Polyphenol biofortification of tea leaves by exposure to ozone

Shoko Watanabe[1], Takeshi Ogawa[1,2], Eiji Kobayashi[3], Yoriyuki Nakamura[3], Tomoko Kobayashi[4], and Hirokazu Kobayashi[1]

1: Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526 Japan
2: Local Collective Promotion Office, Food Science Center, The Shizuoka Organization for Creation of Industries, 44-1 Otemachi, Aoi-ku, Shizuoka 420-0853 Japan
3: Tea Research Center, Shizuoka Prefectural Research Institute of Agriculture and Forestry, 1706-11 Kurasawa, Kikugawa, Shizuoka 439-0002 Japan
4: K-E Corporation, 625-7 Anahara, Shimizu-ku, Shizuoka 424-0301 Japan
Correspondence email: hirokoba@u-shizuoka-ken.ac.jp

Summary

Plants suffer from environmental stresses, resulting in change of metabolites in which regulation of gene expression may be involved. It is speculated that plants may accumulate antioxidant substances to protect themselves against the oxidative stress, when they are exposed to ozone (O_3). Leaves of tea (*Camellia sinensis* cv. Yabukita) were detached from tea plants such as being harvested, treated with O_3 up to 24 hours, and subjected to time-sequential analysis of polyphenols by UPLS-TOF-MS. The exposure of tea leaves to (1) 3-4 ppm and (2) 0.03 ppm O_3 increased the content of epigallocatechin, epicatechin-gallate, kaempferol, quercetin or myricetin, and significantly increased other 35 compounds.

Introduction

Shizuoka Prefecture produces tea leaves to be manufactured for green tea, and their gross output is the highest in Japan. The higher competitiveness in cultivation and manufacture of tea would be desired. Technology to manipulate functional constituents, flavorous compounds and substances for *umami* (savoriness) during cultivation and manufacture of tea, would be required. We have focused on biofortification of polyphenols invaluable as antioxidants. Tea contains flavonoids such as catechins associated with the antioxidant activity, which may protect humans against lifestyle-related illnesses (obesity, diabetes, hyperpiesia, lipemia, hyperuricemia and cancer), allergy and inflammation, or release them from the illnesses and symptoms (Yoshikawa, 1998; Matsumura, 2002). Ozone (O_3) at lower concentration is produced inexpensively from O_2 in the air by its exposure to ultraviolet light or electric discharge. O_3 is associated with strong oxidation activity. Therefore, we have supposed that exposure of tea leaves to O_3 might result in accumulation of antioxidant compounds such as polyphenols to protect their own cells against O_3.

Materials and methods

Detached tea leaves (5 cm in length) were placed in trays and exposed to 3-4 or 0.03 ppm O_3 generated by O_3 generator (LYON TM-08IRZ, Tamura TECO) in a closed cubic space made of transparent acrylic boards at 20-25^{o}C and 40-70% in humidity. Tea leaves were taken out after exposure to O_3 for 0, 12 or 24 hours, ground with liquid N_2 by mortar and pestle, mixed with H_2O:MeOH=1:1, and sonicated, followed by filtration through Millex-LG (Millipore) and analysis by UPLC-TOF-MS (Waters). The UPLC was done using the column BEHC18 (Waters) with H_2O-acetonitrile (mobile phase) at 0.25 ml/min at 40^{o}C by injection of 3 µl of samples maintained at 4^{o}C. The data of mass and retention time were analyzed by software MarkerLynx and further assigned to compounds by referring to ChemSpider.

Results and discussion

The contents of polyphenols after exposure to 3-4 or 0.03 ppm O_3 for 0, 12 and 24 hours are shown in Figure 1. Flavanols such as epicatechin, epigallocatechin and epigallocatechin-gallate, and flavonols such as kaempferol, quercein and myricetin, accumulated at higher levels by O_3 treatment.

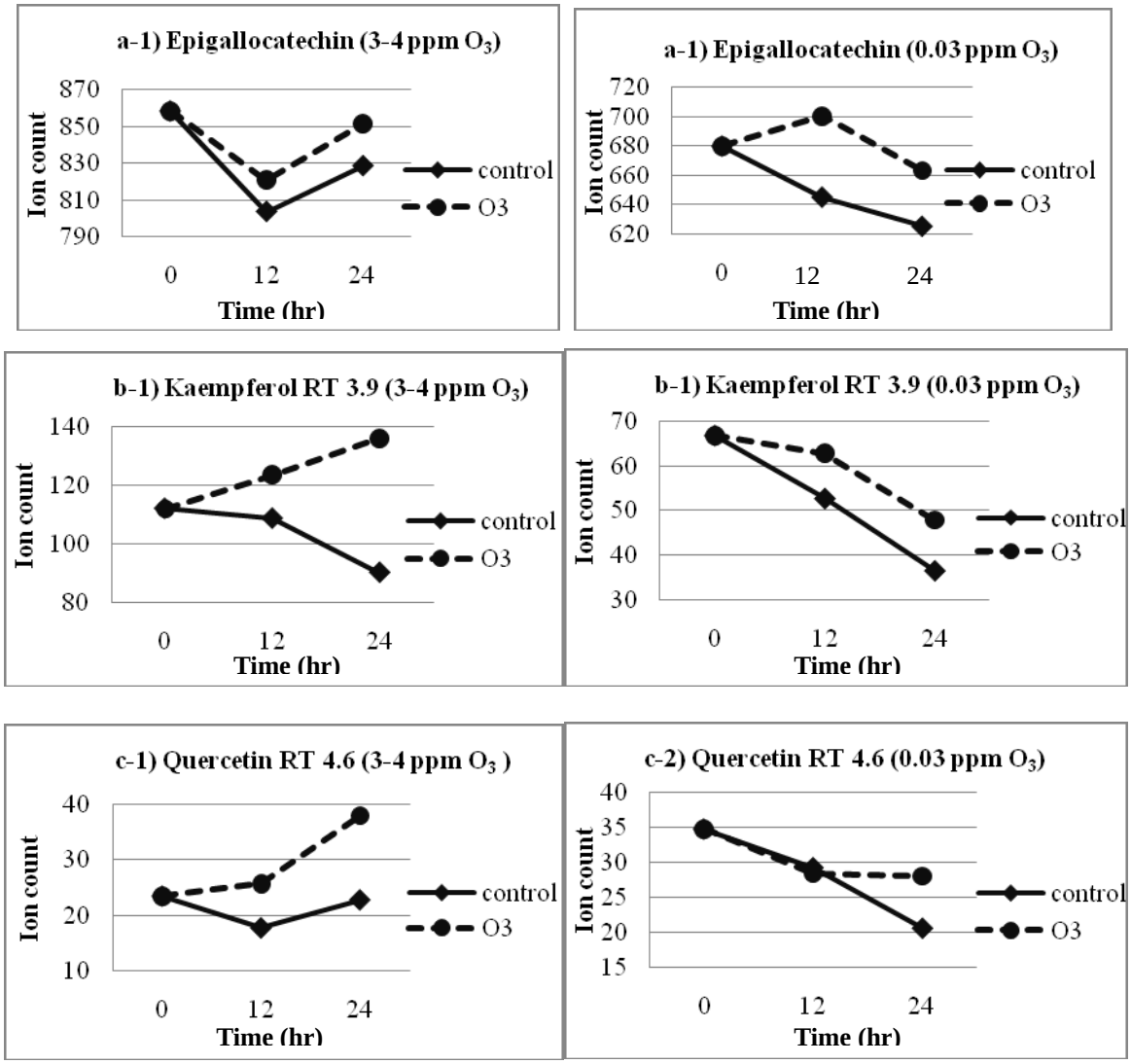

Figure 1. Polyphenols present more abundantly by exposure to O_3 at either 3-4 or 0.03 ppm. Flavonols shown (kaempferol and quercetin) were detected as forms of glycosides. RT, retention time.

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References

Yoshikawa, T., ed. (1998) Medicine of the Flavonoid (in Japanese), Kodansha, Tokyo

Matsumura, K., ed. (2002) Health Science of Tea: New Possibility for Physiological Function, Japan Scientific Societies Press (in Japanese), Tokyo