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Original Research Article

Effect of processing step and condition for Japanese green tea manufacturing process on structural attribute of tea leaves and antioxidant activity of tea infusion

Wei Qin[1], Ryutaro Yamada[2], Takuya Araki[2], Yidi Cai[1], and Yukiharu Ogawa[1*]

[1]*Graduate School of Horticulture, Chiba University, 648, Matsudo, Matsudo, Chiba 271-8510, Japan*
[2]*Institute of Fruit Tree and Tea Science, NARO, 2769 Shishidoi, Kanaya, Shimada, Shizuoka 428-8501, Japan*

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ABSTRACT

Japanese green tea is a highly processed product. The manufacturing process of Japanese green tea consists of six steps which are followed by steaming, coarse kneading, crumpling, secondary kneading, precise kneading and drying. Each step in the process is important to increase the commercial quality of final tea products. However, few studies have focused on the changes in green tea leaf properties during processing. This study aimed to examine the effect of processing at each step on the structural attributes of Japanese green tea leaf. The fresh tea leaves were harvested at experimental field of NARO in Shizuoka in 2017 and continuously processed. The sample tea leaves were collected at each processing step and immediately cooled in a refrigerator. The samples at each step were separately infused by a distilled water at 100 °C for 30 s, and then removed from infusion and placed on a paper towel for drying. A scanning electron microscope (SEM) was used to observe the surface microstructures of sample tea leaves. The processed tea leaves were also infused by a distilled water at 95°C for 5 min to examine the total polyphenol content (TPC), ferric-reducing antioxidant power (FRAP) and free-radical scavenging assay (DPPH). As a result, cracks and wrinkle-like structures on the surface of tea leaves were found and increased with the processing step. TPC, FRAP, DPPH also increased with the processing. The SEM image of tea leaves was also revealed that the surface microstructural changes possibly connected with the TPC and antioxidant activity of green tea infusion. This should be considered that the condition of each processing step could influence on not only the apparent quality of final Japanese green tea product but also the functional properties of green tea infusion.

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* *Corresponding author*:
E-mail address: ogwy@faculty.chiba-u.jp

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INTRODUCTION

Tea had mainly been consumed as a pharmaceutical material from over thousand years ago. Nowadays, the tea infusions such as black tea, oolong tea and green tea become one of the major beverages in the world (Hara et al., 2017. Tanaka, 2012). It is well known that tea infusions have a lot of health benefits. The Japanese green tea, which has traditionally been consumed in Japan from almost one thousand years ago, also has health benefit better than white or black tea (Carloni, 2013), because of its ingredients such as vitamins, minerals, and antioxidants (Higdon and Frei, 2003; Reygaert, 2018; Das and Bhattacharyya, 2013; Cabrera et al., 2006; Yang et al., 2012). There are lots of reports regarding antioxidant properties of green tea infusions, and it is known that the ability to scavenge free radicals are one of the most important advantages of it (Barbosa, 2007; Vinson et al., 1995). The most popular antioxidant in the green tea infusion is catechin, one of the groups of flavonoids involved in polyphenols (Almajano et al., 2008). Therefore, the analysis of molecular structure and biological activities for tea catechins related to health benefit have been studied a lot.

The origin of Japanese green tea manufacturing process technology is also one of the earliest countries. The dried green tea leaves are produced by continuous combination of manufacturing processes which consist of steaming, coarse kneading, crumpling, secondary kneading, precise kneading and drying. Each step of this process affects the content and extraction ability of some components in tea (Shimada et al., 1996). Particularly, the steaming, which aims to kill enzyme activities of the fresh leaves, be softer and be kneaded easier, and steaming conditions affect chemical compositions of green tea that can be recognized as a unique section for the Japanese green tea manufacturing processes compared with the other tea variations (Ohmori et al., 1987). After coarse kneading, the appearance of tea leaf is changed and gradually dried during the processes in which the leaves tend to be easily destructed under the mechanical forces by processing. Thus, it is known that the quality of processed green tea leaves, evaluated by leaf shape and color, aroma and taste of tea infusion, is influenced on the conditions at each processing step (Hara et al., 2017; Kubota et al., 1989; Takayanagi et al., 1984; Furuya et al., 1961). These changes by the processing would also mean the tea quality regarding health benefit could be influenced on the processing conditions. However, the major approaches for studying of tea qualities have been conducted on the extraction of tea ingredients from fresh and/or final tea products including its infusions. Thus, few reports concerning with the effect of processing steps and conditions on the quality of dried tea leaf products and its infusions can be found.

The aim of this study is to examine the effect of processing steps and conditions on the structural attributes of Japanese green tea leaves and antioxidant properties of tea leaf infusions at each processing step.

MATERIALS AND METHODS

Materials

Tea leaves (***Camellia sinensis***, cv. Yabukita) used in this study were harvested by tea picking machine and continuously processed using tea processing facilities at the NARO experimental tea field in Shizuoka, Japan in May 2017.

Sample preparation

Fresh tea leaves (FL) were batch-wise processed using prototype tea processing machine (15K Kawasaki Machinery, Shizuoka, Japan) by steaming (ST), coarse kneading (CK), crumpling (CR), secondary kneading (SK), precise kneading (PK) and drying (DR). The conditions of each processing step are summarized in Table 1. The processed tea leaf samples were picked at the end of each processing step, packed in light-blocking aluminum foil bags, sealed with vacuuming and stored at 4 °C immediately. The fresh leaves were also packed and stored.

Table 1. Processing conditions and moisture content of processed tea leaf samples

Conditions at each processing step			Moisture content[a] (% w.b.)
Condition	Processing temperature (°C)	Processing time (min)	
FL	-	0	77.808±0.009a
ST	100	0.7-0.75	79.226±0.013a
CK	36	0.83-1	48.530±0.014b
CR	-	30	44.008±0.010c
SK	36	30	29.465±0.001d
PK	42	40-60	10.785±0.001e
DR	75	35	5.109±0.001f

[a] Mean ± standard deviation (n = 3). Different letters in the same column indicate significant differences (P < 0.01).

Moisture content

Empty cans and lids for moisture measurement were dried using hot air oven (WFD-400; Eyela, Tokyo, Japan) at 110 °C for 3 h and then cooled down before sample weighing. Approximately two grams of processed tea leaf samples were placed in the can and dried at 110 °C for 5 h. After drying, the can with dried sample was reweighed. The moisture content of the samples was then calculated and shown in wet basis (w.b.).

Microscopy

To investigate the microstructural changes in processed samples, the tea leaf samples were unrolled by immersion in hot water. Four grams of the processed samples were poured in 200 ml of hot water at approximately 100 °C for 30 s. The unrolled tea leaves were separated from the hot water and aligned on a paper towel to remove the moisture for 24 h. The dried tea leaf samples were observed using a scanning electron microscope (SEM, SU1510, Hitachi, Japan).

Preparation of tea infusion

The tea infusion was prepared following Donlao's method (Donlao and Ogawa, 2018). Five grams as dry matter was adjusted for each processed sample based on their moisture content and added hot water at 95 °C up to total mass of 500 g for 5 min (Huang et al., 2013). The infusions were filtered using a filter paper (Whatman no.1, GE Healthcare UK, Buckinghamshire, UK) at 37 °C in a water bath.

Determination of tea infusion characteristics

Total polyphenol content (TPC) of tea infusion was determined according to the Folin-Ciocalteu assay followed by Wang et al. (2000). 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay of the tea infusion was determined following the method of Molyneux (2004) with slight modification. The ferric reducing antioxidant power (FRAP) of tea infusion was determined following the method of Benzie and Szeto (1999).

Statistical analysis

All values are presented as the mean ± standard deviations. One-way analysis of variance (ANOVA) was performed to determine the significance of variables, Tukey's test at a significance level of $P < 0.01$ using R software version 3.4.3 (R Development Core Team, 2009). All analyses were done in triplicates.

RESULTS AND DISCUSSION

Appearances of tea leaves

The appearance of tea leaf samples at each processing step was shown in Fig. 1. The steamed leaves (ST, Fig. 1A) were mostly maintained a leaf structure similar with the fresh leaves (FL), although it was steam-heated. After the step for coarse kneading (CK, Fig. 1B), the tea leaves could not maintain its structure, which was looked like curling. At the crumpling step (CR, Fig. 1C), the leaves tended to be slightly gathered and caked. At the secondary kneading step (SK, Fig. 1D), the caked leaves and/or the agglomerations as shown in Fig. 1C were disappeared, but the curly forms were remained. Since the beginning of the precious kneading (PK, Fig. 1E), a needle-like shape was appeared, which presented as a quality tea leaf. The dried leaves (DR, Fig. 1F) were also looked like PK, however, the moisture was reduced

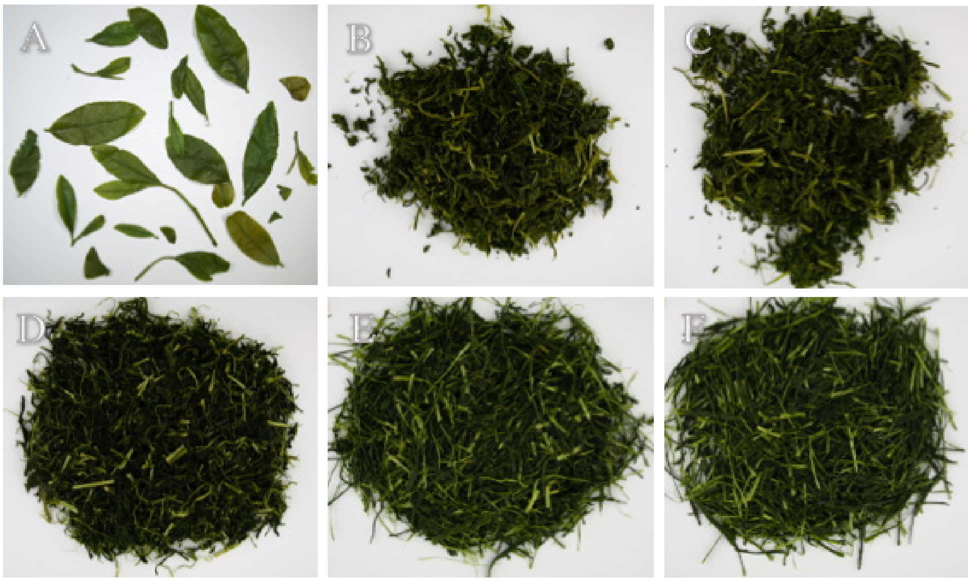

Figure 1. Images of tea leaf samples at each processing step. (A: ST, B: CK, C: CR, D: SK, E: PK, F: DR.)

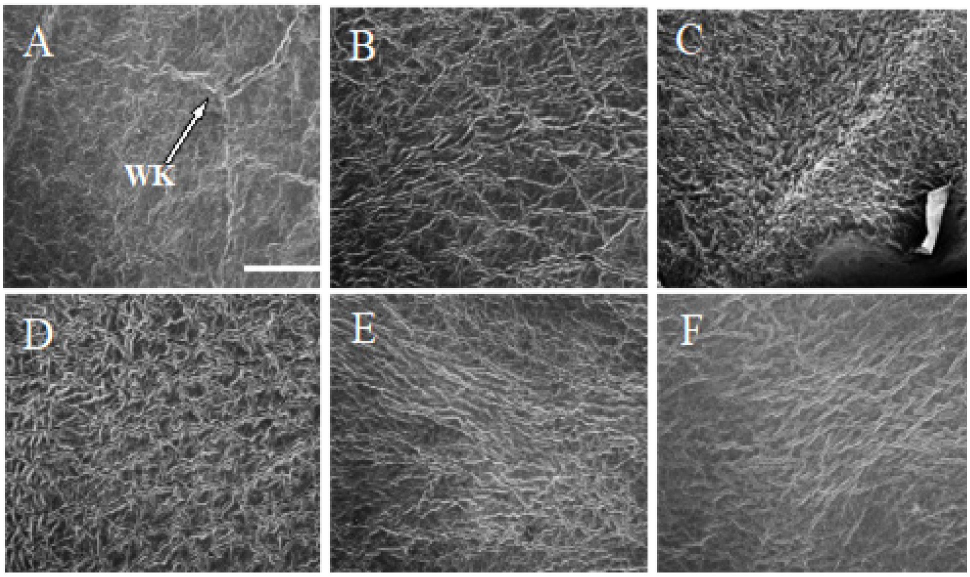

Figure 2. Microstructure of the surface of tea leaf samples at each processing step. (A: ST, B: CK, C: CR, D: SK, E: PK, F: DR. Scale bar shows 500 μm). WK: wrinkle-like structure.

Microstructure changes of tea leaves during Japanese green tea manufacturing process

The surface microstructures of tea leaf sample at each processing step was observed using SEM and the results were shown in Fig. 2. A lot of folds or wrinkle-like structures could be observed on the leaf surface of ST (Fig. 2A). The folds or wrinkle-like structures increased in the leaf surface at the processing steps of CK (Fig. 2B) and CR (Fig. 2C). The microstructures at the steps from SK (Fig. 2D) to DR (Fig. 2F) were looked similar with CR and the structural changes were seemed to be stable. In general, the process of coarse kneading, which was a kind of mechanical processing, would destroy the leaf structure as shown in the appearance change between Fig. 1A and B. Actually, lots of leaves at the coarse kneading step could not maintain its morphological structure. However, there were not so much damages in the microstructures of leaf surface. This suggested that the increase in folds or wrinkle-like structures would be occurred by the moisture loss from leaf tissue.

Total polyphenol content and antioxidant activity of green tea infusion

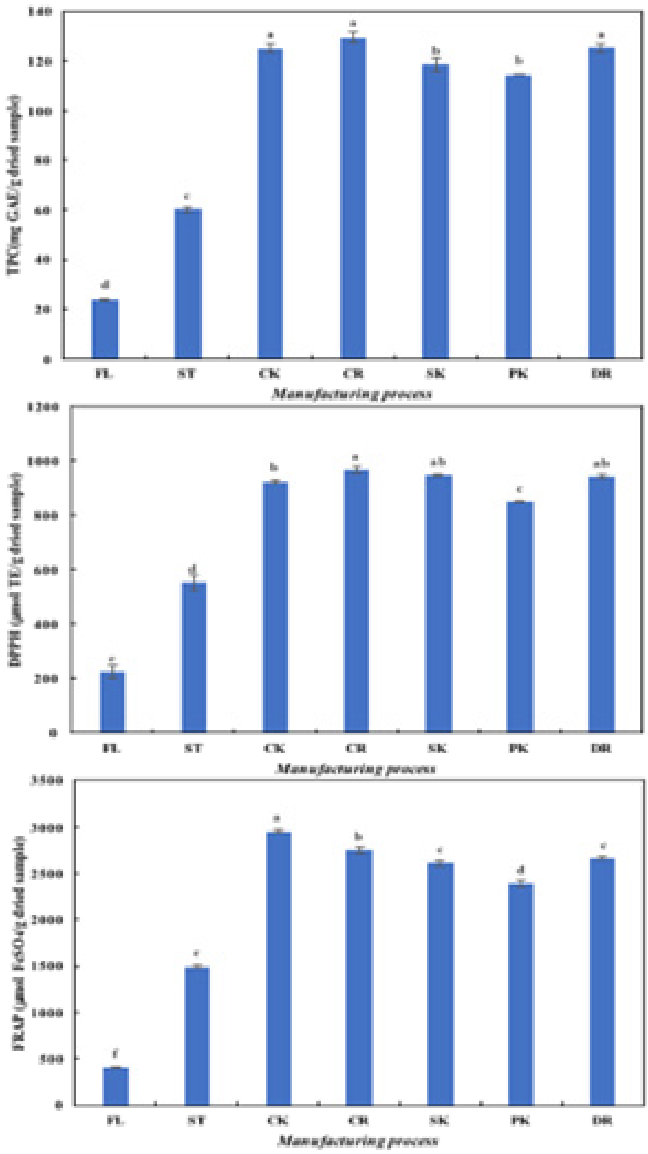

Figure 3. Changes in TPC, DPPH and FRAP values of tea leaf infusions during processing. Error bars represent standard deviations (n = 3). Different letters indicate significant differences ($P < 0.01$).

Fig. 3 depicts changes in TPC, DPPH, and FRAP values of the tea leaf infusions at each processing step. The range of TPC value was from 24 (fresh leaf) to 130 (CR) GAE/g dried sample. The range of DPPH and FRAP values were from 234 to 966 μmol TE/g dried sample and from 399 to 2950 μmol FeSO4/g dried sample, respectively. The value of TPC increased from FL to CK. However, the value mostly showed stable after CK, although the value of DR showed significantly higher than PK. These results almost corresponded to the tendency of changes in observation of the surface microstructures of tea leaf samples. Particularly, the changes in leaf microstructure and increase in dissolution of tea leaf components were found between ST from CK. In contrast, it was found that the value of TPC was increased from FL to ST but it was not corresponded the change of microstructure. This would be due to softened of tea leaves at ST processing, as a result, the components of tea leaf would be extracted easily (Tamura et al., 1987). This trend was also manifested in the change of DPPH and FRAP. These results indicated that the processes for steaming and coarse kneading were significantly important for elution of tea ingredients which mainly showed antioxidant properties in this study. However, the processes after coarse kneading had comparatively lower impact on the antioxidant properties of tea infusion, even though these processes should be needed to increase the total quality of dried tea leaves such as tea leaf appearance. This also indicated that the processing conditions for steaming and coarse kneading could be considered as one of the most important parameters for tea infusion quality.

CONCLUSIONS

The tea leaf appearance and surface microstructure were particularly changed during the processing of steaming and coarse kneading. These structural changes should relate to the changes in total polyphenol content and antioxidant properties of tea leaf infusion. Our results suggested that the quality of tea products could be affected by earlier processing steps in which tea leaf moisture was drastically changed. This finding would contribute to optimizing the tea processing conditions for the better functional quality of green tea.

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