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研究課題名（和文）Study on anti-AD functional marine phytosterols and their sources in dietary seaweeds

研究課題名（英文）Study on anti-AD functional marine phytosterols and their sources in dietary seaweeds

研究代表者

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研究成果の概要（和文）：アルツハイマー病（AD）は、脂質代謝異常が関与する可能性のある神経変性疾患である。植物ステロールはコレステロールの吸収を抑制し、高ＬＤＬコレステロール血症等の脂質代謝異常に機能するといわれるが、海藻由来の植物ステロールに関する研究報告が少ない。本研究では、神経細胞モデルを用いて食用海藻から得られた植物ステロールによる抗AD生物活性を評価した。また、抗AD植物ステロール資源を探求するために様々な食用海藻中に存在する植物ステロールを網羅的に解析し定量した。本研究は、食品由来新規抗AD機能成分として海藻由来植物ステロールを開発しただけでなく、健康に有益な海藻資源の理解を深めたにも役に立つと思われる。

研究成果の学術的意義や社会的意義
It is first time to evaluate marine phytosterols for their anti-AD bioactivity via lipid metabolism, and first time to prepare phytosterols from seaweeds with method optimization, as well as quantitative profiling. This study developed marine phytosterols as new anti-AD functional food components.

研究成果の概要（英文）：Alzheimer's disease (AD) is a serious neurodegenerative disease involved in lipid metabolism disorder, while phytosterols are potential anti-AD components via regulating lipid metabolism. Although terrestrial phytosterols are well studied, marine phytosterols of seaweeds have been ignored for long. In this study, phytosterols were extracted and purified from edible seaweeds using the optimized procedure. Then, they were evaluated for anti-AD bioactivities via lipid metabolism regulation based on nerve cell model. Next, phytosterols in various dietary seaweeds were profiled and determined for exploring the dietary anti-AD phytosterol resources. This study not only developed marine phytosterols as new anti-AD functional components from foods, but also contribute to a better understanding of health-beneficial seaweed resources.

研究分野：食品科学関連

キーワード：Phytosterols　Seaweeds　Alzheimer's disease　Nerrve cells　Lipid metabolism　Quantitative analysis

１．研究開始当初の背景

Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of dementia, and has become one major cause of disability among older people. Accumulating evidence suggests lipid metabolism disorder in the central nervous system. Therefore, improving nervous system lipid metabolism is a promising AD therapeutic strategy.

Phytosterols have been widely known as effective cholesterol-regulating agents in the diet. Studies have shown their beneficial effects on the central nervous system, including the removal of the amyloid precursor protein, the reduction of neuro-inflammation, and the enhancement of remyelination. Recent animal studies also suggested phytosterols as natural anti-AD components through nervous system lipid metabolism improvement. However, while terrestrial phytosterols have been widely studied, comprehensive studies on the structural diversity and biological activity of seaweed phytosterols are relatively limited. Especially, the anti-AD effects of seaweed phytosterols are rarely reported.

Besides, although chemical investigation and bioactivity evaluation of seaweed phytosterols showed their value as functional food components, there are few reports regarding their resource exploration: There is no detailed profiling analysis for understanding the phytosterol characteristics (fingerprints); and for bioactive seaweed phytosterols, there is no quantitative analysis as the fundamental information of functional phytosterol resources. Seaweed foods are popular in Hokkaido, Japan, such as Kombu, Hijiki, and Nori, in which the phytosterols vary along species, environmental factors, cultivation methods, and other factors. But their nutritional values with respect to phytosterol are yet to be explored. Therefore, seaweed phytosterols are newly known as dietary functional components to regulate lipid metabolism, but their further anti-AD potentials, as well as sources in seaweeds, need to be investigated in detail.

２．研究の目的

To hunt for dietary seaweed phytosterols that show anti-AD potential through lipid metabolism regulation, and to investigate their amount in seaweeds.

３．研究の方法

In this study, qualitative and quantitative analysis, as well as in-vitro bioactivity evaluation, will be the major approaches to comprehensively screening the bioactive seaweed phytosterols and targeting their enriched seaweeds.

(1) NMR-based phytosterol analysis

① ^{1}H NMR condition optimization: The parameters for ensuring the feasibility of phytosterol detection were optimized.

② Phytosterol profiling: The NMR signals were assigned via comparison with standards; The whole phytosterols in various kinds and batches of seaweeds were profiled; Chemometrics were performed for understanding marine phytosterol fingerprint variations using multivariate statistical analysis.

③ Phytosterol quantitation: The quantitation method for seaweed phytosterols was developed and validated; Their content in different dietary seaweeds was compared.

(2) In-vitro anti-AD bioactivity evaluation

① Cell culturing and modeling: The PC12 cells as the commonly used neuronal cells were purchased from the JRCB cell bank and were cultured in RPMI 1640 medium. Then, the cells were differentiated by nerve growth factor; The PA-induced AD model in PC12 cells was used to evaluate the in-vitro anti-AD bioactivities of the obtained seaweed phytosterols.

② Cell protection assays: The cell morphology was evaluated using the microscope; The cell damage was tested using the cell counting kit (CCK-8).

③ Cellular lipidomics: The total lipids from the cells were extracted by Folch's method and analyzed using LC/MS under the optimized conditions. The identification of lipid molecular species was conducted on the basis of high-resolution MS and MS/MS characteristics, as well as HPLC behavior with the comparison of the in-house database. The intensity of each lipid species calibrated by internal standards was calculated for amount comparison and further statistical analysis.

4．研究成果

(1) Detection of phytosterols using ^{1}H NMR

The typical ^{1}H NMR spectrum of brown seaweed phytosterols in our study is shown in Figure 1. Such identical and specific NMR signals were fundamental to determining multiple sterols simultaneously.

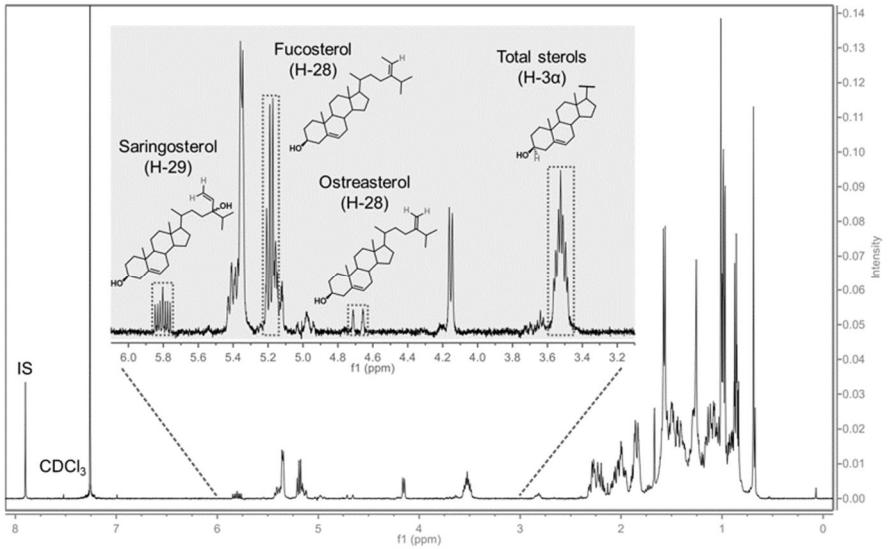

Figure 1. Typical ^{1}H NMR spectrum of phytosterols in brown seaweed sample

(2) Optimization of phytosterol extraction and purification procedures

The current results supported the classic CHCl3-MeOH system (with a one-phase extraction followed by a two-phase partition) was the most suitable solvent system, and CHCl3-MeOH 2:3 was finally selected as the most suitable solvent to extract phytosterols from brown seaweeds (Figure 2).

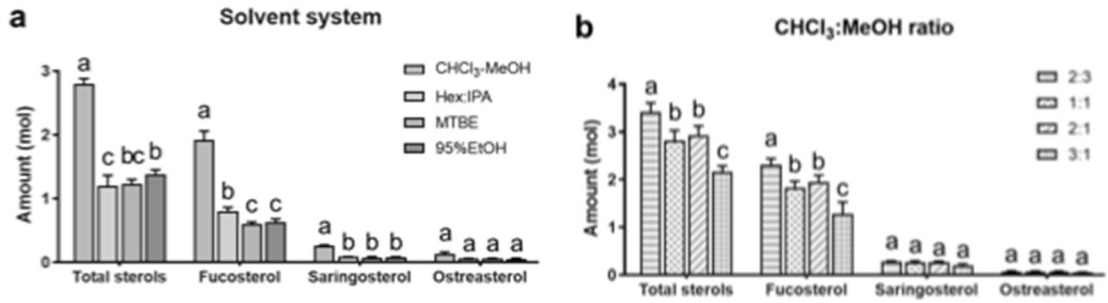

Figure 2. (a) Comparison of different extract solvent systems for extracting Seaweed phytosterols, including $CHCl_3$-MeOH, Hex-IPA, MTBE-IPA, and EtOH-H_2O;
(b) Comparison of different $CHCl_3$-MeOH ratios for extracting phytosterols, including 2:3, 1:1, 2:1, and 3:1 (Bars that do not share similar letters denote statistical significance, by two-way ANOVA)

The central composite design (CCD) was applied to optimize the saponification process for seaweed phytosterols and evaluate the effects of KOH concentration, reaction time, and solution volume. As a result, the final optimal saponification condition was obtained by the numerical expectation function method and was decided as follows: KOH concentration, 1.85 M; reaction time, 14.5 h; solution volume, 1.65 mL (Figure 3). Moreover, the currently optimized method was compared with the typical methods (A and B) reported previously (Figure 4). Our method exhibited much higher efficiency for phytosterol accumulation, which confirmed that seaweed phytosterols could be more effectively achieved through the comprehensive optimization of extraction and purification.

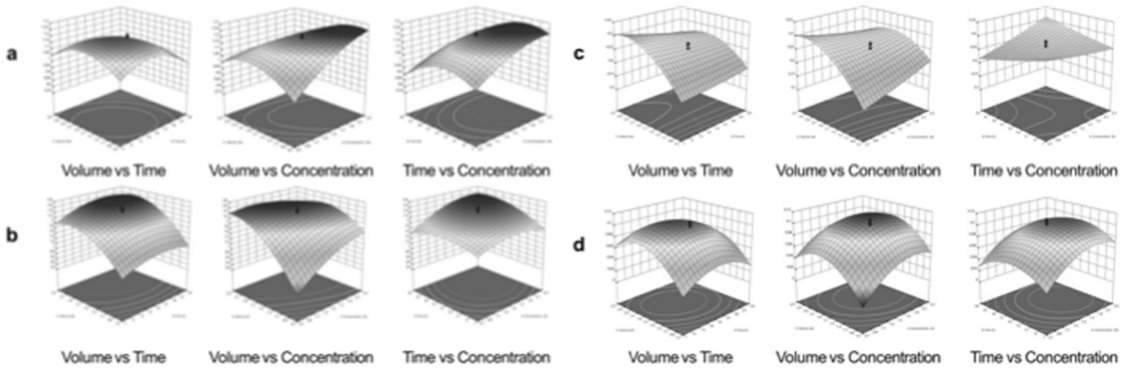

Figure 3. Response surface showing effects of independent variables (X1, concentration of KOH; X2, time of reaction; X3, volume of solution) on total phytosterol (a), fucosterol (b), saringosterol (c), and ostreasterol (d)

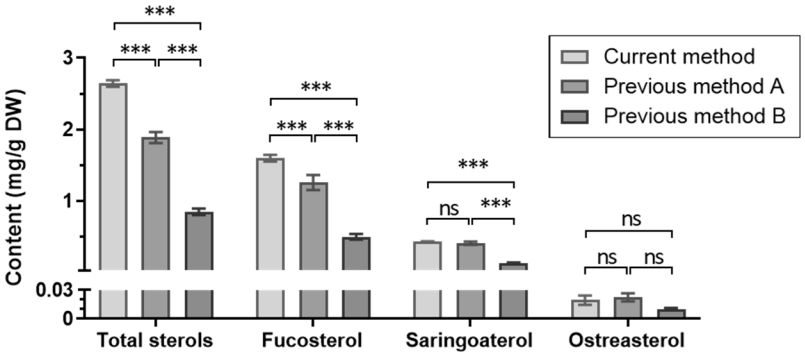

Figure 4. Comparison of currently optimized and previous methods for the obtained content of total sterols, fucosterol, saringosterol, and ostreasterol (*** $P < 0.001$, by two-way ANOVA)

(3) Validation and application of the quantitation method

Method validation was conducted according to JP17, and the data proved that the developed determination method using ^{1}H NMR was reliable and practical for quantitatively investigating phytosterols in edible brown seaweeds.

Then, we applied the established methods to 16 batches of three commonly consumed brown seaweeds collected from Japan, including 4 batches of Hijiki, 4 batches of Wakame, and 8 batches of Kombu. The total phytosterol content varied among different kinds of seaweeds significantly ($P < 0.05$): Hijiki exhibited the highest total sterol amount (2.601 ± 0.171 mg/g DW), followed by Wakame (1.845 ± 0.137 mg/g DW), while Kombu showed the lowest (1.171 ± 0.243 mg/g DW). For each phytosterol constituent, the content of fucosterol expressed the same trend as total sterols, i.e., Hijiki > Wakame > Kombu ($P < 0.05$ for all), while saringosterol did not show significant distinction in the investigated samples (Figure 5).

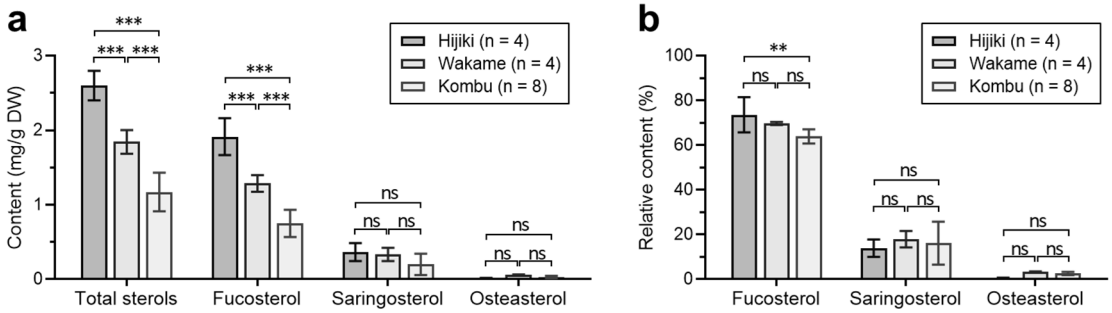

Figure 5. Comparison of the phytosterols in three common edible seaweeds: (a) Content of total sterols, fucosterol, saringosterol, and ostreasterol; (b) Relative content of each phytosterol component (** $P < 0.01$, *** $P < 0.001$, by one-way ANOVA)

(4) Anti-AD potential of seaweed-derived phytosterols

Cell viability significantly decreased to 66.6% ± 19.7% in the PA-treated group compared to the control group, while it significantly increased to 95.4% ± 10.8% in the 10 μg/mL phytosterol-treated group and 110.9% ± 11.4% in the 30 μg/mL phytosterol-treated group. Thus, the cytoprotective effects of phytosterol-treated phytosterols were confirmed (Figure 6).

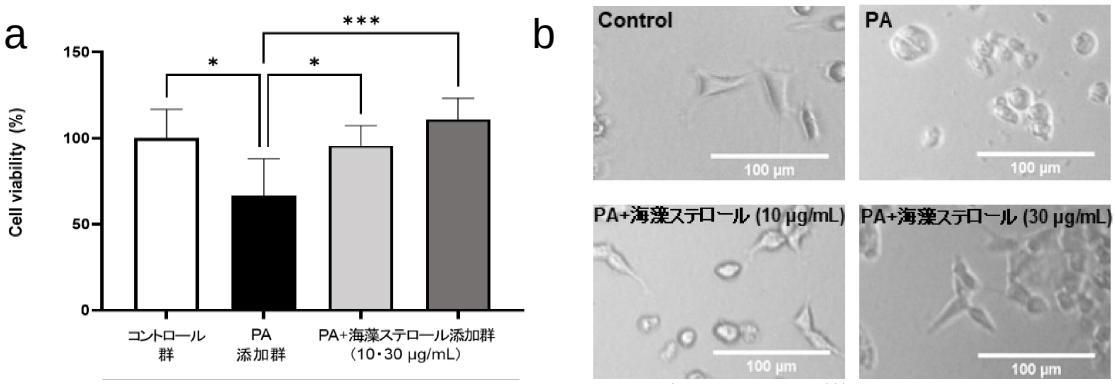

Figure 6. (a) Cell viability in each group (* $P < 0.05$, *** $P < 0.001$); (b) Cell differentiation in each group

The total TG content significantly increased to 11.6 ± 5.3 nmol in the PA-treated group compared to 1.7 ± 0.3 nmol in the control group, while it significantly decreased to 9.4 ± 4.7 nmol in the phytosterol-treated group (Figure 7a). A similar trend was observed for various fatty acyl chains in the TG fatty acid composition assessed by LC-MS/MS (Figure 7b). The results suggested that seaweed phytosterols could inhibit TG accumulation in the cells.

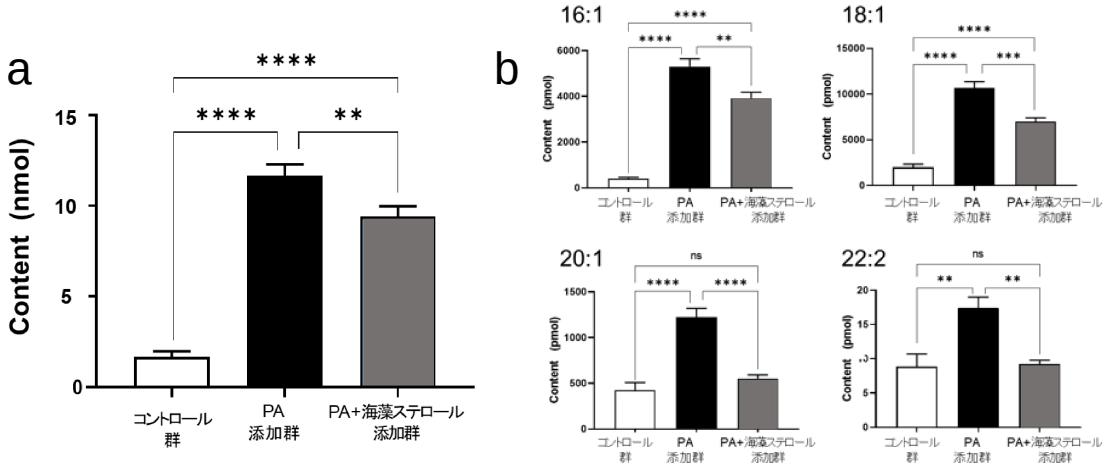

Figure 7. (a) Comparison of total TG content in each group; (b) Representative fatty acyl chains showing a similar trend to the total TG content in each group (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, by one-way ANOVA)

In the PA-treated group, a decrease in cell viability and shrinkage of neurite length were observed. Furthermore, the intracellular lipid analysis revealed the accumulation of TG. Therefore, it is believed that the decrease in cell viability was caused by oxidative stress due to TG accumulation. Additionally, it is suggested that neurite shrinkage was caused by the induction of apoptosis. In contrast, seaweed phytosterols were found to suppress the accumulation of TG. As a result, it is believed that the recovery of cell viability was achieved by inhibiting oxidative stress caused by lipid overloading.

In summary, in this study, phytosterols from seaweeds were extracted and purified using an optimized procedure, and the phytosterols were profiled and determined among different seaweeds. Moreover, the beneficial effects of seaweed-derived phytosterols on AD-modelled PC12 cells were identified.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　1件）

１．著者名 Chen Zhen、Jia Jiaping、Wu Yue、Chiba Hitoshi、Hui Shu-Ping	４．巻 383
２．論文標題 LC/MS analysis of storage-induced plasmalogen loss in ready-to-eat fish	５．発行年 2022年
３．雑誌名 Food Chemistry	６．最初と最後の頁 132320～132320
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.foodchem.2022.132320	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Chen Zhen、Shen Nianqiu、Wu Xunzhi、Jia Jiaping、Wu Yue、Chiba Hitoshi、Hui Shuping	４．巻 12
２．論文標題 Extraction and Quantitation of Phytosterols from Edible Brown Seaweeds: Optimization, Validation, and Application	５．発行年 2023年
３．雑誌名 Foods	６．最初と最後の頁 244～244
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/foods12020244	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Nianqiu Shen, Zhen Chen, Yue Wu, Hitoshi Chiba, Shu-Ping Hui
２．発表標題 Comparison of marine phytosterol amount in edible seaweeds
３．学会等名 第５回FHS国際シンポジウム（The 5th FHS International Conference）（国際学会）
４．発表年 2021年

１．発表者名 Nianqiu Shen, Zhen Chen, Yue Wu, Hitoshi Chiba, Shu-Ping Hui
２．発表標題 Investigation of marine beneficial phytosterol from different seaweeds
３．学会等名 第7回 北大・部局横断シンポジウム（The 7th Hokkaido University Cross-Departmental Symposium）
４．発表年 2021年

１．発表者名 Nianqiu Shen, Zhen Chen, Yue Wu, Hitoshi Chiba, Shu-Ping Hui
２．発表標題 Comparative investigation of health beneficial plant sterols from different algae
３．学会等名 第31回日本臨床化学会北海道支部例会
４．発表年 2021年

１．発表者名 沈 テン秋, 陳 震, Wu Yue,呉 訓智, 千葉 仁志, 惠 淑萍
２．発表標題 Optimization of extraction methods for seaweed phytosterols and evaluation of their neurological bioactivity
３．学会等名 第62回日本臨床化学会年次学術集会
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関