E-E2-03

Novel method of ceramide analysis in biological samples by HPLC after deacylation with ceramidase

Youn-Sun Lee[1], Jae-Myung Yoo[1], Kyong-Mi Choi[1], Yong-Moon Lee[1], Seikwan Oh[2], Tack-Joong Kim[1], Yeo-Pyo Yun[1], Jin-Tae Hong[1], Hwan-Soo Yoo[1*]

[1]College of Pharmacy and CBITRC, Chungbuk National University, Cheongju,
[2]Department of Neuroscience, College of Medicine, Ewha Womans University

Ceramide is involved in cell death as a lipid mediator of stress responses and has been implicated in human diseases such as Alzheimer's, cancer, and atopic dermatitis. To determine the concentration of total ceramide in a diversity of biological samples, we developed a new quantitative method based on added synthetic ceramide and measured endogenous ceramide. N-oleoyl-D-*erythro*-sphingosine (C_{17} ceramide) was added to samples as an internal standard, and lipids were extracted. Ceramide was resolved by thin layer chromatography (TLC), the area with an R_f value corresponding to the ceramide standard was scraped-off, and the ceramide was deacylated by ceramidase to liberate sphingosine. The released sphingosine, representing the ceramide, was measured by high-performance liquid chromatography (HPLC) following fluorescence derivatization. The limit of detection for ceramide was about 1.0-2.0 pmol and the lower limit of quantification was 5.0 pmol. Ceramide recovery was approximately 86-93%. Ceramide concentrations were determined in biological samples including mouse cultured cells, mouse tissues, and mouse and human plasma. The use of an internal standard for the determination of ceramide concentrations in diverse biological samples provides an accurate and reproducible analytical method.

*corresponding author: Tel. 043-261-3215, e-mail: yoohs@chungbuk.ac.kr

E-E2-04

Comparative contents of plant ceramides in agricultural products analyzed by HPLC

Youn-Sun Lee[1], Jae-Myung Yoo[1], Kyong-Mi Choi[1], Yong-Moon Lee[1], Seikwan Oh[2], Tack-Joong Kim[1], Yeo-Pyo Yun[1], Jin-Tae Hong[1], Hwan-Soo Yoo[1*]

[1]College of Pharmacy and CBITRC, Chungbuk National University, Cheongju,
[2]Department of Neuroscience, College of Medicine, Ewha Womans University

Ceramides (CERs) are highly bioactive compounds that affect diverse cellular functions. The objective of this study was to screen the agricultural sources of natural CERs. We developed new analytical method for plant ceramide contents in agricultural products. Samples include chaff, wheat, walnut, apple, soybean, rice, pumpkin, cabbage, onion, chestnut and potato. Total lipids were extracted from samples with ethanol after addition of N-oleoyl-D-*erythro*-sphingosine (C_{17} ceramide) as an internal standard, and then ceramide was resolved by thin layer chromatography (TLC) and deacylated by ceramidase. The released sphingoid bases were derivatized with *o*-phthalaldehyde (OPA) and measured by high-performance liquid chromatography (HPLC). The analytes were phytoceramide (PCer), ceramide (Cer) and dihydroceramide (DCer). PCer contents were ranged from 0.5 to 7.9 mg per kg agricultural products. Cer contents in agricultural products were approximately 0.3 to 3.0 mg per kg agricultural products. Chestnut, chaff and apple had the highest levels of CERs, and wheat, rice and sesame oil had the lowest levels. The sphingolipid contents were highest in chestnut and then chaff. The results indicate that chestnut and chaff may be potential sources for plant CERs.

*corresponding author: Tel. 043-261-3215, e-mail: yoohs@chungbuk.ac.kr