

## Lignan Content of Selected Foods from Japan

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Lignans constitute a group of phytochemicals widely distributed in the human diet. Once ingested, most dietary lignans are metabolized by the gut microflora to enterolactone and enterodiol, also known as enterolignans. Together with isoflavones and coumestrol, enterolignans have been traditionally classified as phytoestrogens, plant-derived compounds that exhibit estrogen-like activity. In addition to a higher intake of vegetables, many studies have suggested that the presence of phytoestrogens in the Japanese diet is one of the factors that might explain the reduced incidence of certain chronic diseases in Japanese populations compared with Western countries. Being a vegetable-based diet, exposure to dietary lignans should be high, but to assess this exposure, a lignan food database is required. Stable isotope-dilution gas chromatography coupled with mass spectrometry was used to quantify six plant lignans in 86 food items commonly consumed in Japan. These data will complement the previous databases and most importantly expand the knowledge of occurrence of lignans in food to Eastern diets.

**KEYWORDS:** Lignans; enterolignans; database; phytoestrogens; enterolactone

### INTRODUCTION

Several studies have demonstrated the association between the intake of plant-derived food and a decreased risk of developing chronic diseases. Dietary polyphenols seem to play an important role in this association (1). Dietary lignans are fiber-related polyphenols (2), and they are therefore present at considerable concentrations in fiber-rich foods, e.g., whole grain products, although the highest concentrations have been found in oilseeds such as flaxseed, sesame seed, and, to a lesser extent, soybean (3–6). Interestingly, after ingestion, most dietary lignans undergo an extensive transformation by the gut microflora, resulting in two major compounds, enterolactone (Enl) and its immediate precursor enterodiol (End), generally called enterolignans. Enl has been studied from two different perspectives: its usability as an exposure biomarker whole-grain intake (7) and its potential role preventing estrogen-dependent diseases (see refs 8 and 9 for review).

It seems now clear that Enl cannot be used as a biomarker of any particular group of food since its precursors are present in most plant foods (3, 10) and could only be used as a biomarker of exposure to lignans. The pioneer work of Mazur and co-

workers measuring secoisolariciresinol (Seco) and matairesinol (Mat) in a wide variety of foods (11–14) constituted the basis of the estimation of lignan intake in different studies (15–20). However, epidemiological trials using this estimation to study the relationship between lignan intake and disease risk revealed contradictory results for breast cancer (21–32), prostate cancer (33–38), and cardiovascular disease (39, 40). The discrepancies found by Thompson and co-workers between the concentrations of Seco and Mat in foods and the Enl production obtained after *in vitro* fermentation (41) led to the identification of other major enterolignan precursors in foods (42). Thereafter, two extensive databases have been published. In these databases, values for Seco, Mat, pinoresinol (Pin), and lariciresinol (Lar) have been collected for selected foods representing Dutch (3) and Canadian (10) diets. Other studies, more limited in the number of samples, have also been published with improved analytical methods and including additional plant lignans (4, 6, 43).

A complete food lignan database is essential to estimate the intake to lignans in a defined population. The aim of this paper was to increase the knowledge on dietary lignan exposure by studying the lignan content of selected foods consumed in Japan. The concentrations of six lignans, namely, Seco, Mat, Pin, Lar, medioresinol (Med), and syringaresinol (Syr), were quantified in 86 food items selected from those commonly consumed in Japan including vegetables, fruits, mushrooms, legumes, roots and tubers, soy-based products, cereal products, and animal products. Additionally, six items were cooked to study the possible losses of lignans during food processing.

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## MATERIALS AND METHODS

**Standards.** Lignan standards including matching  $^{13}\text{C}_3$ -labeled lignans as internal standards were synthesized as previously described (4) by Dr. Botting's group at the School of Chemistry, University of St. Andrews, U.K.

**Sample Pre-treatment.** The lignan content of the selected samples was determined accordingly to a previously reported protocol (4). In brief, after addition of the internal standards, samples were consecutively submitted to alkaline and enzymatic hydrolysis ( $\beta$ -glucuronidase from purified *Helix pomatia*; Sigma-Aldrich, Steinheim, Germany) to release the lignan aglycons. The hydrolyzed extracts were then applied to C18 cartridges (Waters, Milford, MA) and the recovered methanolic extracts purified by means of ion-exchange chromatography using DEAE-Sephadex (Pharmacia Biotech AB, Uppsala, Sweden) in the hydroxyl form. After evaporation to dryness the sample extracts were derivatized using the silylation mixture pyridine/HMDS/TMCS (9:3:1) to be subsequently injected into the chromatographic system. If not specified, all reagents were from major suppliers and with the highest purity available.

**Instrumentation.** The GC-MS system consisted of a chromatograph, Fisons GC 8000 (Fisons Instrumentation, Inc., Milan, Italy), equipped with a BP-1 capillary (12 m  $\times$  0.22 mm i.d., 0.25  $\mu\text{m}$ ) column (SGE International Pty Ltd., Ringwood, Australia) coupled with a Fisons MD 1000 mass spectrometer (Fisons Instrumentation, Inc., Manchester, U.K.). Settings and instrumental conditions were described elsewhere (4).

**Quality Control.** Two control samples consisting of millet (low lignan control) and rye bran (high lignan control) were selected to ensure the precision of the method at both low- and high-lignan concentrations. Control samples were included at the end of each series, and data were accepted when the coefficient of variation values from the controls differ <15% from the mean values previously established (4). Sample dilutions were made, when necessary, to fit the standard curve. The limits of detection for each analyte ranged from 6.16 (Seco) to 16.1 (Pin)  $\mu\text{g}/100$  g on a dry basis. Samples were analyzed in duplicate, and mean values were accepted when the CV < 15%. The accuracy of the method was also guaranteed by the use of the isotope dilution technique by which any possible loss during sample pretreatment is automatically corrected.

## FOOD SAMPLES

**Selection.** Vegetables were selected for analysis on the basis of their relevance and frequency in the Japanese diet. In the case of Japanese samples, different batches were selected at optimal ripening stage and delivered from farmers' cooperatives located in different prefectures of Japan to conform to samples listed in **Table 1**. Animal samples and imported samples were purchased from local markets in Tokyo, Japan.

**Sample Preparation.** Nonedible parts were removed, and samples were prepared as detailed in **Table 1** to allow representative samples. Food samples were analyzed in the form that they are usually consumed, but in some instances, vegetables were further boiled (100 g of sample in 1 L of distilled water for 5 min) to estimate a possible loss of lignans due to food preparation. After individual treatment, samples were all freeze-dried (HETO-FD3, Heto Holten, Allerød, Denmark) and further ground (A11 basic homogenizer; Ika-Werke GmbH & Co., KG, Staufen, Germany) to a homogeneous powder that was kept at  $-20$  °C until analysis. Quality control samples were equally prepared and stored to detect any possible loss during storage.

## RESULTS AND DISCUSSION

Comparison of most of the values reported here with existing data is not possible as this is the first time that major dietary lignans are determined in characteristic foods from Japan. The sample selection was made to complement existing databases (3, 10) and very few items are therefore comparable. In any case,

comparison of similar items from different locations may have little relevance as there are numerous variables that determine the final value. Plant variety and cultivar, ripening state, and analytical protocol used, among others, are the main sources of variation. Comparison with previous papers reporting values for only Seco and Mat are not considered necessary either since its added values represent a minor percentage of total dietary lignans in most foods. We will therefore avoid any major comparison as we are convinced that increasing accumulation of data based on reliable analytical methods is the first step to identify variation factors and the only way to accurately calculate lignan intake in different populations.

A total of six plant lignans were included in the analyses. In **Figure 1**, multiple ion monitoring chromatograms of nonlabeled and labeled ions of both item 5 (perilla leaves) and standards are presented as an example. The amounts of plant lignans in the items selected for the study varied from 0 to 1724  $\mu\text{g}/100$  g (wet basis). In **Table 1**, items are ordered according to different food groups and decreasing levels of plant lignans. From all of the selected plant-derived foods, only mushrooms did not contain plant lignans at detectable levels. As observed earlier (3), the plant lignans Lar and Pin were found at the highest concentrations in most of the items. Lar was the predominant lignan in 44% of the samples, Pin in 19%, and Seco in 15%. Interestingly, Syr was found to be the predominant lignan in 20% of the samples. Mat was only found to be predominant on item 48 (ginger), its concentrations being negligible in most items. Although Mat seems to be labile under the alkaline conditions implied in the analytical protocol (4, 44), the values reported here are accurate because of the use of individual internal standards correcting for any possible loss. It can therefore be confirmed that Mat is a minor dietary lignan, agreeing with previous reports (3, 10).

Lignan values in selected vegetables varied between 18 and 1724  $\mu\text{g}/100$  g (median, 120  $\mu\text{g}/100$  g). The highest concentration corresponded to the stems and leaves of seri (dropwort or Japanese parsley), an edible wild herb belonging to the *Apiaceae* family used as part of the traditional Japanese dish known as seven wild herb gruel (nanakusa-gayu). Asparagus was one of the few items that contained Seco as the predominant lignan, representing more than 70% of the total content (1 mg/100 g). The levels found in this particular sample of asparagus are much higher than those found by other authors (15, 19, 45) and, interestingly, from the ones found in our previous pilot survey of samples from Finnish markets (4). This exemplifies how the values may vary for apparently similar samples even when exactly the same analytical protocol is applied.

The Japanese diet is characterized by a high consumption of roots and tubers. Values in selected items varied between 25 and 954  $\mu\text{g}/100$  g (median, 109  $\mu\text{g}/100$  g). The highest concentration (mainly Lar) was found in wasabi, a root belonging to the *Brassicaceae*, a botanical family known for its high content of lignans (3), and commonly used as a spice for sushi. Other rich sources are lily bulbs, containing almost exclusively Syr and Pin, and burdock root used as a delicacy in Japanese food (mostly Seco).

The content of fruits varied between 31 and 1292  $\mu\text{g}/100$  g (median, 138  $\mu\text{g}/100$  g). The richest source of lignans among fruits was Yuzu, a citrus fruit originating in East Asia whose juice is commonly used as a seasoning. Comparing its lignan content with other citric fruits (items 55 and 56), major differences can be found in their respective Lar contents, 25  $\mu\text{g}/100$  g for navel orange vs 654  $\mu\text{g}/100$  g for yuzu. Values for avocado (item 55), reported here for the first time, are also

Table 1. Lignan Content ( $\mu\text{g}/100\text{ g}$  Wet Basis, Duplicate Analysis) of Selected Foods Consumed in Japan

#	Item	Processing <sup>a</sup>	Origin <sup>b</sup>	%w <sup>c</sup>	Seco	Mat	Lar	Pin	Syr	Med	Total
<b>Vegetables</b>											
1	Seri, stems and leaves Dropwort <i>Oenanthe javanica</i>	Raw, crushing by blender	Shizuoka, JP	66	449	13	746	491	9	15	1724
2	Asparagus <i>Asparagus officinalis</i>	Raw, cutting 2 cm	Nagano, JP	89	743	14	92	122	58	3	1034
3	Komatsuna Japanese mustard spinach <i>Brassica rapa perviridis</i>	Raw, cutting 3 cm	Ibaraki, JP	95	178	-	189	136	32	19	555
4	Kabu, flower buds and stems Turnip rape <i>Brassica rapa</i>	Raw, cutting 2 cm	Fukushima, JP	93	38	-	223	202	18	29	510
5	Shiso, leaves Perilla <i>Perilla frutescens</i>	Raw, crushing by blender	Aichi, JP	89	64	-	123	174	102	46	510
6	Shiso, leaves Perilla <i>Perilla frutescens</i>	Raw, crushing by blender	Chiba, JP	89	138	-	102	118	24	31	414
7	Kikuna Garland chrysanthemum, <i>Chrysanthemum coronarium</i>	Raw, cutting 2 cm	Iwate, JP	96	53	-	49	27	135	28	293
8	Okra <i>Hibiscus esculentus</i>	Raw, removing both ends then cutting 0.5 cm	Kagoshima, JP	89	221	-	10	3	11	-	246
9	Kureson, stems and leaves Watercress <i>Nasturtium nasturtium-aquaticum</i>	Raw, crushing by blender	Shizuoka, JP	90	75	-	102	21	3	3	205
10	Kanpyo Dried gourd <i>Lagenaria siceraria</i>	Raw, cutting 1 cm	Tochigi, JP	16	108	-	78	-	-	-	186
11	Ninniku Garlic <i>Allium sativum</i>	Raw, removing peel then crushing by blender	Aomori, JP	62	55	-	84	45	-	-	184
12	Cauliflower <i>Brassica oleracea</i>	Raw, removing prickle and stem then cutting in quarters	Hokkaido, JP	91	5	-	38	85	26	22	176
13	Saya_endo, immature pods Snap peas <i>Pisum sativum</i>	Raw, removing both ends and string then cutting 1/2	CN	90	-	-	62	97	8	5	173
14	Ashitaba, stems and leaves <i>Angelica keiskei</i>	Raw, cutting 3 cm	Tokyo, JP	93	129	-	9	6	9	1	155
15	Horensou Spinach <i>Spinacia oleracea</i>	Raw, cutting 3 cm	Iwate, JP	94	8	-	110	31	-	-	149
16	Nasu Eggplant <i>Solanum melongena</i>	Raw, removing prickle then cutting 1cm <sup>2</sup>	Fukuoka, JP	94	8	-	40	51	18	15	132
17	Hakusai Chinese cabbage <i>Brassica rapa</i>	Raw, removing prickle then cutting 1cm <sup>2</sup>	Ibaraki, JP	97	25	-	77	17	4	2	126
18	Corn, immature kernels <i>Zea mays</i>	Raw, using whole pieces	Gunma, JP	77	-	-	25	16	74	-	115
19	Horensou Spinach <i>Spinacia oleracea</i>	Boiled, crushing by blender	Gunma, JP	93	13	1	79	15	4	1	113
20	Kyuri Cucumber <i>Cucumis sativus</i>	Raw, removing prickle and stem then cutting 0.2 cm	Fukushima, JP	95	41	-	65	-	-	-	107
21	Corn, immature kernels <i>Zea mays</i>	Boiled, crushing by blender	Aomori, JP	77	7	-	14	-	76	5	103
22	Seiyo_kabocha European pumpkin <i>Cucurbita sp</i>	Boiled, removing seeds and both ends then crushing by blender	Chiba, JP	76	29	3	58	8	-	6	103
23	Celery <i>Apium graveolens</i>	Raw, crushing by blender	Nagano, JP	89	12	-	16	43	10	8	91
24	Yellow paprika Yellow sweet pepper <i>Capsicum sp.</i>	Raw, removing prickle and seeds then cutting in quarters then linearly	NL	92	8	-	79	2	-	-	89
25	Piman Green sweet pepper <i>Capsicum sp.</i>	Raw, removing prickle and seeds then cutting 1.5 cm <sup>2</sup>	Hokkaido, JP	93	5	-	73	6	4	1	89
26	Red paprika Red sweet pepper <i>Capsicum sp.</i>	Raw, removing prickle and seeds then cutting 1.5cm <sup>2</sup>	NL	91	9	-	73	1	-	-	83
27	Ito_mitsuba leaves <i>Cryptotaenia japonica</i>	Raw, crushing by blender	Shizuoka, JP	95	11	6	8	36	16	3	81
28	Cherry tomatoes <i>Solanum lycopersicum var. Cerasiforme</i>	Raw, crushing by blender	Chiba, JP	94	17	-	43	11	6	3	81

Table 1. Continued

#	Item	Processing <sup>a</sup>	Origin <sup>b</sup>	%w <sup>c</sup>	Seco	Mat	Lar	Pin	Syr	Med	Total
<b>Vegetables</b>											
29	Cherry tomatoes <i>Solanum lycopersicum</i> var. <i>Corasiforme</i>	Raw, removing pod then mashing	Fukushima, JP	91	16	-	38	19	3	3	80
30	Seiyo_kabocha European pumpkin <i>Cucurbita</i> sp.	Raw, removing seeds and both ends then cutting 1cm	Nagasaki, JP	76	20	-	29	-	-	-	49
31	Takenoko Bamboo <i>Bambusa</i> sp.	Soaked, then cutting 1 cm <sup>2</sup>	Kagoshima, JP	92	38	-	-	-	3	4	45
32	Tomatoes <i>Solanum lycopersicum</i>	Raw, removing pod then mashing	Fukushima, JP	93	3	-	11	12	7	5	37
33	Nihon_kabocha Japanese pumpkin <i>Cucurbita</i> sp.	Raw, removing peel and both ends then cutting 2 cm	Ishikawa, JP	89	9	-	14	1	-	-	24
34	Myoga spike <i>Zingiber mioga</i>	Raw, crushing by blender	Gunma, JP	94	7	1	-	7	3	1	19
<b>Tubers and roots</b>											
35	Wasabi Japanese horseradish <i>Wasabia japonica</i>	Raw, removing peel then crushing by blender	Shizuoka, JP	84	92	71	659	91	28	12	954
36	Yurine, Lily bulb <i>Lilium tigrinum</i>	Boiled, crushing by blender	Nagano, JP	27	-	-	-	191	415	10	616
37	Gobo Edible burdock <i>Arctium lappa</i>	Raw, removing peel and both ends then cutting 3 cm	Miyazaki, JP	83	251	39	95	74	9	10	479
38	Gobo Edible burdock <i>Arctium lappa</i>	Boiled after removing peel and both ends then crushing by blender	Chiba, JP	85	214	34	68	65	31	19	433
39	Wasabi Japanese horseradish <i>Wasabia japonica</i>	Grated, removing the peel then crushing by blender	Shizuoka, JP	83	37	49	219	16	21	4	347
40	Satsuma_imo Sweet potatoes <i>Ipomoea batatas</i>	Raw, removing peel then cutting 1 cm <sup>2</sup>	Kagawa, JP	69	65	2	79	16	-	-	161
41	Kabu Turnip <i>Brassica rapa</i>	Raw, removing peel then cutting 0.5 cm	Chiba, JP	95	9	-	101	4	33	4	152
42	Daikon Radish <i>Raphanus sativus</i>	Raw, removing peel then cutting 0.5 cm	Aomori, JP	94	-	-	29	43	32	12	116
43	Yurine Lily bulb <i>Lilium tigrinum</i>	Raw, crushing by blender	Nagano, JP	86	1	-	-	58	40	2	102
44	Daikon Radish <i>Raphanus sativus</i>	Boiled after removing peel then crushing by blender	Kanagawa, JP	95	1	-	15	40	29	11	96
45	Renkon East Indian lotus root <i>Nelumbo nucifera</i>	Boiled, removing peel then crushing by blender	Kumamoto, JP	89	3	-	41	33	-	3	80
46	Sato_imo Corm <i>Colocasia esculenta</i>	Raw, removing peel then cutting 0.5 cm	Miyazaki, JP	84	24	1	49	-	-	-	74
47	Renkon East Indian lotus root <i>Nelumbo nucifera</i>	Raw, removing peel then cutting 1 cm <sup>2</sup>	Ibaraki, JP	93	1	-	31	27	-	2	62
48	Syoga Ginger <i>Zingiber officinalis</i>	Raw, removing peel then cutting 0.2 cm	Kumamoto, JP	94	-	16	-	15	5	-	37
49	Yatsugashira Arrowhead <i>Sagittaria trifolia</i>	Raw, removing peel then crushing by blender	Nagano, JP	78	-	-	3	4	20	-	28
50	Nagaimo Japanese yam <i>Dioscorea opposita</i>	Raw, removing peel then cutting 1 cm <sup>2</sup>	Hokkaido, JP	85	13	1	10	-	-	-	24
<b>Mushrooms</b>											
51	Enokitake Winter mushroom <i>Flammulina velutipes</i>	Raw, removing hard tip then cutting 1cm	Nagano, JP	88	-	-	-	-	-	-	0
52	Shiitake <i>Lentinula edodes</i>	Raw, removing hard tip then extruding	Fukushima, JP	88	-	-	-	-	-	-	0
53	Bunashimeji <i>Hypsizia marmoreus</i>	Raw, extruding	Niigata, JP	89	-	-	-	-	-	-	0

Table 1. Continued

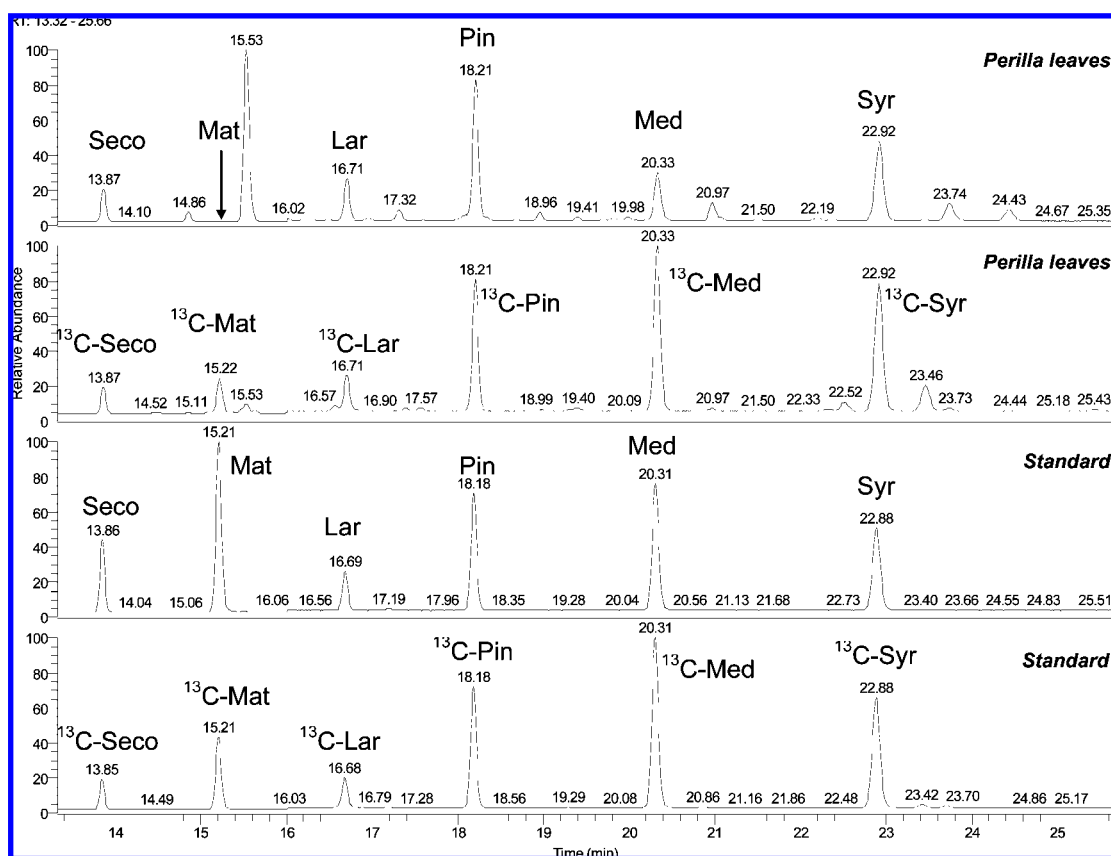
#	Item	Processing <sup>a</sup>	Origin <sup>b</sup>	%w <sup>c</sup>	Seco	Mat	Lar	Pin	Syr	Med	Total
<b>Fruits</b>											
54	Yuzu <i>Bitter orange</i> <i>Citrus aurantium</i>	Raw, removing peel and hard tip then crushing by blender	Shizuoka, JP	77	26	-	192	654	293	125	1292
55	Avocado <i>Persea Americana</i>	Raw, removing peel then crushing by blender	US	74	47	6	31	272	447	241	1046
56	Valencia orange <i>Citrus sinensis</i>	Raw, removing peel then mashing	US	63	56	-	193	51	202	19	521
57	Nevel orange <i>Citrus sinensis</i>	Raw, removing peel then mashing	US	70	14	-	128	24	48	9	225
58	Suika Watermelon <i>Citrullus lanatus</i>	Raw, removing peel and seeds then mashing	Chiba, JP	90	23	-	69	12	42	2	147
59	Pineapple <i>Ananas comosus</i>	Raw, removing peel and core then crushing by blender	PH	87	7	18	24	3	92	1	147
60	Kiwifruit <i>Actinidia chinensis</i>	Raw, removing peel then crushing by blender	NZ	82	106	-	20	13	-	4	144
61	Momo Peach <i>Prunus persica</i>	Raw, removing peel and core then mashing	Yamanashi, JP	87	11	-	38	83	3	5	141
62	Momo Peach <i>Prunus persica</i>	Raw, removing peel and core then cutting 1 cm <sup>2</sup>	Yamanashi, JP	84	16	-	22	94	-	2	135
63	Ichigo Strawberries <i>Fragaria x ananassa</i>	Raw, removing stem then crushing by blender	Chiba, JP	95	51	-	33	21	-	-	106
64	Ume Japanese plum <i>Prunus mume</i>	Raw, removing peel and seeds then mashing	Nagano, JP	85	16	-	20	42	-	1	79
65	Suika Watermelon <i>Citrullus lanatus</i>	Raw, removing peel and seeds then cutting 2 cm <sup>2</sup>	Kanagawa, JP	90	5	-	67	5	1	-	79
66	Ringo Apple <i>Malus domestica</i>	Raw, removing peel and core then crushing by blender	Nagano, JP	84	-	3	55	-	-	-	59
67	Yo_nashi European pear <i>Pyrus communis</i>	Raw, removing peel and core then crushing by blender	Nagano, JP	84	2	-	34	2	14	5	58
68	Banana <i>Musa x paradisiaca</i>	Raw, removing peel then crushing by blender	PH	76	-	-	17	-	19	-	36
69	Nashi Japanese pear <i>Pyrus pirifolia</i>	Raw, removing peel and core then cutting 0.5 cm	Fukuoka, JP	87	7	-	21	-	1	1	31
<b>Legumes</b>											
70	Saya_ingen, immature pods Kidney bean <i>Phaseolus vulgaris</i>	Boiled, crushing by blender	Hyogo, JP	92	58	-	144	37	9	-	249
71	Saya_ingen Kidney bean <i>Phaseolus vulgaris</i>	Raw, cutting 0.8 cm	Fukushima, JP	92	66	-	128	31	8	-	233
72	Ryokuto_moyashi Mung bean sprouts <i>Vigna radiate</i>	Raw, crushing by blender	Fukushima, JP	96	82	1	32	33	-	-	149
73	Saya_endo, immature pods Pea <i>Pisum sativum</i>	Raw, removing both ends then cutting 1/3	Hokkaido, JP	90	-	-	59	50	-	2	111
<b>Soybean-based products</b>											
74	Edamame, immature pods Soybean <i>Glycine max</i>	Frozen, removing the pod then cutting in half	TW	71	67	-	81	33	306	20	509
75	Edamame, immature pods Soybean <i>Glycine max</i>	Raw, removing the pod then cutting in half	Chiba, JP	70	80	-	77	48	105	23	334
76	Edamame, immature pods Soybean <i>Glycine max</i>	Boiled after removing the pod, crushing by blender	Fukushima, JP	69	44	-	58	50	112	24	290
77	Daidzu_moyashi, sprouts Soybean <i>Glycine max</i>	Raw, crushing by blender	Fukushima, JP	92	52	1	69	61	50	13	248
78	Momen tofu Soymilk derivative <i>Glycine max</i>	Raw, dicing	Tokyo, JP	84	14	-	42	24	35	7	124
79	Kinugoshi tofu Soymilk derivative <i>Glycine max</i>	Raw, mashing	Tokyo, JP	94	9	-	15	27	50	13	116



Table 1. Continued

#	Item	Processing <sup>a</sup>	Origin <sup>b</sup>	%w <sup>c</sup>	Seco	Mat	Lar	Pin	Syr	Med	Total
<b>Cereal-based products</b>											
30	Rye_mugi_pan Rye bread (50% rye)	Crushing by blender	Tokyo, JP	45	33	4	47	44	402	28	560
31	Shoku_pan Light bread	Cutting 1cm <sup>2</sup>	Tokyo, JP	32	-	-	20	16	173	9	219
32	Rye_mugi_pan Rye bread (30% rye)	Crushing by blender	Tokyo, JP	84	7	1	18	16	136	10	190
33	France_pan French bread	Crushing by blender	Tokyo, JP	38	-	-	-	-	45	-	45
<b>Animal-derived products</b>											
34	Keiran Hen's egg	Raw, mixing yolk and white	Tokyo, JP	74	-	-	-	-	-	-	0
35	Dasshi_yogurt (Mutou) Skimmed yogurt, unsweetened	As is	Tokyo, JP	76	-	-	-	-	-	-	0
36	Zenshi_yogurt (Katou) Whole milk-yogurt, sweetened	As is	Tokyo, JP	75	-	-	-	-	-	-	0

<sup>a</sup> Sample preparation and processing before freeze-drying and analysis. <sup>b</sup> China (CN), Japan (JP), Netherlands (NL), New Zealand (NZ), Philippines (PH), Taiwan (TW), and United States of America (US). <sup>c</sup> Moisture content.



**Figure 1.** Multiple ion monitoring chromatograms of nonlabeled and labeled ions of both item 5 (Perilla leaves) and standards. Identification of plant lignans: Seco ( $m/z$  560, 13.86 min); Mat ( $m/z$  502, 15.21 min); Lar ( $m/z$  576, 16.69 min); Pin ( $m/z$  502, 18.18 min); Med ( $m/z$  532, 20.31 min); Syr ( $m/z$  562, 22.88 min).

high, Syr being the predominant lignan. In general, our values for fruits are similar or slightly higher, comparing with previous databases (3, 10).

Legumes in general and soybeans in particular are a good source of plant lignans. The total lignan content in selected soybeans was estimated in previous studies as 2 mg/100 g (6). The soy-based products included in this report reflect the lignan profile of soybean although the concentrations are lower due to the higher moisture content. Edemame (immature soybeans) presented the highest concentrations (items 74–76). Tofu products manufactured from soy milk (items 78 and 79) had

the lowest concentrations in this set of samples, in accordance with previous reports (3). The concentration of lignans in soybeans and soy-derived products is highly relevant as soy is considered a staple food in the Japanese diet.

Other frequent ingredients of the Japanese cuisine, such as sesame seed and sesame oil, were not included in this survey since its lignan content has been extensively studied (5, 46, 47). The occurrence of lignans in hen's eggs and dairy products was also studied, but no plant lignan or enterolignan could be traced, agreeing with other databases (10).

**Table 2.** Variation (%) of Lignan Concentrations in Selected Items Due to Boiling<sup>a</sup>

no.	item	Seco	Mat	Lar	Pin	Syr	Med
2	asparagus	-46	-68	0	0	-46	0
4	Kabu turnip rape leaves	-55	0	-49	-71	-50	-68
27	Ito_mitsuba leaves	+65	-37	-29	-32	0	0
49	Yatsugashira arrowhead	0	0	+38	+20	-66	-65
73	Ryokuto_moyashi mung bean sprouts	0	-100	-6	-10	0	-43
77	Daidzu_moyashi soybean sprouts	+16	+70	-29	-16	-23	-22

<sup>a</sup> 100 g of sample boiled for 5 min in 1 L of distilled water.

Variations of lignan levels during food processing occur (Table 2). Items 2, 4, 27, 49, 73, and 77 were submitted to boiling (a necessary step to make them edible), and their lignan content was compared (dry basis) with the raw material. Important losses occur in most of the cases. This may be due to the chemical degradation of the lignan or simply to a diffusion of the compounds to the aqueous environment favored by the high temperature. In some instances, the lignan content of foods increases, which might reflect a higher degree of release from the food matrix or degradations, i.e., Lar to Seco. These results are in agreement with Milder and co-workers, who reported an average 25% decrease in total lignans in selected foods after frying or boiling (3), and highlight the importance of the culinary techniques on the bioavailability of lignans and phytochemicals in general.

Databases containing values for only Seco and Mat underestimate human exposure to plant lignans, as it has been recently proved by Milder and co-workers that concluded that the sum of Lar and Pin accounted for 75% of lignan intake in the Dutch population (48). When more dietary lignans are included in the databases, it becomes clear that some vegetables and fruits are as important sources of lignans as whole grains. Oilseeds such as flaxseed, sesame seed, and, to a much lesser extent, soybeans (3, 4, 6, 10), and not cereal-based products, are the richest sources of lignans. Multigrain breads are a rich source of lignans only if they contain flax or sesame seed since whole rye or wheat bread *per se* contains only moderate amounts of lignans. However, in countries like Finland, rye bread contributes considerably to the intake of lignans because its consumption is relatively high.

The knowledge on the lignan content of foods is constantly expanding as it has been recently pointed out by Smeds and co-workers that increased the number of known dietary lignans to 24 (43). In the present report, six different plant lignans are included in the method. It seems that lignans such as Mat might not be as abundant in food as thought and others, such as Syr, seem to be present at considerable levels in most foods. Other lignans, for instance, sesamin, that have been previously quantified in sesame seeds (5) were not included in the routine measurements since their absence in the foods analyzed so far suggests that it is characteristic of sesame seeds. The lignan 7'-hydroxymatairesinol, predominant in knots of Norway spruce (the richest nondietary source of lignans known to date (49)), was also found in sesame seeds (5) at considerable concentrations and, very recently, quantified in cereals such as wheat and rye (43). More data should be therefore gathered based on appropriate analytical methods in order to define the predominant lignans on the different diets and therefore the relevance of lignans on human health.

In addition to a higher intake of vegetables, many studies have pointed to the presence of phytoestrogens in the Japanese diet as one of the factors that might explain the reduced incidence of chronic diseases among Japanese when compared with Western countries. The Japanese diet is characterized by high consumption of soybean-based products containing unbeatable levels of isoflavones, the most studied phytoestrogen, but being a vegetable-based diet, human exposure to dietary lignans should be high regarding the ubiquity of lignans in plant-based food. The lignan database reported here will complement the current knowledge of lignan occurrence in foods, expanding the knowledge to Eastern diets, specifically Japanese diets. At the same time as the occurrence of plant lignans in different diets is being investigated, efforts should be made to understand the possible transformation of dietary lignans during food processing; also, the human metabolism needs to be elucidated to fully characterize the human exposure to lignans and therefore their relevance to disease prevention.

#### ABBREVIATIONS USED

DEAE, diethylaminoethyl; End, enterodiol; Enl, enterolactone; HMDS, hexamethyldisilazane; Lar, lariciresinol; Mat, matairesinol; Med, medioresinol; Pin, pinoresinol; Seco, secoisolariciresinol; Syr, syringaresinol; TMCS, trimethylchlorosilane.

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