Development of a new method for simultaneous determination of vitamin E eight homologues using LC-MS3 and studies on vitamin E homologues distribution in plants

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Vitamin E, which is a family of fat-soluble vitamins, have eight homologues, namely α-, β-, γ- and δ-tocopherols, and α-, β-, γ- and δ-tocotrienols. Eight homologues share a common structure of chromanol head and phytyl tail. The most well-known function of vitamin E is the chain-breaking antioxidant activity that prevents the cyclic propagation of lipid peroxidation. Antioxidant activity is thought to prevent cardiovascular diseases, neurological diseases and cancers. Vitamin E is most abundant in food oil. Other sources of vitamin E are whole grains and peanut butter, however, except for food source, there is little information about eight homologues of vitamin E in plants. The aim of this study is to elucidate the presence of vitamin E homologues in plants and develop simultaneous method to analyze those homologues LC-MS3 has recently been used in pharmaceutical, biochemical and food chemical fields as a rapid and sensitive strategy for analyzing chemicals. Therefore, LC-MS3 method was applied in this study to measure the four tocopherols and four tocotrienols in plants.

LC-MS3 method to analyze eight kinds of vitamin E homologues consists of 3 steps; 1) Optimize ion spray voltage, curtain gas pressure and ion source gas pressure for protonated molecules, 2) Achieve a complete separation of each homologue using PFP column, 3) Integrate eight optimized ionization parameters into one method. By integrating the most suitable parameters for determinationing four tocopherols and four tocotrienols, simultaneous determination methods was newly developed as a result.

Ion spray voltage at 5500 V, curtain gas pressure at 20 psi and ion source gas pressure at 20 psi is found to be optimal for determining four tocopherols and four tocotrienols simultaneously. In this study, column temperature was set at 40 ℃ and H_2O : methanol ratio was adjusted at 10 : 90 (v/v) to be used as mobile phase. This is the first report on development of a new method for the simultaneous determination of vitamin E homologues by ESI (+) LC-MS3. Compared with LC/MS2 method, this new method has advantages of less interference and higher sensitivity.

The presence of vitamin E homologues in 92 kinds of plant was revealed using LC-MS3. Among vitamin E homologues, only α-tocopherol was detected in all parts of 92 plant species. This result shows that α-tocopherol plays an important role in plant growth. Also, α-tocopherol tends to be detected together with γ-tocopherol. This phenomenon is attributed to the biosynthetic pathway of vitamin E since γ-tocopherol is

the precursor of α-tocopherol in biosynthesis of vitamin E. β-, and γ-tocopherols were detected in medicinal plants and, γ-tocopherol and α-tocotrienol were detected mainly from leaves of native plants of tropical Madagascar. Presence of tocotrienols was confirmed specifically in native plants of tropical Madagascar and subtropical Miyako Island.

Since severe condition is considered to increase stresses for native plants of these areas, environmental stress might have caused the production of tocotrienol. At first, the effect of UV was considered to be a main factor for tocotrienol production. Production of tocotrienol in plant leaf samples was measured *in vitro* after UV radiation. One sample shows the increase of α-tocotrienol after UV B radiation.

Secondly, salt concentration of water was checked for its effect on tocotrienol production in plants. *Sporobolus virginicus* (L.) was cultured in 3.5, 1.75, 0.7, 0.35 and 0.18 % salt solution, and the amount of tocotrienols were measured. The production of α-tocotrienol was not increased when this plant grew under the condition where water contained 1.75, 0.7, and 0.18 % of salt. Soil containing salts, ultraviolet light and temperature changes in tropical area are complex factors that may induce excessive reactive oxygen species (ROS) production in plants. Since α-tocopherol plays a role in protecting oxidative damage caused by ROS, it is considered that plants might produce α-tocopherol and α-tocotrienol as a defense function for excess ROS.

The results obtained in this study will contribute to the research on vitamin E dynamics in plants and the biosynthetic pathway of vitamin E in plants, which have not been elucidated. Furthermore, elucidation of the distribution of vitamin E in foods will help not only the development of nutrition science and dietetics but also to promotion of the human health in the long-term.