

# Change in Ascorbic Acid Contents and Radical Scavenging Activity of Persimmon Leaf Tea in Manufacturing Process

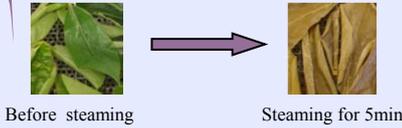
Persimmon (*Diospyros kaki* Thunb.) leaves, calyxes, and branches have long been used in Chinese medicine to stop hiccups and coughing. Because of the abundance of ascorbic acid and polyphenol in persimmon leaves, its consumption as tea and health foods has been increasing in Japan. However, the ascorbic acid and polyphenol contents in persimmon leaf tea are affected by the manufacturing process. We investigated to determine an effective manufacturing process for persimmon leaf tea that retained high antioxidant compounds.

## 【Material and Method】

### (1) Manufacture process of persimmon leaf tea

- Fresh persimmon leaves (approx 300 g flesh weight.)
- Washing (thoroughly in tap water)
- Draining (centrifuged for 1 min)
- Steaming (for 0, 1, 5, 10, 20 min)**
- Drying (machine : for 12 hours at 60 °C)
- Stored at room temperature for up to 370 days

persimmon leaf :collected from 14-year-old orchard grown Japanese persimmon 'Saijo' trees



### (2) Method of analysis of persimmon leaf tea

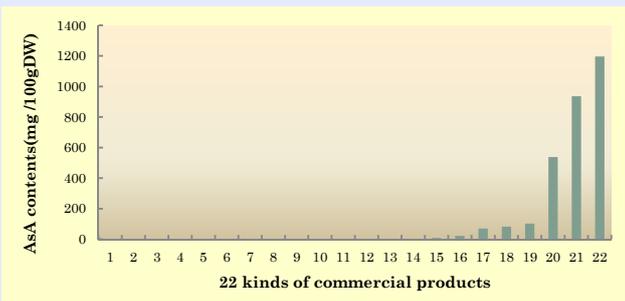
■ **Ascorbic acid analysis**: The samples (200 mg) were extracted with 2% (v/v) metaphosphoric acid. The reduced ascorbic acid (AsA) content of the extract was analyzed using HPLC (HPLC 10A system; Shimadzu Co., Kyoto, Japan) with an Inertsil ODS-2 (4.6 i.d. × 250 mm) column (GL Sciences Co. Ltd., Japan) and a UV-VIS detector (SPD-10AT; Shimadzu Corp.) at 254 nm. The column temperature was maintained at 40 °C. The mobile phase was 1% metaphosphoric acid and the flow rate was 1 ml/min.

■ **Radical scavenging activity assay**: The sample (200mg) was added to 20 ml of ultra pure water, and an extract was obtained by boiling the mixture for 10 min, after filling up to 50ml. Antioxidant activity of the crude extract of persimmon leaf tea was evaluated by DPPH radical scavenging assay. Briefly, a mixture of 70 μL of hot water leaf extract, 70 μL of 100% ethanol aqueous solution (v/v), and 70 μL of 0.2 M MES buffer at pH 6.0 were placed in a 96-well microplate. The reaction was initiated by adding 70 μL of 200 μM DPPH in ethanol. After standing for 20 min at room temperature, the reaction color was measured with a microplate-reader Sunrise-Thermo (TECAN, INC, Salzburg, AUSTRIA) at 540nm.

■ **Plyphenol analysis**: Polyphenol contents of the crude persimmon leaf tea extract was determined according to the Folin method. Briefly, a mixture of 90 μL of hot water leaf extract, 90 μL Folin-Ciocalteu reagent, and 90 μL 10% sodium carbonate were placed in a 96-well microplate. After left standing for 60 min at room temperature, the reaction color was measured with a microplate-reader Sunrise-Thermo (TECAN, INC, Salzburg, AUSTRIA) at 690nm.

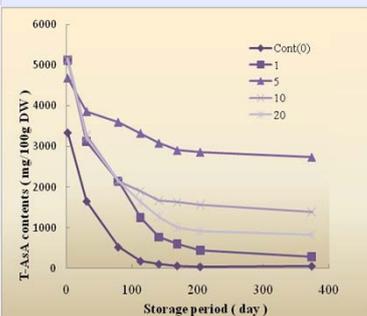
## 【Result and Discussion】

### (1) Comparison of ascorbic acid contents of 22 kinds of commercial products of persimmon leaf tea



The difference of AsA contents of the commercial products were remarkable (0~1,150mg/100g DW).

### (2) Effect of steaming treatment on AsA contents during the stored persimmon leaf tea

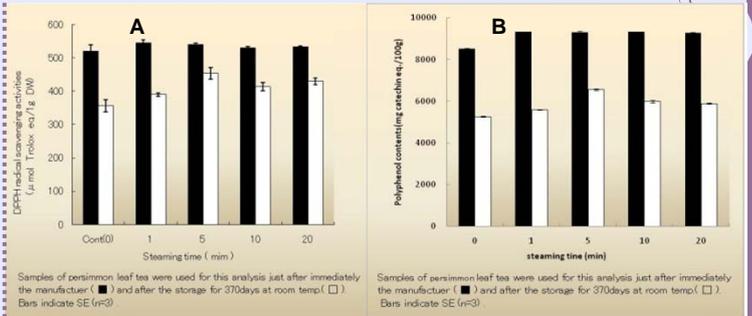


The level of ascorbic acid of non-steamed tea (3,300 mg/100 g DW) after air drying was lower than those of steamed tea (4,700~5,100 mg/100gDW) on day 0, suggesting that steaming process suppressed ascorbic acid oxidase that degrades AsA.

Five min steaming was most effective in retaining AsA level after one year storage.

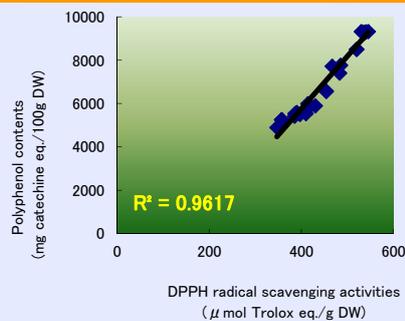
The difference of content of the ascorbic acid of commercial product may be influenced by steaming time.

### (3) Effect of the steaming on radical scavenging activities(A) and polyphenol contents(B) during the stored persimmon leaf tea



The level of radical scavenging activity (A) and polyphenol contents (B) was equivalent irrespective of steaming time on day 0 (■), but five min steaming was most effective in retaining the level of radical scavenging activity and polyphenol contents after one year storage (□).

### (4) Effect of the steaming on polyphenol contents during the stored persimmon leaf tea



There were significant correlations relation between DPPH radical scavenging activity and polyphenol contents ( $R^2=0.9617$ ).

High radical scavenging activity may result from high polyphenol contents.

## 【Conclusion】

Five min steaming of persimmon leaf was an effective manufacturing process in retaining high levels of ascorbic acid, polyphenol and DPPH radical scavenging activity even after one years storage. High radical scavenging activity may in the leaf result from its high polyphenol content.