Food Chemistry 133 (2012) 707–714

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Correlation between leaf age, shade levels, and characteristic beneficial natural constituents of tea (*Camellia sinensis*) grown in Hawaii

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ARTICLE INFO

Article history:
Received 7 October 2011
Received in revised form 8 January 2012
Accepted 24 January 2012
Available online 1 February 2012

Keywords:
Hawaii
Camellia sinensis
Tea
Buds
Young leaves
Theanine
Caffeine
Catechins
HPLC
Shade levels
Antioxidants

ABSTRACT

This study showed the relationship between tea leaf age, bud and first two leaves, and shade levels, on the relative concentrations of six major compounds of tea leaf, namely L-theanine, caffeine, and the major tea catechins; (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (−)-epicatechin gallate (ECG), all of which are reported to have positive effects on human health, as well as at the ferric reducing antioxidant power of bud and leaf extracts. The concentration of L-theanine and caffeine decreased as leaf age increased moving from bud to first and then second leaf, while the concentration of the four catechins increased from the bud to first and second leaves. In most cases this increase was generally relatively small but in the case of EGC it was 7 to 10-fold. Certain chemical components of freshly picked, minimally processed and essentially unoxidised tea may potentially be used as markers for age, quality, authenticity and area of growth.

1. Introduction

"The best tea grows in the forest, on the hills facing the sun, with canopy cover", according to the tea saint Lu Yu in his book the Tea Sutra, also known as Cha Jing (Carpenter, 1974; Yu, 780 BC). For more than 1000 years this simple line has been guiding tea cultivation in East Asia. Yet today, most teas are grown with full exposure to the sun. While enjoying the ease of this mode of monoculture and cheap prices, growers might be sacrificing conditions essential for the production of high quality tea.

In recent years, the consumption of green tea (*Camellia sinensis*) products, particularly as a drink, has become increasingly popular in western cultures because of its reported positive health effects (Dulloo et al., 1999; Venables, Hulston, Cox, & Jeukendrup, 2008; Yamamoto, Juneja, Chu, & Kim, 1997). In contrast to coffee in Hawaii, which has a long history of production of high quality product with worldwide distribution, Hawaiian-grown tea has been produced only in very limited quantities and has failed to enter the global market. However, recent interest has developed in producing high quality teas that can be produced and processed by boutique growers and marketed in conjunction with tourism and regional cuisine (CTAHR, 2011). In order to achieve this end, it is essential to employ analytical methods to determine the effect of cultural practices on levels of desirable natural constituents biosynthesised by the plant.

Tea contains a group of compounds, catechins, which are polyphenols, known for their antioxidant-related effects (Gradisar, Pristovsek, Plaper, & Jerala, 2007; Morley et al., 2005). The major tea catechins are (−)-epigallocatechin gallate (**1**, EGCG), (−)-epigallocatechin (**2**, EGC), (−)-epicatechin (**3**, EC), and (−)-epicatechin gallate (**4**, ECG) (Fig. 1). One of these compounds, EGCG, is often reported as the most abundant compound of this class found in tea leaves (Dalluge, Nelson, Thomas, & Sander, 1998; Lu et al., 2009). Caffeine (**5**) and L-theanine (**6**) (Fig. 1), one of the biosynthetic precursors of catechins like EGCG (Kito, Kokura, Izaki, & Sasaoka, 1968), are two other naturally-occurring substances also reported to have positive health effects. Caffeine is probably best known for its ability to stimulate the central nervous system (Lovett, 2005), while L-theanine is an amino acid that has been found to reduce mental and physical stress (Kimura, Ozeki, Juneja, & Ohira, 2007), to have potential as an immune system stimulant by being able to boost production of anti-bacterial

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doi:10.1016/j.foodchem.2012.01.078

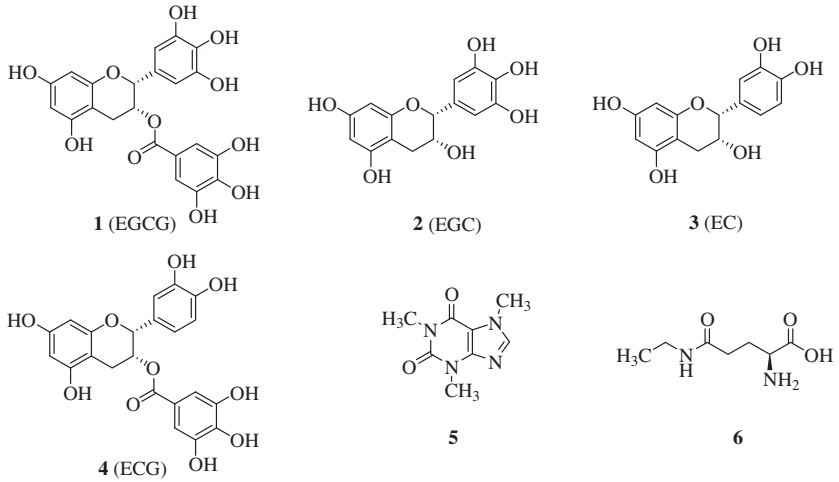

Fig. 1. Chemical structures of epigallocatechin gallate (**1**, EGCG), epigallocatechin (**2**, EGC), epicatechin (**3**, EC), and epicatechin gallate (**4**, ECG), caffeine (**5**) and L-theanine (**6**).

proteins (Kamath et al., 2003), and also be one of the components responsible for the characteristic taste of green tea (Lee et al., 2010). Recent studies of tea leaves have shown there to be a relationship between leaf age and concentrations of natural constituents, based on young leaves being defined as the bud and the two leaves underneath the bud, as compared to other leaves that are considered to be old leaves (Chiu & Lin, 2005; Lin, Juan, Chen, Liang, & Lin, 1996; Lu et al., 2009; Santana-Rios et al., 2001). Three of these studies clearly demonstrated that young leaves contain more antioxidants than older leaves (Chiu & Lin, 2005; Lin et al., 1996; Santana-Rios et al., 2001).

Numerous studies have been conducted to determine how levels of the catechin polyphenols in tea vary according to variety, agricultural conditions and processing (Khokhar & Magnusdottir, 2002; Lee & Ong, 2000; Singh, Ravindranath, & Singh, 1999). However, there have been no similar studies that evaluate changes in polyphenols, caffeine and L-theanine using a single analytical method. This current study aimed to use a high-performance liquid chromatography (HPLC) method to further investigate young freshly picked, minimally processed tea leaves, in order to compare the concentrations of the most abundant polyphenols, EGCG, EGC, EC and ECG (**1–4**), as well as caffeine (**5**) and L-theanine (**6**) in extracts of the bud and the first and the second leaves of selected tea plants. In addition, some prior studies have shown that higher shade levels seemed to increase the L-theanine and caffeine levels in tea leaves (Hirai et al., 2008; Ohta & Harada, 1996; Suzuki & Waller, 1985), while causing a decrease in the catechin (polyphenol) levels (Saijo, 1980). To further investigate these findings for plants used in the current study, a specific selection of leaves was grouped and analysed according to shade levels, in order to determine whether or not a similar trend could be observed. Such findings could have ecological implications arising from the agricultural conditions used in tea production.

A second aim of the study was to assess and confirm that the major catechin (polyphenol) levels in all investigated samples were correlated with antioxidant activity, as measured using the ferric reducing antioxidant power (FRAP) assay. The FRAP activity of pure compounds was also assessed in this study. The FRAP assay was the one selected for use in this study since it is considered a simple, accurate, robust and direct method of assessing the "total" antioxidant activity of a given system, as it has no activity-changing interactions between antioxidants in the system (Benzie & Strain, 1996). It was hoped that the results obtained from this assay would provide definitive evidence of whether compounds implicated in having positive effects on human health are found in the bud in higher concentrations than in the first two young leaves underneath it. Such a finding could be used to the advantage of Hawaiian growers in validating claims that certain tea products are better for human health than others. For example, "silver needle" white tea or Yin Zhen tea contains only the buds and is claimed to be one of the healthiest and purest teas on the market. To the best of our knowledge there has been no study of tea undertaken that has looked specifically at concentrations of the six natural products investigated combined with shade as a variable as reported here, and certainly not of tea grown in Hawaii.

2. Materials and methods

2.1. Study site selection

On the Big Island of Hawaii there are many small tea plantations, as the local geography and climate in certain regions are ideal for growing tea and there are dedicated groups of enthusiastic people prepared to undertake the task of producing some fine locally-grown tea products. For this study it was essential to select a study site that was growing tea in an area that had varying shade levels and was also producing a green tea considered by local growers to be an above average product. After viewing a number of locations, most having tea grown in open fields without any shade and some with too much shade, and discussions with local experts and growers, a site was selected, "Mauna Kea Tea", and the owners were consulted and agreed to allow their plants to be used in this investigation.

2.2. Tea leaf collection

Fresh leaves were collected from "Mauna Kea Tea", a tea plantation on the Big Island of Hawaii. The plantation is located at 46-3870 Old Mamalahoa Highway, Honokaa 96727, Hawaii, USA, and has an average elevation of about 590 m, with atmospheric temperature typically between 10 and 25 °C, and an annual rainfall of 196–245 mm/year, the majority falling between December and April.

The selected study area on the plantation had an approximate area of 300 m^2, and an apparent northeast facing slope. Young seedlings, generated from cuttings so as to maintain varietal characteristics (Variety: Mona Kea #1 (MK1)), were transplanted from nursery into this field, and had never been harvested. The field was mostly open to the sky, except on the northwest and southwest side, where Ohia lehua (*Metrosideros polymorpha*) trees form a