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The fortification of tea with sweeteners and milk and its effect on *in vitro* antioxidant potential of tea product and glutathione levels in an animal model

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ABSTRACT

Several studies have demonstrated that tea flavonoids protect cells and tissues against free radicals which have been implicated in the etiology of oxidative stress-related disease disorders. However, black tea is commonly consumed with additives that could otherwise affect the bioavailability of the active tea molecules. In this study, the biochemical parameters of Kenyan teas were determined and the effect of added milk and sweeteners on the antioxidant activity of Kenyan teas was investigated. The effect of tea antioxidants on glutathione (GSH) was also evaluated *in vivo* in a time series study using Swiss mice. Green teas had the highest levels of total polyphenols, total and individual catechins, while black teas had high levels of total thearubigins, total theaflavins and theaflavin fractions. The antioxidant activity was high in green teas though some of the black teas were as efficacious as the green teas. The addition of milk, sugar and honey significantly ($p < 0.05$) decreased the antioxidant activity of tea in a concentration-dependent manner. Addition of the sweetener, stevia (*Stevia rebaudiana* Bertoni), showed no significant ($p > 0.05$) influence on the antioxidant activity of tea and therefore can be recommended as a preferred sweetener for tea. Significantly ($p < 0.001$) higher levels of GSH were observed in plasma than in other tissues. GSH levels were generally highest 2 h after tea consumption, which indicates the need to repeatedly take tea every 2 h to maximise its potential health benefits.

1. Introduction

Black (aerated) tea is the most consumed beverage globally. It is largely produced in Africa, with Kenya being the largest producer (Abeywickrama, Ratnasooriya, & Amarakoon, 2011; Cabrera, Reyes, & Rafael, 2006; Economic Review of Agriculture, 2007; Kerio, Wachira, Wanyoko, & Rotich, 2012; Mwaura & Ogise, 2007). Black tea is processed from the tender leaves of *Camellia sinensis.* Fresh tea leaves are rich in catechins which form the principal polyphenolic compounds in green (non-aerated) tea, while black teas are rich in oxidation products of the catechins, called theaflavins and thearubigins (Frei & Higdon 2003; Karori, Wachira, Wanyoko, & Ngure, 2007). Tea-derived polyphenols have been extensively studied as promising substances in disease prevention (Cabrera et al., 2006; Jianbo et al., 2011). For example, the chemo-preventive activity of tea against cancer has been demonstrated in several body organs, such as colon, stomach and lung (Sharangi, 2009; Wang et al., 2010). Regular consumption of tea has also been linked with the protection against harmful UV radiation, and maintenance of skin structure and functions (Heinrich, Carolyn, Silke, Hagen, & Wilhelm, 2011). Tea catechins and theaflavins have been implicated in the protection against cardiovascular and kidney ailments through several mechanisms (Shinichi, 2008). Tea catechins also decrease glucose production and hence regulate glucose and insulin concentrations in the blood (Wu, Juan, Hwang, Hsu, & Ho, 2004). Tea has been demonstrated to ameliorate inflammatory conditions due to its anti-parasitic, anti-heamolytic and anti-oxidant properties (Karori, Wachira, Wanyoko, & Ngure, 2008). Regular consumption of black tea has been associated with low risks of cognitive impairment and has been shown to delay or prevent the onset of dementia (Chen et al., 2010; Ng, Lei, Mathew, Ee, & Keng, 2008). Tea polyphenols with the galloyl moiety have particularly been demonstrated to inhibit the entry of the HIV virus by binding to the gp41, which is essential for the HIV-1 attachment to the target cells (Shuwen et al., 2005). The fluoride present in tea prevents dental decay by inhibiting the demineralization of protective coat enamel by both pathogenic microorganisms and commensal organisms (Hamilton-Miller, 2001).

The above health effects of tea are largely ascribed to its antioxidant properties, which enable it to protect cells against oxidative damage caused by free radicals (Gupta, Siddique, Beg, Ara, & Afzal,

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2008). Free radicals are important in normal physiological processes but they have also been associated with the etiology of chronic and degenerative diseases, including atherosclerosis, Alzheimer's and Parkinson's disease, diabetes, cardiovascular diseases, cancer, allergies and premature aging (Svetli, Guy, & Heidar-Ali, 2010). Endogenous antioxidant defence mechanisms are not always sufficient to fully scavenge free radicals from the body and dietary components that have antioxidant properties, such as tea flavonoids, are therefore necessary to supplement the activity of endogenous antioxidants. One of the major endogenous antioxidants in the body is glutathione (GSH). GSH has many functions, one of which is the protection against oxidative damage by scavenging free radicals and peroxides produced during normal cellular respiration, which would otherwise oxidise proteins, lipids and nucleic acids (Giustarini, Rossi, Milzani, Colombo, & Dalle-Donne, 2004; Moskaug, Harald, Myhrstad, & Rune, 2005).

Though numerous studies have been carried out to determine the health benefits associated with antioxidant properties of tea, these efforts have largely been directed towards the biological activity of green tea with very little effort on black tea, which is the principle product of Africa and which is also widely consumed in the world. Black tea is largely consumed with substantial amounts of milk and added sweeteners, such as sugar, stevia and honey. The effects of milk and added sweeteners on the antioxidant activity of tea have hardly been investigated. This study aimed to establish whether addition of these secondary additives affects the potential health benefits that can be derived from the antioxidative potency of tea. It is envisaged that evidence of the effects of these ingredients on the antioxidant activity of tea will improve preparation and consumption habits, and promote tea as a functional beverage and contribute to its increased consumption.

2. Materials and methods

2.1. Tea samples and reagents

Leaf samples from five tea cultivars (AHP S15/10, TRFK 303/577, TRFK 6/8, BBK35 and TRFK 306/1) were sourced from the Tea Research Foundation of Kenya (TRFK) 0°26́S, 37°15́E). Refined white cane sugar, produced and packaged by Mumias Sugar Company, fresh cow's milk, processed and packaged by the Kenya Co-operative Creameries Ltd (KCC) and honey, processed and packaged by Baraka Agricultural Institute, were used in this study. The milk was ultra-heated with no additives or preservatives. Stevia powder was bought from a local chemist shop.

2.2. Sample preparation

2.2.1. Processing of tea samples

Tea was manufactured in a miniature tea factory at the TRFK, Kericho, Kenya. Black teas were manufactured using physical wither for 18 h to attain a moisture content of 50–65%. Aeration was carried out for 2 h at 24 °C and the leaf fired in a fluid bed drier at 120 °C for 20–25 min. Green teas were manufactured by steaming the leaf for 1 min, crushing, tearing and curling and finally firing in a fluid bed drier at 120 °C. The processed teas were packaged into polythene bags and stored at room temperature prior to further analysis.

2.2.2. Determination of dry matter content

Five grammes (5 g) each of the tea products were weighed to the nearest 0.001 g, placed in pre-weighed aluminium dishes and dried in an oven (Oven Memmert, UND300, Germany) at 103 ± 2 °C for 16 h to constant weight. Percentage dry matter (DM) content for each sample was calculated from the weight differences of the dried and pre-dried tea samples.

2.3. Preparation of extracts

2.3.1. Extraction of polyphenols and catechins

Tea samples of coarse granular structure were ground to fine powder. A sample of 0.2 ± 0.001 g of tea was weighed into a graduated extraction tube (10 ml) and 5 ml of 70% hot water/methanol extraction mixture added using a dispenser (Dispensette Brand Germany), stoppered and mixed on a vortex mixer (Rotamixer, Huck andTucker, England). The mixture was immediately placed in a water bath at 70 °C and incubated for 10 min. After incubation, the mixture was removed from the water bath, cooled and centrifuged at 3500 rpm for 10 min (Heraeus Sepatech, Germany). The supernatant was decanted in a graduated tube. The extraction step was repeated and both extracts were pooled and the volume adjusted to 10 ml with cold 70% methanol.

2.3.2. Extraction of theaflavins and thearubigins

Nine grammes (9 g) of coarse tea sample were weighed, and placed in a 500 ml thermos flask and 375 ml of hot distiled water added .The mixture was then agitated, using a mechanical shaker for 15 min. The tea infusion was then filtered through cotton wool into a flat-bottomed beaker and allowed to cool to room temperature.

2.4. Analysis of total polyphenols

The Folin–Ciocalteau reagent method was used to determine total polyphenols as per the International Organisation for Standardization (ISO) 14502-1 (ISO 14502-1, 2005). From the sample extract, 1 ml was pipetted into a 100 ml volumetric flask and made up to the mark with distiled water. One millilitre of the diluted sample was complexed with 5 ml of 10% Folin–Ciocalteau's phenol reagent and 4 ml of 7.5% sodium carbonate solution. The mixture was stoppered, vortexed and the optical densities (OD) measured after 1 h in 10 mm length cells against distiled water, using a digital grating spectrophotometer at 765 nm (model Cecil CE 393). The total polyphenol content was expressed as gallic acid equivalents (GAE), in g/100 g.

2.5. Analysis of catechins

Catechin analysis was done (HPLC) according to ISO 14502-2, 2005. Reverse phase HPLC was employed. A Shimadzu LC AT HPLC machine with SIL 20A auto sampler, SPD-20 UV–visible detector with a class LC 10 chromatography work station and A Luna TM 5 μM C6, 250 mm × 4.6 i.d (Phenomenex, Tolerance, CA, USA) column with a Reodyne pre-column filter 7335 model was used. One millilitre (1 ml) of the extract (2.3.1 above) was diluted to 5 ml with stabilizing solution (10% v/v acetonitrile with 500 μg/ml EDTA and ascorbic acid), filtered through a 0.45 μm membrane filter and 20 μl were injected into the machine. A gradient elution was carried out using mobile phases A and B: mobile phase A (acetonitrile/acetic acid/double distiled water-9/2/89 v/v/v), and mobile phase B (acetonitrile/acetic acid/double distiled water-80/2/18 v/v/v). The mobile phase composition for a binary gradient condition started at 100% solvent A for 10 min, then over 15 min a linear gradient to 60% mobile phase A, 32% mobile phase B and held at this position for 10 min. The condition was reset to 100% mobile phase A and allowed to equilibrate for 10 min before the next sampling. The flow rate of the mobile phase was 1 ml per minute and the temperature of the column was set at 35 ± 0.5 °C. The identification of individual catechins was carried out by comparing the retention times and UV-absorbance of sample peaks with peaks