Direct Analysis of Esterified Docosahexaenoic Acid Oxidation Products with Liquid Chromatography-Tandem Mass Spectrometry

I. Kusumoto

Food Function Analysis Laboratory, Graduate School of Agricultural Science, Tohoku University (468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0845, Japan)

S. Kato

Food Function Analysis Laboratory, Graduate School of Agricultural Science, Tohoku University (468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0845, Japan)

K. Nakagawa*

Food Function Analysis Laboratory, Graduate School of Agricultural Science, Tohoku University (468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0845, Japan)

Abstract:

Background: Docosahexaenoic acid (DHA) plays a vital role in numerous physiological functions, particularly in the domains of brain and cognitive health, visual acuity, and cardiovascular function. Due to its six double bonds, DHA is highly prone to oxidation (e.g., during food processing, storage, and cooking). Oxidation of DHA not only diminishes the nutritional quality of food but also produces potentially harmful lipid oxidation products. Generally, oxidized lipid derivatives, including those of DHA, contribute to various inflammatory diseases. For DHA in particular, accumulating evidence suggests that certain DHA oxidation products play a distinct role in resolving inflammation, which may directly account for the documented health benefits of DHA intake. Therefore, understanding DHA oxidation is essential for preserving the nutritional quality of DHA-rich foods and for exploring the role of its oxidation products in disease pathology. Currently, direct analytical methods for DHA oxidation products in esterified forms (e.g., phospholipid) remain scarce, despite the fact that DHA is predominantly found as esterified in food and biological systems. In the present study, we addressed the analytical limitation by constructing liquid chromatography-mass spectrometry (LC-MS/MS) methods to directly analyze esterified DHA oxidation products, including DHA hydroperoxide in phosphatidylcholine (PC), triacylglycerol (TG), and cholesteryl ester (CE).

Experimental procedures: Mackerel fillet and rat whole brains were selected as representative food and biological samples, respectively. Target molecular species – PC and TG for mackerel fillet and PC and CE for rat brains – were determined based on lipid composition [1,2]. A hydroperoxide isomeric mixture for each target was prepared via photo-oxidation and used as a reference to optimize the multiple reaction monitoring (MRM) conditions. Optimized LC separation conditions were coupled with the established MS/MS analysis and applied to the actual samples, including mackerel fillet and whole brains of Sprague Dawley rats.

Results and discussion: PC 16:0/22:6 and TG 18:1_18:1_22:6 were selected as targets for food analysis based on their abundance in mackerel, as determined through LC-MS/MS analysis. For biological analysis, PC 16:0/22:6 and CE 22:6 were targeted according to reports on DHA distribution among lipid pools in the rat brain [2]. MS/MS analysis of the reference mixture for each target exhibited fragment ions corresponding to the hydroperoxide group positions, consistent with our previous observations on free DHA hydroperoxide (DHA; OOH) [3]. The optimized LC-MS/MS method demonstrated high sensitivity, as confirmed by the detection of all targeted DHA; OOH isomers in raw

mackerel. Further investigations are underway to analyze oxidized DHA in rat brains. The LC-MS/MS method offers potential applicability in exploring DHA oxidation in both food and biological systems, providing a foundation for developing regulatory measures to control DHA oxidation.

Keywords:

Lipid oxidation; DHA; Nutritional value; Hydroperoxide; LC-MS/MS.