HOKKAIDO UNIVERSITY

Title	STUDY ON SEAWEED CHLOROPHYLLS AND LIPIDS : DISTRIBUTION, BIOAVAILABILITY, AND FUNCTIONALITY [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(水産科学)
Dissertation Number	甲第14289号
Issue Date	2020-12-25
Doc URL	https://hdl.handle.net/2115/80600
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	Eko_Susanto_abstract.pdf, 論文内容の要旨

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学位論文内容の要旨

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学 位 論 文 題 目

STUDY ON SEAWEED CHLOROPHYLLS AND LIPIDS: DISTRIBUTION, BIOAVAILABILITY, AND FUNCTIONALITY

（海藻のクロロフィルと脂質に関する研究：分布,生体利用および機能性）

Chapter 1. (Introduction). Seaweeds are a large group of 28,792 species divided into three phyla by botanists based on pigments distributions, mainly chlorophylls (Chls) and carotenoids. Chlorophylls and carotenoids, both abundant in seaweeds, are important pigments in the photosynthesis system. Among both pigments, carotenoids are well-known to positively contribute to human health; however, Chls, which are regarded as the most abundant pigment in nature, have not been paid much attention due to their low bioavailability. Currently, Chls are paid attention to apply for the treatment of cancer and anti-inflammatory diseases. Although the role of CDs in the prevention of chronic diseases in humans is still unclear and their anti-cancer, photodynamic therapy, and anti-inflammation effects are limited, it is important to study their compositions, bioavailability, and bio-functionality in seaweeds as major components. Therefore, this study aimed to investigate the composition, in-vitro bioavailability, and functionality of isolated CDs generated from seaweeds. In this study, bioavailability and the suppressive mechanism for intracellular stress such as ROS and MDA in HepG2 cells were investigated by using pure CDs.

Chapter 2. The study analyzed the various lipid components of 15 species of seaweeds belonging to the Chlorophyta, Ochrophyta, and Rhodophyta phyla collected in tropical (Indonesia) and temperate (Japan) climates. Analyses were performed on multiple components, including Chls, carotenoids, n-3, and n-6 polyunsaturated fatty acids (PUFAs), as well as alpha-tocopherol (α-Toc). The results showed that Chls and carotenoid contents varied among phyla with different sampling locations. Chl a and Chl b were the major Chls in Chlorophyta, while Chl a and Chl c were the main Chls in Ochrophyta, with a dominant Chl a in Rhodophyta. Additionally, β-carotene and fucoxanthin (Fx) were detected as major seaweed carotenoids; β-carotene was present in all species in a variety of ranges, while Fx was mainly found in Ochrophyta and not at all in Chlorophyta. The TL content and FAs composition were strongly affected by sampling location, with TL and n-3 PUFAs levels higher in temperate seaweeds than in tropical ones. The major n-3 PUFAs in different phyla, namely, eicosapentaenoic acid (EPA) and stearidonic acid SDA in Ochrophyta, α-linolenic acid (ALA) and SDA in Chlorophyta, and EPA in Rhodophyta, were major fatty acids in temperate seaweeds. The CDs and carotenoids are associated with various health benefits, such as antioxidant activities,

while n-3 PUFAs are essential nutrients that positively influence human nutrition and health. However, among the phytochemicals, CDs are less exploited; therefore, the following chapters discuss their potential.

Chapter 3. CDs need to be satisfied with the bioavailability in the human body to maximize their health benefits. To date, their characteristics have not been fully elucidated; therefore, this study addressed absorptions of 6 CDs with different structures—Chl a, Chl b, Chl c2, Phy a, Phy b, and Pheo a—in dCaco-2 cells. The present study demonstrated that the absorption of CDs was completed in the following order: Phy a>Pheo a>Chl c2>Phy b>Chl a>Chl b. However, to determine their characteristics in different concentrations and temperatures, the four most absorbable CDs (Phy a, Pheo a, Chl c2, Phy b) were used. Phy a showed a passive diffusion mechanism, while other CDs indicated that saturations in higher concentrations were associated with facilitated uptake. Therefore, to better characterize the CDs, only Phy b and Pheo a were used for bi-direction study, in which they demonstrated efflux ratios of 3.07 and 1.3, respectively. The efflux ratios showed that ABC efflux transporters are majorly involved in the CDs absorptions, with the ABCB1 and ABCG2 involved in the Pheo a efflux from dCaco-2 cells, while ABCG2 and ABCG5/ABCG8 were found in the Phy b efflux activity. Furthermore, in the case of the CDs absorption, the presence of NPC1L1, SR-B1, and CD36 inhibitors reduced the absorption of Pheo a, while NPC1L1 and CD36 inhibitors lowered the Phy b absorptions. These results imply that Phy b and Pheo a absorptions are partly mediated through the lipophilic transporter and are modulated based on the mRNA expressions.

Chapter 4. This chapter examined the biological activity of different structures of CDs to reduce oxidative stress in human hepatocellular carcinoma (HepG2) cells stimulated by H_2O_2. The primary endpoint of this study was the ability of different CDs to reduce and protect HepG2 cells from the excessive amount of ROS with an increase in antioxidant status in both chemical and cellular-based activities. Furthermore, there was a significant ($p<0.05$) effect of reducing ROS and MDA in HepG2 cells through the modulation defense enzymes, with the pretreatments of CDs denoting the protective effects of HepG2 damaged by H_2O_2.

In conclusion, the present study shows seaweeds are potent bioresources for functional Chls and lipids. The variation of seaweed lipid bioactives such as CDs distributed according to seaweed phyla rather than geographical location. This study provides evidence that the various CDs from seaweeds showed different absorption rates in dCaco-2 cells. The different structures of CDs displayed the different mechanisms of each CD absorption, which are partly facilitated by intestinal transporter. Moreover, CDs originated from seaweeds showed the antioxidant activity and protected HepG2 cells against oxidative stress induced by H_2O_2. More effort should be done to make clear the biological activities of CDs from seaweeds, judging from the high content of CDs in the seaweeds lipids, they are expected to have potential for the use of functional food materials.