioxidant activity of equal combination of bush tea and black tea and also to ascertain if blending two teas has any effect on polyphenols, antioxidant activities and tannin content.

4.2 Materials and methods

Chemicals and materials: Black tea was obtained from Mukumbani Tea Estate (22°53'60S 30°25'0E 727 m.a.s.l) and bush tea was obtained from Hazeyview (25°1'60S 31°7'0E 524 m.a.s.l). Folin-Ciocalteau, methanol, sodium carbonate (anhydrous), potassium persulfate, 2,2'azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (Steinheim, Germany). Tannic acid was obtained from Saarchem-Holpro Analytical (Pty) Ltd (Johannesburg, South Africa) while vanillin and sodium dihydrogen orthophosphate (anhydrous) were obtained from Associative Chemical Enterprise (Pty) Ltd (Johannesburg, South Africa). Deionized water was used in all analyse, and all other chemical and solvents were all of analytical grade.

Sample preparation: Bush tea was harvested and dried under the shade and finally ground into powder. Black tea and bush tea were brewed using a water bath at different temperatures and duration. An amount of 6 g mixture (3 g bush tea and 3 g black tea), was measured and poured into 1000 ml beaker and 600 ml of distilled water was added. The teas were then brewed for 30°C for 3 minutes, 30°C for 10 minutes, 60°C for 3 minutes, 60°C for 10 minutes, 90°C for 3 minutes and 90°C for 10 minutes respectively. The tea was then dried in an

oven at 80°C until thoroughly dried. The dried tea was packaged and stored for one week at -1°C in a refrigerator.

Determination of total polyphenol content: The Folin Ciocalteau method (Singleton *et al*., 1965) as modified by Waterman *et al*. (1994) was used to determine total phenols in combined black tea and bush tea extracts. This method is based on the reducing power of phenolic hydroxyl groups (Hahn *et al*., 1984) which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. Tea extracts (2 g) were reconstituted in 30 ml of methanol solvent and an aliquot (0.5 ml) of the reconstituted sample was used to determine total phenols as described in section 3.2.

Determination of tannins: The vanillin HCl method of Prince *et al*. (1978) was used for the determination of tannins. This method is based on the ability of flavoids to react with vanillin in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. Tea extracts (2 g) were reconstituted in 30 ml of methanol solvent and an aliquot (0.5 ml) of the reconstituted sample was used to determine total phenols as described in section 3.2.

Determination of antioxidant activity: Antioxidant activity of the extracts was determined using trolox equivalent antioxidant capacity (TEAC) assay (Awika *et al*., 2004). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the $ABTS^{+}$ radical cation chromogen in relation to that of trolox, while the water soluble vitamin E analogue is used as an antioxidant standard. Tea extracts (2 g) were reconstituted in 30 ml of methanol solvent and an aliquot (0.5 ml) of the reconstituted sample was used to determine total phenols as described in section 3.2.

4.3 Statistical Analysis

The experiment was replicated three times and all measurements were done in duplicate. Data were subjected to analysis of variance (ANOVA), computed using SAS (2003). General Linear Model procedure and the Least Significant Difference test was used to identify differences among the means.

4.4 Results and Discussion

Yield of methanol extract from combined bush tea and black tea: The powder, yield extraction and concentration of black and bush tea are shown in Table 1.3. The amount of tea powder extracted increased from 1.09 g to 1.31 g with increasing temperature and duration. At 30°C for 3 minutes 1.05 g was obtained which was significantly lower than 1.09 obtained from tea boiled for 10 minutes. Tea powder increased to 1.15 g and 1.18 g for 3 minutes and 10 minutes at 60°C and these values were significantly different from each other. The highest tea powder was obtained at 90 °C for 10 minutes (1.31 g) and was significantly greater than 1.27 g obtained from tea boiled for 3 minutes.

Yield extract increased from 17.90% to 22.24% from tea boiled for 30°C for 10 minutes and tea boiled at 90°C for 10 minutes. Tea brewed at 30°C for 3 minutes and 10 minutes contained 17.90% and 18.32% of yield extract. It further increased to 19.32% and 19.66% at 60°C for 3 and 10 minutes. At 90°C 21.83% and 22.24% were obtained which were not significantly different from each other.

Tea concentration also increased with temperature and duration of time. At 30°C for 3 and 10 minutes 2.50 mg/ml and 3.57 mg/ml are obtained which were significantly different from each other. At 60°C 4.91 mg/ml and 5.08 mg/ml were obtained which were significantly different from each other. Highest amount of tea concentration was obtained from tea boiled at 90°C for 10 minutes (7.82 mg/ml) which was significantly different from 6.65 mg/ml for tea boiled for 3

minutes. The increase in powder, yield extraction and concentration is related to the amount of polyphenols, catechins present in either bush tea or black tea.

Table 1.3 Yield of methanol extract from combined bush and black tea at different soaking conditions

Tea solution (mg/ml)	Treatments		Powder (g)	Yield extract (%)	Concentration
1	30°C	3 min	1.05^{f}	17.90^{c}	2.50^{e}
2		10 min	1.09^{e}	18.32^{c}	3.57^{d}
3	60°C	3 min	1.15^{d}	19.32^{b}	4.91^{c}
4		10 min	1.18^{c}	19.66^{b}	5.08^{c}
5	90 °C	3 min	1.27^{b}	21.83^{a}	6.65^{b}
6		10 min	1.31^{a}	22.24^{a}	7.82^{a}
Standard error			0.005	0.185	0.270

Means within each column followed by different letters are significantly different ($P<0.005$).

Effects of soaking conditions on polyphenol content of combined bush tea and black tea: Total phenols are shown in Table 1.4. Tea boiled at 90°C for 3 minutes had significantly ($p<0.05$) higher total phenols than tea boiled at different temperatures. Total polyphenols decreased with a decrease in temperature and duration. At 90°C for 3 minutes 2.64 mg/100 g total polyphenols was achieved which was significantly different from 2.11 mg/100g from of tea boiled for 10 minutes at the same temperature. Polyphenols continued to decrease with a decrease in temperature and duration and at 60°C 1.85 mg/100g was obtained which was significantly different from 1.72 mg/100g obtained from tea boiled for 10 minutes. At 30°C for 3 minutes 1.42 mg/100g polyphenols was obtained which were not significantly different from 1.39mg/ml obtained at tea boiled for 10 minutes at the same temperature. However there was a decrease in total polyphenols after combining the two teas. For example, black tea yielded 7.68

mg/100 g total polyphenol at 90°C for 3 minutes (Table 1.2) but black tea and bush tea yielded only 2.64 mg/100 g total polyphenols. This suggests that some reaction that reduces polyphenols took place when the two teas were mixed.

Table 1.4: Yield extract of total polyphenols, antioxidants activity and tannin content.

Treatments	Total polyphenols (mg/100 g)	Antioxidant activity (µmol/g)	Tannin (mg/100 mg)
1 90°C 3 min	2.64[a]	2.48[a]	1.80[a]
2 10 min	2.11[b]	2.45[a]	1.50[a]
3 60°C 3 min	1.85[c]	1.44[b]	1.03[c]
4 10 min	1.72[d]	1.43[b]	0.87[d]
5 30°C 3 min	1.42[e]	0.40[c]	0.86b
6 10 min	1.39[e]	0.35[c]	0.63[e]
Standard error	0.011	0.019	0.012

Means within each column followed by different letters are significantly different ($P<0.005$).

Effects of soaking conditions on antioxidant activity of combined bush tea and black tea: Antioxidants decreased with decreases in temperature and duration. Higher antioxidant activity was achieved at 90°C for 3 (2.48µmol/g) and 10 (2.45µmol/g) minutes which were not significantly different from each other. Antioxidant activity continued to decrease and at 60°C for 3 and 10 minutes 1.44 µmol/g was achieved which was not significantly different from 1.43 µmol/g obtained when tea was boiled for 10 minutes. Lowest antioxidant activity was

achieved at 30°C for 3 (0.40 µmol/g) and 10 (0.35µmol/g) minutes which were not significantly different from each other.

Effects of soaking conditions on tannin content of combined bush tea and black tea: Tannin content decreased with a decrease in temperature and duration. At 90°C for 3 minutes 1.80 mg/100 g tannin was achieved which is not significantly different from 1.50 mg/100 g obtained when tea was boiled for 10 minutes. Tannin content continued to decrease and at 60°C for 3 minutes 1.031 mg/100 g which was significantly greater than 0.87 mg/100 g obtained when tea was boiled for 10 minutes. Lowest tannin was obtained at 30°C for 10 minutes (0.63 mg/100 g) which was significantly different from 0.86 mg/100 g obtained from tea boiled for 3 minutes. High content of polyphenol and TEAC radical scavenging activity is associated with the presence of tannin in both black and bush tea.

In conclusion, combining the two tea types causes decrease in total polyphenol content, antioxidant activity and tannin content. Tea boiled at 90°C for 3 minutes contained higher levels of polyphenol and antioxidants than tea boiled at 30°C for 3 minutes. It is, however, difficult to make comparison to other research since blending bush tea and black tea is not documented.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Discussion and conclusions of methods used

A number of methods are used to determine phenolic compounds, and the choice of a method depends on the type of phenolic compound to be determined. The Folin-Ciocalteu method (Singleton *et al*., 1965) as modified by Waterman *et al*. (1994) was used in this study for the determination of total phenols. Determination of total phenols quantifies the total concentration of phenolic hydroxyl groups present in a sample, regardless of the specific molecules in which the hydroxyl groups occur (Waterman *et al*., 1994). The Folin-Ciocalteu method was chosen for this purpose due to its wide applicability for biological materials and its simplicity to use in the lab (Waterman *et al*., 1994).

The Vanillin-HCl method (Price *et al*., 1978) was used for the determination of tannins due to its specifity in quantifying the tannins (Deshpande, Cheryan and Salankhe, 1986). It is also reported to be reproducible. The vanillin reagent (4% HCl in methanol and 0.5% vanillin in methanol) reacts with flavanols with a single bond between C-2 and C-3 of the C ring for basic flavonoid structure in the presence of HCl to give a bright red colour that is determined spectrophotometrically. Since tannins are condensation products of flavan-3-ols and flavan-3, 4-diols, both with a single bond between C-2 and C-3 of the C ring, they give a positive reaction with vanillin (Gupta *et al*., 1980).

The TEAC assay is one of the widely used screening methods for antioxidant activity. This study used a modification of the assay as described by Awika *et al*. (2004). The assay measures the relative ability of an antioxidant to scavenge the 2,2'-azinobis (3-ethyl-benzothiazoline-6 sulfonic acid) radical ($ABTS^{\bullet+}$) generated in aqueous phase, as compared with trolox (water soluble vitamin E analogue) standard. The degree of decolorization reflects the extent of the scavenging of

the $ABTS^{\bullet+}$ radicals and is determined spectrophotometrically at 734 nm. The assay is applicable for assessing antioxidant capacity of single compounds, food components, and food extracts as well as biological systems (Van den Berg *et al*., 2000), and is rapid, simple and relatively cheap and has good repeatability.

5.2 Discussion and conclusions of study results

The results of this study showed that black tea polyphenols, antioxidant activity and tannin content decrease with a decrease in temperature, and at each temperature declined with duration. At 90°C for 3 minute 7.68 mg/100 mg of total polyphenols was achieved which declined to 5.50 mg/100 mg at 30°C for 10 minutes. This tendency can be attributed to release of polysaccharides at 90°C.

Black tea was then combined with bush tea equally (50:50) and effects of soaking temperature were investigated. Combining two teas caused a decrease in polyphenol, antioxidant and tannin content. Similarly, more polyphenol, antioxidant activity and tannin were obtained at higher temperature (90°C for 3 minutes) than at 30°C for 3 minutes.

This study showed that combined black tea and bush tea and solely black tea had large TEAC radical scavenging activity. The antioxidant activity of the tea solution increased with an increase in soaking temperature and soaking duration. It is recommended that 3-min soaking temperature at 90 °C of black tea and combined bush tea and black tea can be used due to the highest total polyphenols and antioxidant activity. This tea will offer more health benefits to consumers.

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APPENDICES

1.1 Analysis of variance of black tea powder

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.66871111	0.13374222	4.72	0001
Error	12	0.06493333	0.00541111		
Corrected Total	17	0.73364444			

1.2 Analysis of variance of black tea yield extract

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	176.4927611	35.2985522	155.64	0001
Error	12	2.7214667	0.2267889		
Corrected Total	17	179.2142278			

1.3 Analysis of variance of black tea concentration

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	24.78820000	4.95764000	49576.4	0001
Error	12	0.00120000	0.00010000		
Corrected Total	17	24.78940000			

1.4 Analysis of variance of black tea polyphenols

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12.44969444	2.48993889	3704.04	0001
Error	12	0.00806667	0.00067222		
Corrected Total	17	12.45776111			

1.5 Analysis of variance of black tea antioxidant activity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	17.15484444	3.43096889	2278.87	0001
Error	12	0.01806667	0.00150556		
Corrected Total	17	17.17291111			

1.6 Analysis of variance of black tea tannins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	13.08071111	2.61614222	3797.63	0001
Error	12	0.00826667	0.00068889		
Corrected Total	17	13.08897778			

1.7 Analysis of variance of black and bush tea powder

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	0.15220000	0.03044000	304.40	0001
Error	12	0.00120000	0.00010000		
Corrected Total	17	0.15340000			

1.8 Analysis of variance of black and bush tea yield extract

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	48.10065000	9.62013000	92.77	0001
Error	12	1.24440000	0.10370000		
Corrected Total	17	49.34505000			

1.9 Analysis of variance of black and bush tea concentration

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	56.76071111	11.35214222	51.84	0001
Error	12	2.62786667	0.21898889		
Corrected Total	17	59.38857778			

4.10 Analysis of variance of black and bush tea polyphenol

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3.30502778	0.66100556	555.99	.0001
Error	12	0.01426667	0.00118889		
Corrected Total	17	3.31929444			

4.11 Analysis of variance of black and bush tea anti oxidants

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	13.11111111	2.62222222	2395.94	.0001
Error	12	0.01313333	0.00109444		
Corrected Total	17	13.12424444			

4.12 Analysis of variance of black and bush tea tannin

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	5	2.92844444	0.58568889	1158.51	.0001
Error	12	0.00606667	0.00050556		
Corrected Total	17	2.93451111			