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# Quantitative Analysis of Isoflavones and Lignans in Sea Vegetables Consumed in Korea Using Isotope Dilution Gas Chromatography-Mass Spectrometry

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**Abstract** The phytoestrogens including isoflavones (genistein, daidzein, biochanin A, formononetin, and glycitein), coumestrol, and lignans (secoisolariciresinol, matairesinol, and anhydrosecoisolariciresinol) were quantified in edible sea vegetables from Korea. Sea vegetable samples were collected based on domestic consumption data. After hydrolysis of phytoestrogen glycosides in prepared samples, aglycones of phytoestrogens were extracted with diethyl ether and analyzed with isotope dilution gas chromatography-mass spectrometry in selected ion monitoring mode (ID-GC-MS-SIM). Total samples included 19 samples representing eight species. Most of the samples showed rather low concentrations, ranging from not determinated to 79.2 μg/kg for isoflavones and from 106.4 to 694.8 μg/kg for lignans. The daily intake of phytoestrogen from sea vegetables, estimated from the present data and domestic consumption data, was about 0.13 μg/day for isoflavones and 2.0 μg/day for lignans. When we compared these results with those from legumes, sea vegetables would not be considered the major source of phytoestrogens in the Korean diet.

**Key words:** sea vegetables, phytoestrogens, isoflavones, lignans, ID-GC-MS

## Introduction

Sea vegetables are popular food throughout Asian countries. More than 50 species of sea vegetable are eaten as fresh or dried vegetables, or used as food additives in Korea (1). Recently there has been increasing interest in using sea vegetables for human and animal nutrition (2), as they contain substantial amounts of polysaccharides (3, 4), lipids (5), proteins (2, 5), and minerals (6). Moreover, many studies have shown that sea vegetables contain various bioactive compounds showing antioxidant (7), antitumor (7, 8), or immune modulation activity (9), even though the responsible compounds are not clearly defined.

Phytoestrogens have been suggested to have beneficial effects for the prevention of cardiovascular disease and osteoporosis due to their antioxidative, estrogenic, and vascular effects (10-12). However, their effects on cancers of reproductive tissues and the endocrine system are still controversial (12). The typical human diet contains diverse phytoestrogens with structural similarities to estrogens. These phytoestrogens are classified as isoflavones or lignans (11). Isoflavones are contained mainly in legumes, whereas lignans are widely distributed in many types of plants: nuts, legumes, grains, vegetables, fruits, and teas (13-17). While the distribution of lignans is varied widely, chemical analyses of lignans in diet have not been adequately assessed. Most phytoestrogen analyses have been confined to isoflavones. Despite the importance of sea vegetables in Korean diet, phytoestrogen analysis in sea vegetables has yet to be reported.

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In this study, both isoflavones and lignans were simultaneously quantified using isotope dilution gas chromatography-mass spectrometry in the selected ion monitoring mode (ID-GC-MS-SIM). This technique is appropriate for quantifying a large range of foods with different matrixes by adding the deuterated standard chemicals as internal standards prior to extraction. Nineteen samples of commonly consumed species of sea vegetables were subjected to analysis to offer useful information to assess phytoestrogen intake in Korea.

## Materials and Methods

**Sample preparation** Nineteen samples of eight commonly consumed species were selected based on average daily consumption data (18). Sea vegetables were obtained from local markets in several areas to reflect usual consumption patterns. Fresh samples were freeze-dried using an Ilsin Engineering FD5508 lyophilyzer (Yangju, Korea). Other dried and freeze-dried samples were ground, passed through 60-mesh sieve, and stored at -20°C until analyzed.

**Reagents** The reagents used in this study were of analytical grade or better. Methanol and ethyl acetate were purchased from Rathburn Chemicals Ltd. (Walkerburn, Peeblesshire, Scotland). Pyridine, toluene, glacial acetic acid, sodium hydroxide, diethyl ether, and hydrochloric acid were obtained from E. Merck AG (Darmstadt, Germany). Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) were from Pierce Chemical Co. (Rockford, IL, USA). Activated charcoal was obtained from Sigma Chemical Co. (St. Louis, MO, USA). *Helix pomatia* juice was purchased from Biosepra IBF/Sepracor (France).

**Standards** Natural and deuterated standards for the isoflavones including genistein, daidzein, formononetin, biochanin A, and glycitein, coumestrol, and the lignans including secoisolariciresinol (SECO), anhydrosecoisolariciresinol (ANHSEC), and matairesinol (MAT) were provided by Dr. Herman Adlercreutz (Clinical chemistry, University of Helsinki, Finland).

**Extraction and purification** Extraction and purification were carried out using the method of Mazur *et al.* (14). In Brief, 50 mg of dried and milled sea vegetable was rehydrated in 0.5 mL of $H_2O$. Deuterated internal standards of isoflavones were added and the isoflavone glycosides were hydrolyzed by enzyme (*Helix pomatia* juice) for 2 hr at 60°C. *Helix pomatia* juice was treated with activated charcoal before use to get rid of any phytoestrogens known to be present. Hydrolyzed isoflavones were extracted twice with 5 mL diethyl ether and separated by freezing the aqueous phase in an ethanol-dry ice bath. To the thawed aqueous phase containing the remaining lignan glycosides, 6 M HCl was added to a final concentration of 2 M and hydrolyzed for 2.5 hr at 100°C. After neutralization and addition of the deuterated internal standards, hydrolyzed lignans were extracted as described above (14). The combined ether phase, including isoflavones and lignans, was evaporated and resolubilized in MeOH. The extract was purified by Lipidex 5000 and DEAE-$OH^-$ Sephadex, then isoflavones and lignans were separated by QAE-$Ac^-$ Sephadex (14). Each fraction was subjected to GC-MS analysis (14).

**GC-MS analysis** Each dried fraction was derivatized using 0.2 mL of silylation reagent, pyridine:HMDS:TMCS (9:3:1), for 30 min at room temperature and was analyzed by the GC-MS (Fnnigan GC8000, MS1000 quadrupole mass spectrometer, autoinjector AS800, and data system Windows 3.11 Mass Lab release 1.4). A bonded phase BP-1 vitreous silica column (0.2 mm × 12.5 m, SGE) was used and helium was used as a carrier gas. The oven temperature was kept at 100°C for 1 min and then was increased by 30°C/min to 280°C. The concentrations of isoflavones and lignans were calculated by comparing the peak area ratio of selected ions for phytoestrogen to corresponding deuterated internal standard from samples with that from external standards (Fig. 1) (14). All analyses were duplicated for each sample, and mean values were used to express the range of concentrations of phytoestrogens in each species.

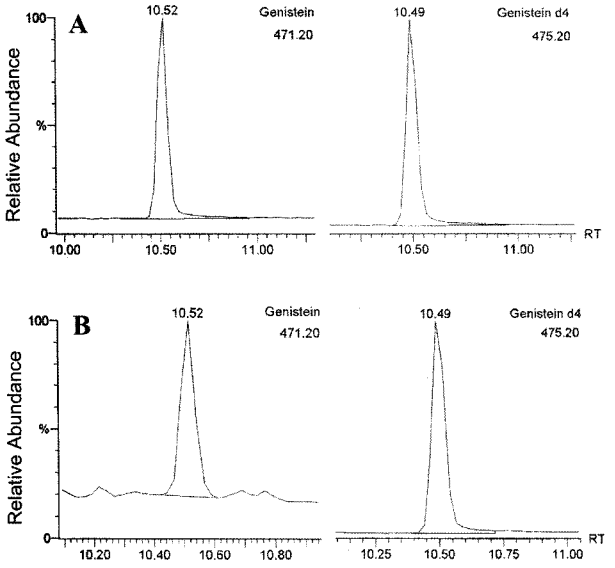


**Fig. 1. GC-MS-SIM chromatograms of genistein (m/z=471.2) and d4-genistein (m/z=475.2) in a standard (A) or a sample (B).** Each component shows a retention time of 10.52 or 10.49.

## Results and Discussion

Concentrations of isoflavones, coumestrol, and lignans were determined in 19 samples from eight species of sea vegetables. Table 1 shows the list and classification of selected species and their daily consumption data used for sample selection (18, 19). Two species of green algae, four species of brown algae, and two species of red algae were collected in local markets and included in analysis. Five isoflavones (genistein, daidzein, formononetin, biochanin A, and glycitein), coumestrol, and three lignans (ANHSEC, SECO, and MAT) were analyzed. ANHSEC is formed during acid hydrolysis from SECO, so the values were added to the SECO values.

Isoflavones showed rather low concentrations (Table 2). Only the samples in which the area ratio of peak to noise was higher than 3 were subjected to calculation of concentration. Figure 1 shows a representative selected ion chromatogram for genistein in a standard or sample. Genistein was present in most of the samples analyzed and ranged from not determinated to 79.2 μg/kg. Sea fusiforme showed slightly higher level of genistein than other species, but there were no outstanding differences in genistein content among species. Daidzein, the major isoflavone in plant with genistein, was found in sea fusiforme, celyon moss, and sea staghorn. Daidzein was not found in highly consumed species such as sea mustard, sea tangle, and laver. Biochanin A, precursor of genistein, was observed in a sample of sea mustard but not in any other samples. Most isoflavones and coumestrol except genistein were shown only in a few samples. Formononetin and glycitein were not found in any of the samples.

In a recent report studying the Korean exposure level of isoflavone, overall isoflavone intake was estimated as 14.88 mg/day/person: 7.32 mg genistein, 5.81 mg daidzein, and 1.75 mg glycitein (20). Soybeans and traditional soy foods (*tofu*, soybean paste, and soybean sprouts) contributed to more than 94% of the total isoflavone intake of the Korean population (20). The intake level of isoflavones from sea vegetables was estimated using data on isoflavone content from the present study and sea vegetable consumption data from the Korean Ministry of Health and Welfare (Table 1) (18). The consumption data expressed as raw sea vegetable was converted into dry weight based on the water content reported in The 7th Recommended Dietary Allowances for Koreans from The Korean Nutrition Society (21). The estimated phytoestrogen intake from sea vegetables was extremely low, as low as 0.088 μg/day for genistein and 0.13 μg/day for total isoflavones. This level seems to be negligible compared to

**Table 1. Sea vegetable varieties commonly consumed in Korea and analyzed by GC-MS**

| Division | Scientific name | Common name | Local name | Daily intake[1](g/day) | |
|---|---|---|---|---|---|
| | | | | Dry sea vegetable | Wet sea vegetable |
| Brown algae | *Undaria pinnatifida* | Sea mustard | Miyeok | 0.5 | 4.8 |
| | *Laminaria japonica* | Sea tangle | Dasima | 0.4 | 0.8 |
| | *Sargassum fulvellum* | Gulf weed | Mojaban | 0.0[3] | -[2] |
| | *Hizikia fusiforme* | Sea fusiforme | Tot | 0.0[3] | 0.0[3] |
| Red algae | *Porphyra tenera* | Laver | Gim | 1.3 | 0.1 |
| | *Gelidium amansii* | Ceylon moss | Umutgasari | 0.0[3] | -[2] |
| Green algae | *Enteromorpha linza* | Sea lettuce | Parae (Ipparae) | 0.1 | 1.1 |
| | *Codium fragile* | Sea staghorn | Cheonggak | -[2] | -[2] |

[1]Report on 2001 National Health and Nutrition Survey (18).
[2]Daily intake was not reported but are popular items in local markets.
[3]Although the reported intake was zero, they were included due to their importance in Korean diet.

**Table 2. Isoflavone and coumestrol content in various sea vegetables[1]**

| Common name | n[2] | Genistein | Daidzein | Formononetin | Biochanin A | Glycitein | Coumestrol |
|---|---|---|---|---|---|---|---|
| Sea mustard | 4 | 10.1-68.6 (30.1) | nd[3] | nd[3] | nd[3]-34.8 (34.8) | nd[3] | nd[3] |
| Sea tangle | 3 | nd[3]-5.7 (5.7) | nd[3] | nd[3] | nd[3] | nd[3] | nd[3] |
| Gulf weed | 1 | 13.0 | nd[3] | nd[3] | nd[3] | nd[3] | nd[3] |
| Sea fusiforme | 1 | 79.2 | 15.6 | nd[3] | nd[3] | nd[3] | nd[3] |
| Laver | 4 | 21.6-43.8 (35.2) | nd[3] | nd[3] | nd[3] | nd[3] | nd[3] |
| Ceylon moss | 1 | 59.8 | 43.0 | nd[3] | nd[3] | nd[3] | 3.7 |
| Sea lettuce | 3 | nd[3]-32.5 (24.9) | nd[3] | nd[3] | nd[3] | nd[3] | nd[3] |
| Sea staghorn | 2 | nd[3]-74.8 (74.8) | nd[3]-29.4 (29.4) | nd[3] | nd[3] | nd[3] | nd[3]-1.4 (1.4) |

[1]Results are means of duplicate measurements of each sample and presented as a range of values (μg/kg). Values in parenthesis are means of each species. The samples, in which chemicals were not determinated, were excluded from the calculation of means.
[2]Number of sample.
[3]Not determined by ID-GC-MS because of the low concentration. A ratio of peak to noise lower than 3 was not subjected to calculation of concentration.

that from soybeans and soy products (20). This is probably because of much lower concentrations of isoflavones in sea vegetables as well as lower average intake of sea vegetables than soy-related products.

Only limited studies have been done on lignans in sea vegetables and there was no comparable quantitative data to assess lignan intake in Korea. In this quantitative analysis (Table 3), lignans showed an even distribution among the sea vegetable species ranging from 391.6 to 694.8 μg/kg for total SECO and 106.4 to 137.2 μg/kg for MAT. Laver and sea mustard, the two most commonly consumed sea vegetables in Korea, showed somewhat high mean levels of total SECO and MAT. However, this data was not notably different from that from other species. It was probably because lignans are found in a wide range of plants (17) and there were no significant differences in structure and composition among sea vegetable species (1). The lignan intake levels from sea vegetables were estimated to be 1.7 μg/day for total SECO and 0.38 μg/day for MAT. However, in a Canadian study using fermentation methods, a much higher concentration of mammalian lignans, 900±247 μg/100 g, was found in dried sea vegetables (*mekuba* and *hijiki*) (22). In addition, a recent report by Hernandez *et al.* (23) showed that the

**Table 3. Lignan content in various sea vegetables[1]**

| Common name | n[2] | Total SECO[3] | MAT |
|---|---|---|---|
| Sea mustard | 4 | 475.6-617.7 (527.8) | 115.2-137.2 (122.1) |
| Sea tangle | 3 | 391.6-494.1 (445.3) | 106.4-117.1 (111.0) |
| Gulf weed | 1 | 533.2 | 126.5 |
| Sea fusiforme | 1 | 524.6 | 119.9 |
| Laver | 4 | 507.4-694.8 (566.4) | 116.5-133.7 (123.5) |
| Ceylon moss | 1 | 428.7 | 112.8 |
| Sea lettuce | 3 | 481.6-588.8 (521.9) | 115.7-120.6 (117.5) |
| Sea staghorn | 2 | 458.2-473.5 (465.9) | 113.1-113.8 (113.5) |

[1]Results are means of duplicate measurements of each sample and expressed as a range of values (μg/kg). Values in parenthesis are means of each species.
[2]Number of sample.
[3]Total amount of anhydrosecoisolariciresinol and secoisolariciresinol.

mammalian lignan, enterodiol, was positively associated with cervical squamous intraepithelial lesions (SILs). A positive association was also reported for sea vegetable intake (ogo or limu sea vegetable), which suggested that sea vegetables could be a source of lignan (23). To identify

the presence of other lignans (isolariciresionl, lariciresinol, syringaresinol, and pinoresinol), some sea vegetable samples were analyzed using HPLC equipped with a Coularray detector. However, no obvious peak for precursor lignans was detected in our analysis (data not shown). When comparing these results with other mammalian lignan data, there were possibilities of unknown precursor lignans in sea vegetables. The method of Mazur *et al.* (14), which measured only three lignans, may have been unable to completely detect these compounds.

Many researchers are intensively studying the beneficial effects of phytoestrogens on hormone-related chronic diseases (11, 12). They have also suggested their applications to estrogen replacement therapy in postmenopausal women. However, the phytoestrogens may modulate the hormonal balance and act as endocrine-disrupting chemicals (24-26). Many studies have focused on the physiological function of phytoestrogens to resolve this problem. To evaluate their effects on the human body, first of all, the exposure level and concentration in food samples are essential information. Although a lot of data has been published in this regard, foreign data may not be applicable due to the variations in plant variety, geographic location, climate, and storage conditions (27). Moreover, currently available data on phytoestrogens in food has shown variations among extraction methods and sample species with different matrix (28). Various internal standards such as coumarin and equilenin have been developed for quantitative analysis of phytoestrogens (17, 29). However, there are limitations in applying them to every class of phytoestrogens, even though these chemicals are structurally similar to phytoestrogens. In the present study, quantification was done by ID-GC-MS (13). Corresponding synthesized deuterated standards for each compound used in this study represented the best method for the correction of losses during the procedure (14).

In conclusion, the estimated intake levels of isoflavones and lignans from sea vegetables are much lower than those used in *in vivo* and *in vitro* investigations showing endocrine modulation. Thus, it is suggested that sea vegetables are negligible as a phytoestrogen source in the Korean diet. Nevertheless, these results can be used in the evaluation of phytoestrogen levels in the Korean diet encompassing a broad spectrum of food items as well as in the estimation of intake levels of high end-consumers. This data may also be used to find the differences between Korean and Western diets, which are highlighted for their different incidence of sex hormone-related cancers (30).

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## References

1. Lee IK, Shim SC, Cho HO, Rhee CO. On the component of edible marine algae in Korea. J. Korean Agric. Chem. Soc. 14: 213-220 (1971)
2. Wong KH, Cheung PCK. Nutritional evaluation of some subtropical red and green seaweeds. Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. Food Chem. 72: 11-17 (2001)
3. Jiménez-Escrig A, Sánchez-Muniz FJ. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. Nutr. Res. 20: 585-598 (2000)
4. Do JR, Kim EM, Koo JG, Jo KS. Dietary fiber contents of marine algae and extraction condition of the fiber. J. Korean Fish. Soc. 30: 291-296 (1997)
5. Sánchez-Machado DI, López-Cervantes J, López-Hernández J, Paseiro-Losada P. Fatty acid, total lipid, protein and ash contents of processed edible seaweeds. Food Chem. 85: 439-444 (2004)
6. Rupérez P. Mineral content of edible marine seaweeds. Food Chem. 79: 23-26 (2002)
7. Kim SJ, Woo S, Yun H, Yum S, Choi E, Do J, Jo J, Kim D, Lee S, Lee TK. Total phenolic contents and biological activities of Korean seaweed extracts. Food Sci. Biotechnol. 14: 798-802 (2005)
8. Hiroishi S, Sugie K, Yoshida T, Morimoto J, Taniguchi Y, Imai S, Kurebayashi J. Antitumor effects of Marginisporum crassissimum (Rhodophyceae), a marine red alga. Cancer Lett. 167: 145-150 (2001)
9. Castro R, Zarra I, Lamas J. Water-soluble seaweed extracts modulate the respiratory burst activity of turbot phagocytes. Aquaculture 229: 67-78 (2004)
10. Choi J, Ryu H, Chung J, Park J, Hwang J, Shin D, Lee S, Ryang R. Antioxidant property of genistein: inhibitory effect on HOCl induced protein degradation, DNA cleavage, and cell death. Food Sci. Biotechnol. 14: 399-404 (2005)
11. Tham DM, Gardner CD, Haskell WL. Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. J. Clin. Endocrinol. Metab. 83: 2223-2235 (1998)
12. Anderson JJB, Anthony M, Messina M, Garner SC. Effects of phyto-oestrogens on tissues. Nutr. Res. Rev. 12: 75-116 (1999)
13. Liggins J, Bluck LJC, Runswick S, Atkinson C, Coward WA, Bingham SA. Daidzein and genistein content of fruits and nuts. J. Nutr. Biochem. 11: 326-331 (2000)
14. Mazur W, Fotsis T, Wähälä K, Ojala S, Salakka A, Adlercreutz H. Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. Anal. Biochem. 233: 169-180 (1996)
15. Mazur WM, Duke JA, Wähälä K, Rasku S, Adlercreutz H. Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans. Nutr. Biochem. 9: 193-200 (1998)
16. Mazur WM, Wähälä K, Rasku S, Salakka A, Hase T, Adlercreutz H. Lignan and isoflavonoid concentrations in tea and coffee. Br. J. Nutr. 79: 37-45 (1998)
17. Meagher LP, Beecher GR. Assessment of data on the lignan content of foods. J. Food Comp. Anal. 13: 935-947 (2000)
18. Korean Ministry of Health and Welfare. 2001 National Health and Nutrition Survey. Korean Ministry of Health and Welfare, Seoul, Korea. pp. 284-286, 296 (2002)
19. Ministry of Education and Human Resources Development. Illustrated encyclopedia of fauna and flora of Korea. Vol. 8. Marine Algae. Ministry of Education and Human Resources Development, Seoul, Korea. (1968)
20. Kim JS, Kwon CS. Estimated dietary isoflavone intake of Korean population based on National Nutrition Survey. Nutr. Res. 21: 947-953 (2001)
21. The Korean Nutrition Society. Recommended Dietary Allowances for Koreans, Seventh reversion. The Korean Nutrition Society, Seoul, Korea. pp. 380-385 (2000)
22. Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignan production from various foods. Nutr. Cancer 16: 43-52 (1991)
23. Hernandez BY, McDuffie K, Franke AA, Killeen J, Goodman MT. Plasma and dietary phytoestrogens and risk of premalignant lesions of the cervix. Nutr. Cancer 49: 109-124 (2004)
24. Bennette HW, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterranean clover pastures in western Australia. Aust. J. Agric. Res. 22: 131-138 (1946)
25. Kanno J, Kato H, Iwata T, Inoue T. Phytoestrogen-low diet for endocrine disruptor studies. J. Agric. Food Chem. 50: 3883-3885 (2002)
26. Phipps WR, Martini MC, Lampe JW, Slavin JL, Kurzer MS. Effect of flax seed ingestion on the menstrual cycle. J. Clin. Endocrinol.

Metab. 77: 1215-1219 (1993)
27. Thompson LU, Rickard SE, Cheung F, Kenaschuk EO, Obermeyer WR. Variability in anticancer lignan levels in flaxseed. Nutr. Cancer 27: 26-30 (1997)
28. Liggins J, Grimwood R, Bingham SA. Extraction and quantification of lignan phytoestrogens in food and human samples. Anal. Biochem. 287: 102-109 (2000)
29. Wilkinson AP, Wähälä K, Williamson G. Identification and quantification of polyphenol phytoestrogens in foods and human biological fluids. J. Chromatogr. B 777: 93-109 (2002)
30. Wu AH, Ziegler RG, Nomura AM, West DW, Kolonel LN, Horn-Ross PL, Hoover RN, Pike MC. Soy intake and risk of breast cancer in Asians and Asian Americans. Am. J. Clin. Nutr. 68: 1437S-1443S (1998)