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Original Research Article

Antioxidant activity of different white teas: Comparison of hot and cold tea infusions☆

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ABSTRACT

The study investigates the antioxidant characteristics of various white teas steeped in either hot or room-temperature water in relation to grade of tea and brewing conditions. Antioxidant activity, chelating activity, total phenol (TPC), flavonoids (TFC), theaflavins and individual catechin content were examined. The results confirm that extraction of tea leaves carried out with water at room temperature leads to the formation of infusions particularly rich in compounds with antioxidant activity. In fact, for all the white teas studied, cold infusions had a higher content of phenols (4.77–7.63 mmol/L Gallic Acid Equivalents, GAE), flavonoids (1.47–2.53 mmol/L Catechin Equivalents, CE) and catechins (441.5–1328.2 μg/mL) compared to hot infusions (1.43–4.02 mmol/L GAE, 0.70–1.13 mmol/L CE, 83.4–534.8 μg/mL, respectively). The same trend was also observed for antioxidant activities examined using the ABTS assay (cold: 17.09–34.23; hot: 5.26–17.07 mmol/L Trolox Equivalents) and by monitoring the effects of the infusions on LDL oxidation (lag time, cold: 172.4–271.2; hot: 88.4–145.9 min). A general trend in antioxidant activity and in polyphenolic compound content can be delineated between Chinese teas, i.e. Bai Mu Dan ≥ Xue Ya ≥ White Lung Ching > Anji Needle Mao Feng > Yhin Zhen Bai Hao and between African teas, i.e. White Salima Peony > Thyolo Bsp > Bvumbwe Bsp. Concerning metal chelating activity, all the white teas displayed similar levels (0.3–0.6 mmol/L EDTA Equivalents) with no significant differences between the hot and cold infusions (except Bvumbwe Bsp and Thyolo Bsp). This paper contains key information on the antioxidant properties, TPC, TFC, and individual catechin content of several white teas commercially available and the outcomes suggest that preparing tea infused in room temperature water for approximately 2 h may constitute an alternative tea beverage potentially richer in healthful bioactive compounds compared to the more commonly consumed hot tea infusions.

1. Introduction

Tea is an extremely popular beverage around the world and can be served hot or ice cold. White, green, oolong, black teas, among the most commonly consumed teas, come from the leaves and buds of the *Camellia sinensis* (L.) (family Theaceae) plant and are categorized by variations in harvesting, processing, and associated degree of oxidation of polyphenols in fresh tea leaves (Sharangi, 2009; Unachukwu et al., 2010). Recently in the United States and Europe, white tea has been receiving increasing attention. White tea, a very rare and valuable type of tea native to China, is produced in very small quantities because the leaves are collected only at dawn during a few days in the spring when the buds are still closed. The traditional method used for white tea processing is to spread out the leaves to dry under the sun; during a lengthy drying process, in which the structure of the leaf cell is kept intact and not broken through any external physical interferences such as curling or twisting, the tea becomes slightly "oxidized". This oxidation converts small amounts of catechins, which have been described as potent antioxidants, into theaflavins and thearubigins, responsible for the characteristic aroma and color of black and oolong teas (Obanda et al., 2004).

The name "white tea" originates from the silky white fuzz that covers the immature leaves and buds, and it has a delicate, sweet flavor that differs from green tea's grassy taste. Because of its taste, white tea is becoming an increasingly common beverage. It is commercially available in several grades: Silver Needle and White Peony are the main ones, but several other grades with different trade names can be found. Silver Needle (traditional name Bai Hao Yinzhen), is one of the most famous and expensive white teas produced from the cultivar Bai. It is made only from the unopened

☆ Dedicated to Professor Lucedio Greci on the occasion of his 72nd birthday.

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buds of the plant with no leaves. Its color is silver-white, with long, small needles as the name implies. The first step of the processing method is sun drying of the buds on sieves or drying mats for one whole day, followed by baking over a slow fire until fully dried. The resulting drink is light yellow with a delicate flavor. White Peony (traditional name Bai Mudan) is produced from the vegetative apex of the plant, from the bud and one or two leaves; it is processed with the two simple steps of withering (sun drying/airing/low-temperature) and basket drying. Tea brewed from these leaves has a light golden-brown color and a pleasing roasted aroma. Lastly, Snowbud is a rare and organic white tea from coastal China developed in the 1980s. It is composed of only the newest leaves and buds, all gathered and dried in the early days of spring; its clear infusion offers a fresh green note with a complex sweet and savory flavor and aroma (He-Yuan, 2008; Hilal and Engelhardt, 2007).

White tea is marketed as possessing a wide range of health benefits, such as antioxidant, antimicrobial, and anticancer effects. In fact recent studies have shown that white tea exerts neuroprotection against hydrogen peroxide-induced toxicity in PC12 cells (López and Calvo, 2011), induces lipolytic activity and inhibits adipogenesis in human subcutaneous (pre)-adipocytes (Sohle et al., 2009), increases the antioxidant capacity of plasma and some organs in mice (Koutelidakis et al., 2009), has potent antimutagenic activity in the Salmonella assay (Santana-Rios et al., 2001), suppresses intestinal tumorigenesis in mice (Orner et al., 2003) and inhibits pancreatic lipase activity in vitro (Gondoin et al., 2010). Moreover, its ability to promote strong and elastic skin and alleviate inflammation and rheumatoid arthritis has led to an increased interest in this tea type (Thring et al., 2009). Concerning the levels of catechins, total polyphenols and total antioxidant activity, in general white tea is not significantly different from green tea (Karori et al., 2007; Rusak et al., 2008) even if some authors have found higher mean levels of some catechins and gallic acid in white than in green teas (Unachukwu et al., 2010).

In a previous study, we analyzed different types of tea (white, black, green, oolong) prepared as a hot infusion at 90 °C for 7 min or as a cold one at room temperature for 2 h (Venditti et al., 2010). The results obtained from the analysis of the antioxidant activity of these infusions showed that white tea, unlike black, oolong and green tea, exhibited a greater activity when steeped for 2 h in water at room temperature. We therefore wanted to verify if this feature could be common to most white teas, whose beneficial effects are often attributed to their antioxidant activity and which has been little studied to date. Accordingly, in this study a batch of 8 white teas was analyzed: 5 from China (*Sinensis* variety) and 3 from Malawi (Africa), 1 of which is from the *Sinensis* variety, while the other 2 are from hybrids bred in Malawi that contain variable amounts of this latter variety. Among the studied teas, some are of common grades, while others are new or very rare. The teas were prepared according to the typical brewing method for white tea (70 °C for 5–7 min) and at room temperature (20–25 °C) for 2 h, and antioxidant activity, chelating activity and overall content of polyphenols, flavanols, and catechins (HPLC) were determined.

2. Materials and methods

2.1. Chemicals and equipment

All reagents, including phenolic compounds used as standards for HPLC analysis and solvents of highest purity available, were purchased from Sigma–Aldrich Chemical Co. (Milan, Italy) and used as received. Ultrapure water was used throughout and obtained from a Milli-Q system from Millipore (Milford, MA, USA), except for preparation of teas where commercially available bottled mineral water (VERA Nestle – S. Pellegrino S.p.A., Milan, Italy) was used.

Spectrophotometric measurements were recorded on a Varian Cary 50 UV-visible spectrophotometer (Agilent Technologies Italia S.p.A., Milan, Italy) or on a microplate reader (Synergy HT, Biotek, Winooski, VT, USA).

HPLC analyses were performed on a liquid chromatograph equipped with a 321 Solvent pump and a 234 Autoinjector (Gilson Inc., Middleton, WI, USA) and an AD20 Absorbance Detector (Dionex Corporation, Sunnyvale, CA, USA). A C18 guard column and a μ-Bondapack C18 pre-packed column (3.9 mm × 300 mm) were used for separation (Waters Corporation, Milford, MA, USA).

2.2. Tea samples and preparation

A set of 8 white tea samples were analyzed which included 5 from China: Bai Mu Dan (BMD) a White Peony, Xue Ya (XY) a Snow Bud, White Lung Ching (WLC) a very rare tea, Anji Needle Mao Feng (ANMF) sold as a white tea although it undergoes the typical processing steps of green tea, and Yhin Zhen Bai Hao (YZBH) a Silver Needle, all commercially available; 3 came from a private estate in Malawi (Africa) and were produced from different clonal varieties: White Salima Peony contains about 50–60% *Sinensis* var. with high catechins and theanine (WSP), Bvumbwe Bsp (BB) contains about 30% *Sinensis* var. with high catechins while Thyolo Bsp (TB) is 100% *Sinensis* var. The 2 latter teas are new and similar to a White Peony.

Cold tea infusions were prepared by adding 20 mL of water at room temperature to 0.5 g of tea and leaving the infusions to stand at room temperature (20–25 °C) for 2 h, agitating manually every 30 min. Hot tea infusions were prepared by adding 20 mL of water at 70 °C to 0.5 g of tea and leaving to infuse for 7 min. Both hot and cold infusions were then filtered through Whatman paper filters (43–38 μm) and diluted appropriately with water according to each specific assay. Throughout this study, the terms cold and hot tea are used to express these two infusion types.

2.3. Total phenol content (TPC)

Total phenol content in the tea infusions was determined using Folin–Ciocalteu reagent (Singleton et al., 1999). To 1.975 mL of distilled water, 0.125 mL of Folin–Ciocalteu reagent followed by 0.025 mL of tea previously diluted 5-fold, or appropriately diluted gallic acid standard ethanolic solution, or water as blank, were added and mixed. After 10 min, 0.375 mL of 20% Na_2CO_3 were added, mixed and samples were left for 2 h at room temperature in the dark. Absorbance was read at 760 nm and the results were expressed as mmol/L Gallic Acid Equivalents (GAE) using the linear regression value obtained from the gallic acid calibration curve.

2.4. Total flavonoid content (TFC)

The total flavonoid content in the tea infusions was measured using a colorimetric assay according to the method of Gursoy et al. (2009) with some modifications. Briefly, 0.05 mL of tea infusion or (+)-catechin standard ethanolic solution appropriately diluted in water, or water as blank, were added to 1.35 mL of distilled water. After mixing, 0.05 mL of 5% $NaNO_2$ followed by 10% $AlCl_3$ (0.05 mL) were added and mixed and samples were left for 10 min at room temperature in the dark. Absorbance was read at 415 nm and the results were expressed as mmol/L Catechin Equivalents (CE) using the linear regression value obtained from the catechin calibration curve.

2.5. Total theaflavin content (TTC)

The total theaflavin content in the tea infusions was determined according to the Flavognost method (Hilton, 1972). Tea infusions