LWT - Food Science and Technology 89 (2018) 648–656

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Impacts of processing conditions on digestive recovery of polyphenolic compounds and stability of the antioxidant activity of green tea infusion during *in vitro* gastrointestinal digestion

Natthawuddhi Donlao[a,b], Yukiharu Ogawa[c,*]

[a] School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand
[b] Tea Institute, Mae Fah Luang University, Chiang Rai 57100, Thailand
[c] Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo 271-8510, Japan

ARTICLE INFO

Keywords:
Green tea
Processing conditions
Process severity
In vitro digestion
Antioxidant

ABSTRACT

Green tea samples were produced traditionally with different roasting temperatures (200 and 300 °C) and drying temperature (80, 120 and 160 °C). Total polyphenol content (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging ability, and ferric reducing antioxidant power (FRAP) were evaluated in dried tea leaves and tea infusions, and in tea infusions during *in vitro* gastrointestinal digestion. Colors of tea infusions and methanol extracts of dried tea leaves were measured. In dried tea leaves, TPC, DPPH and FRAP ranged from 150 to 173 mg GAE/g dried sample, 1245–1454 μmol TE/g dried sample, and 2976–3370 μmol $FeSO_4$/g dried sample, respectively, whereas in tea infusions, these values ranged from 88 to 130 mg GAE/g dried sample, 691–930 μmol TE/g dried sample, and 1905–2609 μmol $FeSO_4$/g dried sample, respectively. Greater process severity resulted in a greater loss of TPC and antioxidant activity in the samples. Processing conditions also altered colors of all tea extracts. Polyphenols in tea infusions were relatively stable throughout digestion, however, reductions in DPPH and FRAP values after digestion ranged from 16.0% to 25.7% and 15.5%–31.0%, respectively. These results indicate that processing conditions affected ability of antioxidants in samples to withstand digestion.

1. Introduction

Tea is an infusion of dried leaves of the tea plant (*Camellia sinensis*) (Higdon & Frei, 2003; Koo & Noh, 2007; Record & Lane, 2001), and it is the most popular beverage in many countries around the world (Astill, Birch, Dacombe, Humphrey, & Martin, 2001; Chan, Lim, & Chew, 2007; Dou, Lee, Tzen, & Lee, 2007; Guo et al., 1999; Liang, Lu, Zhang, Wu, & Wu, 2005; Okello, McDougall, Kumar, & Seal, 2011; Wang, Kim, & Lee, 2000). The differences in chemical composition among tea products are generally attributable to plant variety, growth conditions, and processing (Astill et al., 2001; Ravichandran & Parthiban, 2000). Based on the degree of fermentation occurring during processing, teas are divided into three major categories: green (non-fermented), oolong (partially fermented), and black (fully fermented) (Almajano, Carbó, Jiménez, & Gordon, 2008; Cabrera, Artacho, & Giménez, 2006; Cabrera, Giménez, & López, 2003; Dou et al., 2007; Liang et al., 2005). In East Asia, tea has been consumed as an everyday beverage and as a therapeutic aid in many illnesses medicine for thousands of years (Cabrera et al., 2003, 2006; Sato et al., 1989; Yen & Chen, 1995). Interest in the health benefits of tea, including its role in the prevention of some diseases, has increased, and numerous studies have sought to explain the health-related properties of tea and tea products (Higdon & Frei, 2003). The antioxidant activity and free radical–scavenging capacity of tea polyphenols have been reported as being among the most important benefits of tea (Almajano et al., 2008). The major polyphenols in tea are catechins (Dreosti, 2000; Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007), and the compounds in this group have the most powerful antioxidants (Vinson, Dabbagh, Serry, & Jang, 1995).

Polyphenolic compounds are considered to be key components of tea due to their health benefits; however, their content can be affected greatly by processing (Friedman, Levin, Choi, Lee, & Kozukue, 2009; Gadow, Joubert, & Hansmann, 1997; Higdon & Frei, 2003). Fermentation of oolong and black teas generally reduces the number of catechins in the resulting products (Ananingsih, Sharma, & Zhou, 2013; Cabrera et al., 2003). For this reason, green tea has a significantly higher catechin content (Ananingsih et al., 2013; Cabrera et al., 2003; Chan et al., 2007; Guo et al., 1999) and stronger antioxidant activity than oolong and black teas (Gadow et al., 1997; Manzocco, Anese, &

* Corresponding author.
E-mail address: ogwy@faculty.chiba-u.jp (Y. Ogawa).

https://doi.org/10.1016/j.lwt.2017.11.051
Received 4 September 2017; Received in revised form 24 November 2017; Accepted 24 November 2017
Available online 29 November 2017
0023-6438/

Nicoli, 1998). Although green tea is an excellent source of green tea catechins (GTCs), results from several studies indicate that GTCs are absorbed only partially in rat and human intestines (Zhu, Zhang, Tsang, Huang, & Chen, 1997). Two important concepts related to the absorption of antioxidants are bio-accessibility, or the amount of antioxidants released from the food matrix and presented to the small intestine; and bio-availability, or the amount of antioxidants passing through the cell membrane and available for use within the cell (Wootton-Beard, Moran, & Ryan, 2011). The absorption of polyphenolic compounds is considered to be limited due to their chemical structures; moreover, the factors involved in the bio-availability of polyphenols are their stability under gastrointestinal conditions and their bio-accessibility (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). These may also be limiting factors for the bio-availability of these compounds and their efficacy in terms of providing the desired beneficial health effects.

From the perspective of health benefits and for comparative purposes, knowledge of the amount and activity of antioxidants in green tea after processing would be useful. However, such information does not accurately reflect the potential health effects of the tea product; the determination of antioxidant activity after green tea has undergone digestion would be more useful. Most research on green tea processing has focused on treatments to enhance the quality of the dried tea leaves making up the final product. Little is known about whether processing affects the quality of green tea infusions, or ready-to-drink teas. Several researchers have applied the *in vitro* digestion technique to evaluate the stability of individual antioxidants from various foods, meals, and supplements (Ryan & Prescott, 2010), but no study to date has provided information regarding the effects of processing on the stability of antioxidant activity of tea infusions. Apart from all health benefits of tea drinking, green tea has been traditionally enjoyed as a beverage throughout history; consequently, strict sensory evaluations for appearance and flavor have been applied to tea grading. Among various evaluation parameters, color is one of the most important attributes for the tea quality. Especially, the tea infusion color seems to be much more important in ready-to-drink green tea or bottled green tea beverages (Wang, Park, Chung, Baik, & Park, 2004).

Thus, this study was conducted to investigate the effects of green tea processing conditions on the digestive recovery of polyphenolic compounds and the stability of the antioxidant activity of green tea infusions at various stages of *in vitro* gastrointestinal digestion. Characterization of the compounds behaviors during simulated digestion provides valuable information for future research on the impacts of manufacturing practices on the bio-accessibility and bio-availability of green tea polyphenols. In addition, the change of the tea infusion color by different processing conditions was also studied.

2. Materials and methods

2.1. Materials

Tea plants (*C. sinensis* var. *sinensis*) were grown on the Boon Rawd Farm tea plantation, Chiang Rai Province, Thailand. Young tea shoots (usually the top three leaves of each branch) were harvested in October 2015. The leaves were packed in plastic mesh bags and transferred immediately to the processing plant at Mae Fah Luang University, Chiang Rai Province, Thailand.

2.2. Sample preparation

The samples were subjected to traditional commercial processing, which involves withering, roasting, rolling, and drying. The fresh tea leaves were spread out on bamboo trays and allowed to wither at room temperature for 12 h, then in sunlight for 2 h. The tea leaves were then roasted in a drum roaster (Yuan Chang Machinery, Taoyuan, Taiwan) at 200 °C or 300 °C for 6 min. The roasted leaves were then rolled using a rolling machine (Yuan Chang Machinery) for 10 min; tea leaves were cut and twisted in this step. After the rolling process, each tea leave was dried in an electric convection hot-air dryer (Kluay Nam Thai, Bangkok, Thailand) at different temperatures (80 °C, 120 °C, and 160 °C) until the moisture content was reduced to < 8 g/100 g wet basis (w.b.) Processing conditions applied to tea leaves are summarized in Table 1. The dried samples were packed immediately in plastic bags and hermetically sealed. The samples were ground in a mill (NM-200; Nakasa, Osaka, Japan) and then passed through 5-mm mesh. The ground samples that passed through the mesh were packed in aluminum foil bags and stored at 4 °C.

Table 1
Processing conditions applied to tea leaves and drying times required to obtain the final moisture content.

Processing conditions			Drying time (min)	Moisture content[a] (g/100 g)
Conditions	Roasting temperature (°C)	Drying temperature (°C)		
R200D80	200	80	65	4.90 ± 0.05a
R200D120		120	45	3.06 ± 0.17c
R200D160		160	20	3.36 ± 0.17c
R300D80	300	80	60	4.78 ± 0.15a
R300D120		120	35	3.89 ± 0.31b
R300D160		160	18	3.88 ± 0.11b

[a]Mean ± standard deviation (n = 3). Different letters in the same column indicate significant differences (P < 0.05).

2.3. Determination of moisture content

Empty aluminum can and lid were dried in a hot-air dryer at 105 °C for 3 h and transferred to desiccator to cool. The dried empty can and lid were weighed. The tea sample (~1 g) was filled in the moisture can and dried at 105 °C for 24 h in a hot-air dryer (WFD-400; Eyela, Tokyo, Japan). After drying, the can and its dried sample were reweighed. The moisture content of the dried tea leaves following each processing condition was expressed on a wet basis (w.b.), that is, as the water mass in grams per 100 g of the total mass.

2.4. Methanol extraction

Several researchers have recommended the use of methanol for the extraction of polyphenolic compounds from plant tissues, and methanol has been reported to be the most suitable solvent for such extraction from tea leaves (Chan et al., 2007). Thus, we used methanol solution to extract the compounds contained in the dried green tea leaves. Ground dried green tea samples (1 g each) were placed in test tubes. Twenty milliliters of 70% (v/v) methanol (Wako Pure Chemical, Osaka, Japan) at 70 °C were added to each test tube to start the extraction process, and the mixture was then mixed thoroughly on a tube mixer (Trio TM-2F, As One, Osaka, Japan). The test tubes were then incubated at 70 °C for 10 min in a temperature-controlled water bath (T-25, Thomas Kagaku, Tokyo, Japan). After 5 and 10 min incubation, the samples were mixed further on the tube mixer. The samples were then allowed to cool at room temperature. They were centrifuged using a centrifuge machine (Model 2800, Kubota, Tokyo, Japan) at 1800 × g for 10 min and the supernatant was carefully decanted and collected. The extraction and centrifugation steps were repeated and the supernatant liquids were combined. The combined extract was transferred to a 50-ml volumetric flask and adjusted to 50 ml with cold 70% (v/v) methanol.

2.5. Preparation of green tea infusion

Green tea infusion was prepared according to the method described by Manzocco et al. (1998) with some modification. Briefly, green tea sample (5 g) was mixed with 500 ml 95 °C water for 5 min, with