**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)

Fresh persimmon leaves collected from 14-year-old orchard grown Japanese persimmon 'Saijo' trees

Before steaming Steaming for 5 min

(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 Inertisil 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 (200 mg) was added to 20 ml of ultrapure water, and an extract was obtained by boiling the mixture for 10 min, after filling up to 50 ml. 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 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 540 nm.

- **Phyphenol analysis**: Phyphenol 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 of 0.2% Sodium carbonate, and 90 μl of 0.2% sodium carboxymethyl cellulose 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 690 nm.

The difference of content of the commercial products were remarkable (6~1,150 mg/100g DW).

The level of radical scavenging activity (A) and polyphenol contents (B) was determined 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 (□). There were significant correlations between DPPH radical scavenging activity and polyphenol contents (R²=0.9617). High radical scavenging activity may result from high polyphenol contents.

**Result and Discussion**

(1) Comparison of ascorbic acid contents of 22 kinds of commercial products of 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/100 g DW) 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

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

**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 year’s storage. High radical scavenging activity may in the leaf result from its high polyphenol content.