JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

1,3-β-Glucan Quantification by a Fluorescence Microassay and Analysis of Its Distribution in Foods

YUAN-TIH KO* AND YU-LING LIN

Department of Nutrition, China Medical University, 91 Hsueh-Shih Road, Taichung, Taiwan 40421

The objective of this study was to establish analytical approaches to quantify 1,3-β-glucan (1,3-β-G) in foods. Six food categories including legumes, cereals, tubers, vegetables, fruits, and mushrooms and 17 total items were tested. An extraction procedure was designed to prepare food cold-water soluble, hot-water soluble, cold-alkaline soluble, and hot-alkaline soluble fractions. A fluorescence microassay based on aniline blue dye, which bound specifically to 1,3-β-G, was developed to measure its content in the food fractions. Curdlan was used as standard to construct the 1,3-β-G calibration curve, and a linear correlation within a 14 μg/mL concentration range was obtained. This microassay displayed selectivities among various 1,3-β-G species. Biologically active ones such as pachyman and yeast glucan possessed much stronger fluorescent signals than others such as laminarin and barley glucan. Possible fluorescent interference from food proteins was estimated. This assay tolerated up to 50% of bovine serum albumin in 10 μg/mL curdlan. Analysis of the four food fractions showed that besides the well-known lentinan-containing shiitake, popular foods such as celery, chin-chian leaves, carrot, and radish contained nearly 20% 1,3-β-G in their total sugar. Soybean dry weight contained 0.8% 1,3-β-G, which was twice the amount compared to shiitake. Snow mushroom dry weight had the highest 1,3-β-G content, at 2.5%, and was rich in both water (0.67%) and alkaline soluble (1.87%) forms. In conclusion, this dye-binding fluorescence microassay in conjunction with the extraction procedure can be applied in the prescreening of potential foods rich in functional 1,3-β-G.

KEYWORDS: 1,3-β-Glucan; aniline blue; fluorescent spectroscopy

INTRODUCTION

1,3-β-Glucan (1,3-β-G) constitutes one of the structural macromolecules in the cell wall of higher plants (wound-induced sugar on the plant new cell wall, callose), yeasts (located in the budding scar), and mushrooms (e.g., lentinan). It also plays a role as a storage polysaccharide in some brown agar (laminarin), euglenoids (paramylon), and fungi (pachyman). Furthermore, it is an extracellularly secreted polysaccharide of bacteria (e.g., curdlan from Agrobacteria) and filamentous fungi (schizophyllan and scleroglucan) (*1*).

Various structures of 1,3-β-G were found. Some are composed of a 1,3-β-linked glycolytic backbone and exist as a single-chain linear conformer such as pachyman, curdlan, paramylon, and laminarin, whereas some from fungal sources belong to diverse shapes of 1,3-β-G main chain with different degrees of 1,6-β-glucan branches and length such as lentinan, schizophyllan, and yeast glucan (*2*). Cereal 1,3-β-Gs are mixed linked glucans (MLG; oat and barley glucan), which contain not only the above two linkages together but also intramolecular 1,4-β-glycosyl linkages. 1,3-β-G of the same chemical origin particularly, however, may display single-helix, triple-helix, or random coil form based on three-dimensional structures (*3*). These conformations are known to be related in an interconvertable manner in aqueous solution under certain physical and chemical treatment as indicated by the studies of schizophyllan (*4*), scleroglucan (*5*), and laminarin (*6*).

The health functions of 1,3-β-G have attracted much attention in recent years. Besides being a source of dietary fiber, it is linked with certain biomedical effects such as host defense potentiator (HDP) (*7*), antitumor (*8*, *9*), anti-infective (*10*), and immunostimulator (*11*). Zymosan, for example, 1,3-β-G of the cell wall biopolymer from *Saccharomyces cerevisiae* identified 36 years ago, was used as a biological response modifier (BRM) that stimulates tumor rejection with regulatory effects on the immune system. Well-identified foods containing 1,3-β-G with biological functions are cereals, yeasts, and fungi (mushrooms). Oat bran and barley MLGs are considered to be health foods that have proven to reduce total cholesterol and low-density lipoprotein (LDL) levels of hypercholesterolemia patients (*12*). Evaluation of the effect on serum lipids of a yeast-derived β-glucan fiber in obese, hypercholesterolemic men showed their LDL−cholesterol concentrations declined by 8% at week 8 of consumption (*13*). Oat β-glucan (Oβ-G) also demonstrated immunomodulatory activities (*14*). In addition, a structure−function relationship of 1,3-β-G was well demonstrated in various studies (*11*). Among 1,3-β-G conformers, the single-

* Author to whom correspondence should be addressed (telephone 011-886-4-2205-3366, ext. 3326; fax 011-886-4-22062891; e-mail irisko@mail.cmu.edu.tw).

10.1021/jf0354085 CCC: $27.50
Published on Web 05/04/2004

helix and triple-helix have been considered to be the biologically active forms.

The above health benefits of the naturally occurring 1,3-β-Gs have been proved, so we were encouraged to identify new food sources. A fluorescent dye, aniline blue, was used in this study to label 1,3-β-G. It has long been recognized as a staining dye to localize callose in plants. Its active constituent is an impurity of the commercially available dye (*15*). The chemical structure of the responsible fluorochrome was sodium 4,4′-[carbonylbis(benzene-4,1-diyl)bis(imino)]bis(benzene sulfonate), designated sirofluor (*16*). 1,3-β-G of single-helix conformation preferentially reacts with aniline blue in solution (*6*) and is eventually labeled, whereas the unlabeled dye is decolorized. Only the stably bound form of 1,3-β-G−dye complexes exhibits fluorescence. The specific binding of aniline blue to 1,3-β-G has been well illustrated elsewhere (*17*).

Using previous observations as a basis, we hypothesized that functional 1,3-β-G in food can be differentially solubilized and specifically quantified and used to identify new sources. The objectives of this study, therefore, were to establish a convenient extraction procedure and a simple aniline blue-induced 1,3-β-G fluorescence assay. Then these analytical approaches were applied to the screening of foods potentially rich in 1,3-β-G. We successfully constructed a reproducible calibration curve using curdlan as standard to measure 1,3-β-G distribution in the food extracts. Some 1,3-β-G-containing foods were identified and compared with lentinan-containing shiitake.

MATERIALS AND METHODS

Materials. Amylopectin, aniline blue (C.I. 42780, CAS 28983-56-4), barley glucan (G6513), bovine serum albumin (BSA), cellulose (C6288), glucose, glycogen (G8751), laminarin (L9634), mushroom glucan (G8902), phenol, pullulan (P4516), sulfuric acid, yeast glucan (G5011), and common reagent grade chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Curdlan (YWN9300) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Pachyman (B23359) was purchased from Calbiochem-Novabiochem Corp. (San Diego, CA). Glycine was purchased from Bio-Rad (Hercules, CA). Six categories of raw food samples including legume seeds (soybean, mung bean, and Indian bean), cereals (adlay seeds and oat), vegetables (celery, chin-chian leaves, and Chinese cabbage), tubers (radish, carrot, and taro), fruits (banana, apple, and pear), and mushrooms (*Shiitake pileus*, snow mushroom, and Juda's ear) were purchased from a local fresh food market.

Fractionation of Food Extract. Food samples were fractionated into cold-water soluble, hot-water soluble, cold-alkaline soluble, and hot-alkaline soluble fractions. Because they have both dry and hydrous forms, to be consistent, only initial treatments were different. For dry samples such as legume seeds or cereals, 10 g of sample was first ground into a fine powder by a Braun coffee bean grinder in 10 s bursts at 20 s intervals three times, followed by passage through a 400 mesh sieve. Then 3 g of powdered sample was mixed with 80 mL of cold PBS buffer (pH 7.2) and transferred into a small beater vessel. The vessel was submerged in an ice−water jacket chamber (Biospec Products, Bartlesville, OK) and blended for 20 s three times at 4 °C. For hydrous samples such as vegetables, tubers, fruits, and mushrooms, 10 g of sample was first chopped and mixed with 40 mL of cold PBS buffer, followed directly by homogenization in the above blender for 30 s at 1 min intervals three times at 4 °C to consistency. Each homogeneous sample was thus centrifuged (Hitachi CR-21) at 5000*g* and 4 °C for 10 min. The supernatant was collected while the pellet was added with another 40 mL of PBS to repeat the extraction. The resulting combined supernatant was the cold water extract (fraction I).

The pellet was further extracted with 40 mL of hot deionized water by autoclaving at 121 °C for 30 min. After cooling to room temperature, the mixture was centrifuged the same way as above except at 25 °C, and the resulting supernatant was the hot-water extract (fraction II). The pellet was then subjected to alkaline extraction with 40 mL of 1 N NaOH by mixing at 100 rpm for 2 h in a cold room. After centrifugation as described above at 4 °C, the supernatant was the cold-alkaline extract (fraction III). Finally, the pellet was extracted with another 40 mL of 1 N NaOH at 65 °C in a water bath for 2 h, followed again by centrifugation except at 25 °C. The supernatant was thus the hot-alkaline extract (fraction IV). The final pellet residues were collected and together with the original minced samples were dried at 105 °C to constant weight to obtain their mass value. The above four extracted fractions were stored at −20 °C for further analysis. Prior to quantification, the frozen fractions were thawed on ice first and then heated to 50 °C for 8 min with occasional vortexing followed by putting on ice for sampling.

Total Sugar Determination. Total sugars were determined according to a phenol−sulfuric acid colorimetric method (*18*).Two hundred microliter sample fractions were first mixed with 5% phenol (w/v in water) solution and then with concentrated sulfuric acid solution in a volume ratio of 1:1:5. The reaction mixtures were incubated for 30 min at room temperature, and the optical densities of the orange-yellow color produced were measured at 490 nm. The calibration curve was constructed by using glucose as standard.

Microassay for 1,3-β-G. The fluorescence dye-binding microassay for 1,3-β-G quantification was adapted from a procedure by Shedletzky et al. (*19*). The extracted food fraction or glucan sample was first properly diluted 10−50-fold with1 N NaOH to a final volume of 300 μL in a 1.5 mL microcentrifuge tube. Next, 30 μL of 6 N NaOH was added and incubated at 80 °C for 30 min. The tube of denatured glucan conformers was immediately put on an ice bath. A 630 μL dye mix (40 volumes of 0.1% aniline blue in water, 21 volumes of 1 N HCl, and 59 volumes of 1 M glycine/NaOH buffer, pH 9.5) was mixed into the tube, which was then incubated at 50 °C for 30 min to form 1,3-β-G−fluorochrome complex. The unbound fluorescent dye was further decolorized at room temperature for 30 min, and the fluorescence intensity was measured with a fluorescence spectrophotometer (Hitachi F-2000). The fluorescence of the 1,3-β-G−aniline blue complex was measured at an emission wavelength of 502 nm (20 nm slit width) with an excitation wavelength of 398 nm (20 nm slit width). The dilution factor for tested samples was determined empirically to obtain fluorescence intensity within the calibration range. Each sample was performed in duplicate.

Curdlan was used as 1,3-β-G standard to construct the calibration curve. The binding specificity of the commercial aniline blue dye to 1,3-β-G was examined by comparing the aniline blue induced fluorescent intensities among various glucans. Tested glucans included yeast glucan, pachyman, mushroom glucan, pullulan, barley glucan, laminarin, glycogen, and cellulose. A range of diluted glucan concentrations were prepared in 1 N NaOH, and each 300 μL was taken for the above dye reaction. The effect of simulative protein on the fluorescent assay was tested by adding final concentrations of 1, 5, 10, and 30 μg/mL BSA in 4 or 10 μg/mL curdlan solutions in a total volume of 300 μL. These formulated solutions were then subjected to dye reaction as described above. Besides, protein concentration in each fraction was determined according to the Bradford method (*20*) using protein assay standard II dye reagent concentrate kit from Bio-Rad (Hercules, CA).

RESULT AND DISCUSSION

Fluorescence Spectra of Aniline Blue Dye. To adapt the assay that measured 1,3-β-G reaction product for glucan synthase activity (*19*), we first examined the binding stability of the commercial aniline blue to curdlan in the dye mix. The fluorescence spectra of the aniline dye existing as a free form and as its bound form with curdlan were compared in the presence of water or the dye mix (**Figure 1**). Curdlan was used as standard because of its homogeneous nature from fermentation and because it can be specifically stained by aniline blue (*21*). We also maintained the same proportion between the dye mix and the sample as described previously (*19*) particularly because the fluorescence intensity of aniline blue−glucan complexes can be affected by ionic strength (*22*), pH, and

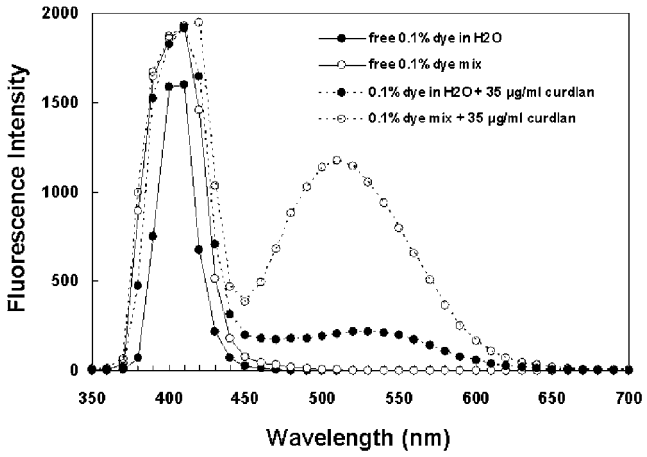

Figure 1. Fluorescence spectra of the free and bound forms of aniline blue.

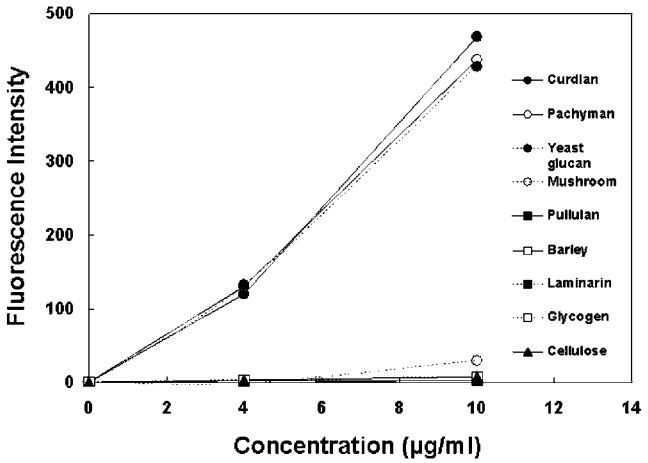

Figure 2. Specificity of the fluorescence dye-binding detection of aniline and various β-glucans.

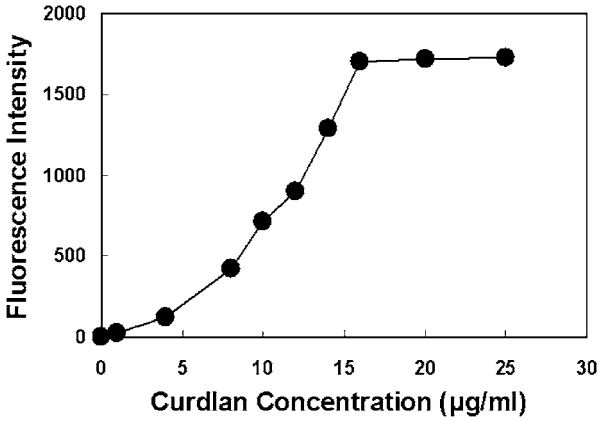

Figure 3. Aniline blue dye-binding saturation curve of curdlan standard.

temperature, as was well demonstrated by Young and Jacobs (*3*). **Figure 1** showed that if 0.1% aniline dye was in either water or dye mix as free form, both cases exhibited only excitation peaks at a wavelength of ~400 nm and emitted no fluorescence at higher wavelength range. However, if 0.1% aniline dye was mixed with 35 μg/mL of curdlan in the dye mix, then it apparently exhibited both excitation and emission spectra. If 0.1% aniline dye was mixed with 35 μg/mL of curdlan in water, however, there was only one-fifth of the fluorescence intensity of its presence in the dye mix. Therefore, the fluorochrome itself does not fluoresce in aqueous solution, but it does quite strongly upon binding with 1,3-β-G. The composition of the dye mix is essential for curdlan to form a stable complex with aniline dye. These observations were consistent with those made previously by Young et al. (*6*), which indicated that 1,3-β-G opens the triple-helical conformation at alkaline pH at NaOH concentrations >0.25 M and that only its single-helix-rich portion is capable of forming a complex with glucan. The maximal excitation wavelength (Ex) was observed at 398 nm, and the maximal emission wavelength (Em) was observed at 502 nm, which were set as measuring condition for our studies.

Specificity of the Fluorescence Assay. The specificity of the fluorescence binding of aniline blue with 1,3-β-G in our assay mixture was demonstrated when we compared various glucan sources within a 10 μg/mL concentration range. Curdlan, pachyman, pullulan, and laminarin are linear 1,3-β-G. Mushroom glucan and yeast glucan are 1,3-β with branched 1,6-β glucans. Barley glucan is MLG. Glycogen, a 1,3-α with 1,6-α branched glucan, and cellulose, a 1, 4-β glucan, served as negative controls. **Figure 2** shows that curdlan, pachyman, and yeast glucan exhibited significant and similar quantitative correlation signals. Laminarin showed a very weak signal, whereas mushroom, pullulan, barley glucan, glycogen, and cellulose showed almost background signals. We found that different degrees of fluorescence exerted by different 1,3-β-G are indeed dependent on structural and conformational differences. Only those containing common specific 1,3-β-G structure exerted similar fluorescence profiles. The evidence for the poorer fluorescence signal of laminarin, pullulan, and barley glucan was consistent with the report by Wood and Fulcher (*22*). The results of our negative control glucans were again consistent with the previous report of Evans and Hoyne (*16*). Because pachyman (*9*) and yeast glucan (*10*) were proven to be biologically active 1,3-β-Gs, the specificity of our microassay assay therefore indicated the ability to selectively identify the presence of aniline blue-induced 1,3-β-G with a conformation similar to that of those functional species.

1,3-β-Glucan Quantification by the Fluorescence Assay. To quantify 1,3-β-G in food samples, we constructed a calibration curve of curdlan. **Figure 3** shows that there is a linear correlation of the fluorescence intensity of curdlan concentration in the range of 0−18 μg/mL until a saturation plateau is reached. We used the lower 0−10 μg/mL range to extrapolate 1,3-β-G concentration for our food samples. Foods contain proteins, which may interfere with fluorescence; legumes are especially rich in proteins, ~20−40% (*23*). Soybean contains up to 42% proteins, which are mainly globulins and albumins; therefore, we tested the influence of protein on the aniline fluorescence measurement using BSA as simulative proteins. Curdlan at 4 and 10 μg/mL concentrations was mixed with 1−30 μg/mL of BSA (percent of BSA in curdlan was 10−300%).

Figure 4 shows that the presence of 1−5 μg/mL BSA in 10 μg/mL curdlan did not affect the fluorescence intensity. However, when 5 μg/mL BSA was in 4 μg/mL curdlan, the intensity increased 28%. The intensity was not affected when 5 μg/mL of BSA was with 10 μg/mL of curdlan. It seemed that the fluorescence intensity from BSA will be an interfering factor if its concentration is higher than that of curdlan. When 10 μg/mL BSA was added in 10 μg/mL curdlan, the fluorescence intensity increased 2-fold. Therefore, protein is an interfering factor in the fluorescence of 1,3-β-G−aniline blue complex as predicted, but 10−50% (1−5 μg/mL range) of BSA in 10 μg/mL curdlan had no effect on the calibration.

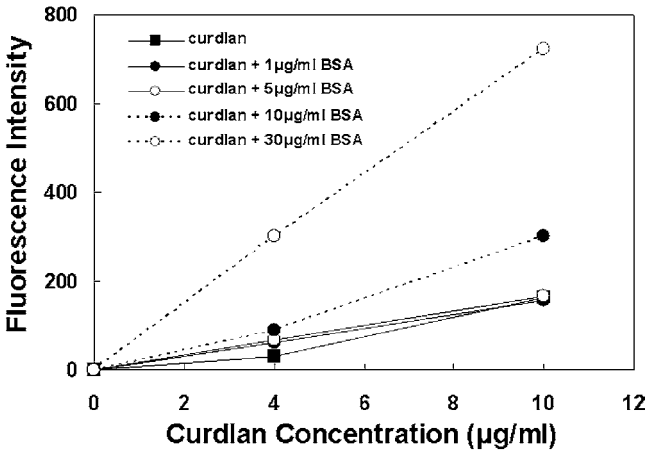

Figure 4. Influence of protein on curdlan quantification by the fluorescence method.

We further measured the protein concentration in the food fractions to make sure their values were below the allowable protein concentration of the assay. Results showed that their protein content was in the range of 0.02−0.21 μg/mL, which was 25-fold less than the limit for fluorescence assay (5 μg/mL); therefore, protein should not be an interference factor in their fluorescence measurements.

Quantification of 1,3-β-G Content in Food Samples. The method of glucan preparation is crucial to obtaining a group of populations with similar solubilities and conformations and to identify biologically reactive 1,3-β-Gs. Sequential extraction with hot water, cold NaOH, and hot NaOH solution was a common procedure used in studies such as *Cryptoporus volvatus* (*24*) and *Agaricus blazei* (*25*). A study in which it was attempted to prepare soluble forms of 1,3-β-G from the yeast *Saccharomyces cerevisiae* was also reported recently (*26*).

We thus designed a procedure to collect four extract fractions of 17 food samples from six categories. 1,3-β-G concentration in each fraction was measured by the fluorescence assay, and its content in food was calculated according to a curdlan calibration curve. The percentage of 1,3-β-G in the sample dry weight for each fraction is summarized in **Table 1A**, and its percentage in total sugar is summarized in **Table 1B**. **Table 1** also shows the total and subtotal of 1,3-β-G in water soluble (fraction I plus fraction II) and alkaline soluble fractions (fraction III plus fraction IV). The profiles of 1,3-β-G distribution in all food samples are shown in **Figure 5**. We found the structure recovery procedure for frozen 1,3-β-G to be important. The method we used was to pretreat the thawed food extract by

Table 1. 1,3-β-Glucan Content of the Extracted Fractions in Food

(A) Dry Weight

	mean ± SD (%)						
sample	fraction I	fraction II	total water extract	fraction III	fraction IV	total alkaline extract	total
soybean	0.37 ± 0.18	0.13 ± 0.04	0.50 ± 0.23	0.23 ± 0.10	0.10 ± 0.04	0.32 ± 0.14	0.82 ± 0.37
mung bean	0.09 ± 0.03	0.09 ± 0.03	0.18 ± 0.06	0.12 ± 0.02	0.07 ± 0.02	0.19 ± 0.04	0.37 ± 0.10
Indian bean	0.08 ± 0.04	0.05 ± 0.01	0.13 ± 0.05	0.01 ± 0.01	0.02 ± 0.03	0.03 ± 0.04	0.16 ± 0.01
adlay seeds	0.08 ± 0.03	0.09 ± 0.02	0.18 ± 0.05	0.13 ± 0.04	0.07 ± 0.00	0.20 ± 0.03	0.38 ± 0.09
oat	0.05 ± 0.02	0.17 ± 0.06	0.22 ± 0.09	0.15 ± 0.03	0.05 ± 0.00	0.19 ± 0.04	0.41 ± 0.12
Chinese cabbage	0.03 ± 0.03	0.06 ± 0.13	0.09 ± 0.11	0.00 ± 0.00	0.06 ± 0.14	0.06 ± 0.14	0.15 ± 0.03
celery	0.06 ± 0.00	0.05 ± 0.00	0.11 ± 0.00	0.07 ± 0.00	0.06 ± 0.02	0.13 ± 0.02	0.24 ± 0.02
chin-chian	0.33 ± 0.11	0.12 ± 0.04	0.45 ± 0.08	0.04 ± 0.02	0.04 ± 0.06	0.08 ± 0.08	0.53 ± 0.01
carrot	0.09 ± 0.00	0.14 ± 0.02	0.23 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.27 ± 0.04
radish	0.12 ± 0.01	0.19 ± 0.03	0.31 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.08 ± 0.01	0.39 ± 0.00
taro	0.02 ± 0.01	0.07 ± 0.02	0.09 ± 0.01	0.03 ± 0.01	0.16 ± 0.23	0.19 ± 0.22	0.28 ± 0.21
banana	0.19 ± 0.04	0.06 ± 0.00	0.25 ± 0.04	0.05 ± 0.00	0.05 ± 0.00	0.10 ± 0.00	0.35 ± 0.02
apple	0.11 ± 0.00	0.05 ± 0.00	0.16 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.22 ± 0.00
pear	0.08 ± 0.01	0.07 ± 0.02	0.15 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.21 ± 0.01
Shiitake pileus	0.00 ± 0.01	0.07 ± 0.02	0.01 ± 0.01	0.24 ± 0.02	0.12 ± 0.01	0.36 ± 0.25	0.37 ± 0.01
snow mushroom	0.09 ± 0.01	0.58 ± 0.05	0.67 ± 0.04	1.03 ± 0.35	0.84 ± 0.13	1.87 ± 0.48	2.54 ± 0.52
Juda's ear	0.08 ± 0.01	0.05 ± 0.13	0.13 ± 0.15	0.01 ± 0.13	0.14 ± 0.03	0.05 ± 0.16	0.28 ± 0.25

(B) Total Sugar

	mean ± SD (%)				
sample	fraction I	fraction II	fraction III	fraction IV	total
soybean	0.61 ± 0.28	0.22 ± 0.04	0.37 ± 0.14	0.16 ± 0.06	1.36 ± 0.52
mung bean	0.11 ± 0.04	0.10 ± 0.03	0.14 ± 0.02	0.07 ± 0.01	0.42 ± 0.10
Indian bean	0.11 ± 0.04	0.07 ± 0.01	0.01 ± 0.01	0.03 ± 0.04	0.22 ± 0.01
adlay seeds	0.11 ± 0.04	0.12 ± 0.04	0.16 ± 0.06	0.10 ± 0.01	0.49 ± 0.13
oat	0.06 ± 0.02	0.19 ± 0.06	0.16 ± 0.03	0.05 ± 0.00	0.46 ± 0.11
Chinese cabbage	0.06 ± 0.02	0.13 ± 0.09	0.00 ± 0.00	0.13 ± 0.21	0.31 ± 0.10
celery	4.79 ± 0.47	3.52 ± 0.18	5.46 ± 0.42	4.29 ± 1.43	18.05 ± 0.80
chin-chian	10.65 ± 3.85	3.91 ± 1.02	1.16 ± 0.63	1.22 ± 1.82	16.94 ± 0.37
carrot	6.34 ± 0.20	10.53 ± 0.01	1.72 ± 0.32	1.32 ± 0.69	19.91 ± 0.81
radish	6.02 ± 0.02	9.06 ± 1.51	3.13 ± 0.02	0.90 ± 0.31	19.11 ± 1.16
taro	0.06 ± 0.01	0.21 ± 0.03	0.09 ± 0.00	0.47 ± 0.27	0.80 ± 0.26
banana	4.28 ± 0.37	1.35 ± 0.00	1.13 ± 0.01	1.13 ± 0.06	7.88 ± 0.31
apple	1.46 ± 0.04	0.66 ± 0.01	0.40 ± 0.03	0.40 ± 0.02	3.04 ± 0.11
pear	0.76 ± 0.01	0.66 ± 0.07	0.28 ± 0.01	0.28 ± 0.02	1.98 ± 0.04
Shiitake pileus	0.21 ± 0.02	0.39 ± 0.04	12.18 ± 0.01	6.35 ± 0.03	19.13 ± 0.04
snow mushroom	0.21 ± 0.01	1.25 ± 0.11	2.23 ± 0.75	1.82 ± 0.29	5.47 ± 0.01
Juda's ear	0.08 ± 0.02	0.05 ± 0.15	0.01 ± 0.14	0.15 ± 0.03	0.30 ± 0.33

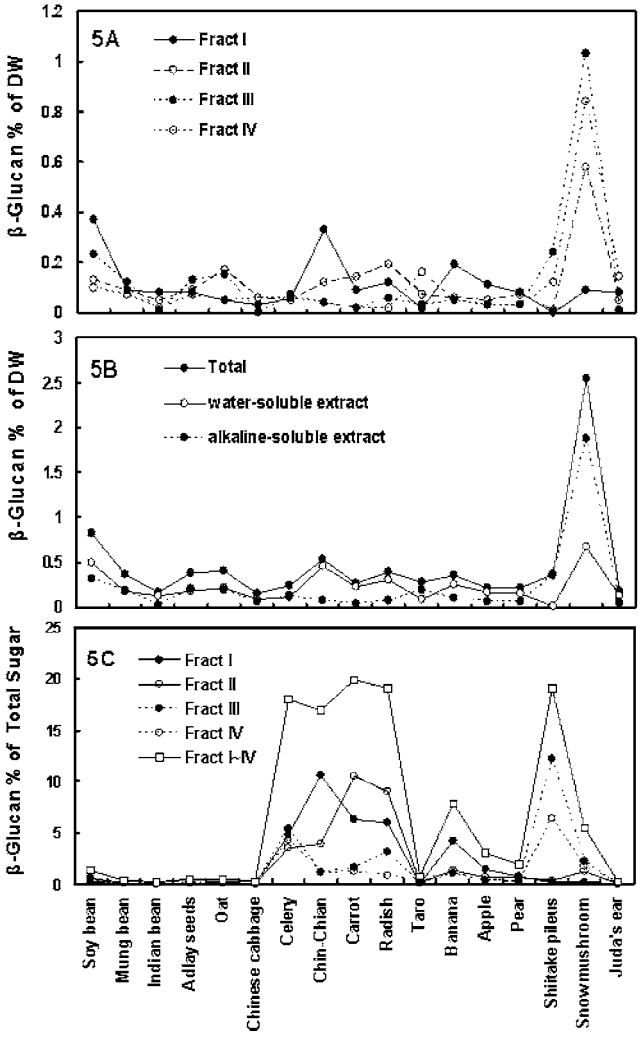

Figure 5. 1,3-β-G distribution in the food extracted fractions: (**A**) 1,3-β-G content of the four fractions in the food dry weight; (**B**) 1,3-β-G content of the water (fractions I and II) and alkaline (fractions III and IV) food extracts in the food dry weight; (**C**) 1,3-β-G distribution of the four fractions in the food total sugar.

reheating it to 50 °C for 8 min with vortex and then putting it on ice before withdrawing homogeneous aliquots for aniline binding analysis. Without this reheating step, 1,3-β-G contents were underestimated by up to 50%.

When total 1,3-β-G in food dry weight was compared, the legume group contained 0.16−0.82%, and soybean was rich in the cold-water soluble form (0.37%, fraction I) (**Figure 5A**,**B**). Oat in the cereal group contained 0.41%. The vegetable group contained 0.15−0.53%, and chin-chian was rich in the cold-soluble form (0.33%, fraction I). The tuber group contained 0.27−0.39%, and radish was the highest. The fruit group contained 0.21−0.35%, and banana was the highest. The mushroom group contained 0.28−2.54%, and snow mushroom showed the most abundant content (2.54%) and was rich in hot-water soluble (0.58%, fraction II), cold-alkaline soluble (1.03%, fraction III), and hot-alkaline soluble forms (0.84%) (fraction IV). Total 1,3-β-G in snow mushroom (2.54%) is 7.5-fold higher than in the well-known 1,3-β-G (lentinan)-containing *Shiitake pileus* (0.37%). In addition, total 1,3-β-G in soybean (0.82%) was twice the amount of that in *Shiitake pileus*.

When subtotals of water-extract and alkaline-extract 1,3-β-G were compared, there were many more water-soluble than alkaline-soluble forms in all fruits, carrot, and radish. Snow mushroom (0.67%) contained the most water soluble 1,3-β-G, followed by soybean (0.5%), chin-chian (0.45%), and radish (0.31%). Snow mushroom (1.87%) again contained the most alkaline soluble 1,3-β-G, followed by *Shiitake pileus* (0.36%) and soybean (0.32%). Most 1,3-β-G in *Shiitake pileus* was in alkaline soluble forms (0.36/0.37%) and so was that in snow mushroom (1.87/2.54%).

When 1,3-β-G contents in total sugar were compared, **Table 1B** and **Figure 5C** showed that 1,3-β-G consisted of 17−20% total sugar in celery, carrot, radish, chin-chian, and *Shiitake pileus*. Nearly 11% total sugar of chin-chian was cold-water soluble 1,3-β-G (fraction I). 1,3-β-G distribution in total sugar of celery was in all forms. An average of 10% total sugar of carrot and radish was hot-water soluble 1,3-β-G (fraction II). Besides, banana contained more 1,3-β-G than apple and pear and was rich in cold-water soluble form (4.28/7.88%, fraction I).

Plants and fungi are the main food supplies of carbohydrates and dietary fiber, and are good sources for health functional 1,3-β-Gs. Much work has been done with the biological functions of 1,3-β-G. For 1,3-β-G used as host defense potentiator (HDP), it is mediated through specific receptors on target cells. 1,3-β-G receptors on human monocytes are of phagocytic receptors with ligand specificity for yeast and fungal carbohydrate polymers (*7*). The mechanism of tumoricidal β-glucans has been used for immunotherapy. High molecular weight 1,3-β-Gs such as schizophyllan, lentinan, krestin, grifolan, and pachyman exhibit immunomodulatory activities (*9*). 1,3-β-G as BRM has demonstrated that molecular conformation, degree of branching, molecular size, and solubility are important determinants for biological activity and that M_r (100000−200000 g/mol) glucans with degree of branching of 0.2−0.33 are most active (*11*). 1,3-β-G ligand, PGG-glucans from *S. cerevisiae*, bind with specific receptors on target cells to cause subsequent complement activation, phagocytosis, lysosomal enzyme release, and release of prostaglandin, thromboxan, and leukotrienes to promote nonspecific anti-infective function (*10*). *In vitro* studies of 1,3-β-G binding to a lectin domain within complement receptor type 3 (CR3) also performed in an animal model (*27*). A number of specific 1,3-β-G receptor binding sites were widely expressed and located on the cell membranes of human neutrophils and macrophages (*28*). All of the above studies made us believe that similar health mechanisms can fit into the same functional 1,3-β-G group if new sources can be found.

Here we demonstrated a systematic approach to quantify 1,3-β-G content in raw food materials by a specific aniline blue fluorescent binding assay. Some food items in this study found to possess high 1,3-β-G contents are candidates as functional 1,3-β-G sources. Their 1,3-β-G content values can be used as references, but not the absolute values, because variation should exist when they are grown in various geographical areas and soil conditions and from different cultivars. Our results, however, indicated that they may contain potent 1,3-β-G species and are currently under investigation.

ACKNOWLEDGMENT

We thank Yi-Ching Li for technical assistance.

LITERATURE CITED

(1) Stasinopuolos, S. J.; Fisher, P. R.; Stone, B. A.; Stanisich, V. A. Detection of two loci involved in (1−3)-β-glucan (curdlan) biosynthesis by *Agrobacterium* sp. ATCC31749, and comparative sequence analysis of the putative curdlan synthase gene. *Glycobiology* **1999**, *9*, 31−41.

(2) Mizono, M.; Minato, K.; Tsuchida, H. Preparation and specificity of antibodies to an anti-tumor β-glucan, lentinan. *Biochem. Mol. Biol. Int.* **1996**, *39*, 679−685.
(3) Young, S.-H.; Jacobs, R. R. Sodium hydroxide-induced conformational change in schizophyllan detected by the fluorescence dye, aniline blue. *Carbohydr. Res.* **1998**, *310*, 91−99.
(4) Yanaki, T.; Itoh, W.; Tabata, K. Correlation between the antitumor activity of schizophyllan and its triple helix. *Agric. Biol. Chem.* **1985**, *50*, 2415−2416.
(5) Mueller, A. M.; Raptis, J.; Rice, P. J.; Kalbfleisch, J. H.; Stout, R. D.; Ensley, H. E.; Browder, W.; Williams, D. L. The influence of glucan polymer structure and solution conformation on binding to (1−3)-β-D-glucan receptors in a human monocyte-like cell line. *Glycobiology* **2000**, *10*, 339−346.
(6) Young, S. H.; Dong, W. J.; Jacobs, R. R. Observation of a partially opened triple-helix conformation in 1,3-β-glucan by fluorescence resonance energy transfer spectroscopy. *J. Biol. Chem.* **2000**, *275*, 11874−11879.
(7) Czop, J. K.; Kay, J. Isolation and characterization of β-glucan receptors on human mononuclear phagocytes. *J. Exp. Med.* **1991**, *173*, 1511−1520.
(8) Miura, T.; Ohno, N.; Miura, N. N.; Adachi, Y.; Shimada, S.; Yadomae, T. Antigen-specific response of murine immune system toward a yeast β-glucan preparation, zymosan. *FEMS Immunol. Med. Microbiol.* **1999**, *24*, 131−139.
(9) Vetvicka, V.; Thornton, B. P.; Ross, G. D. Soluble β-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. *J. Clin. Invest.* **1996**, *98*, 50−61.
(10) Wakshull, E.; Brunke-Reese, D.; Lindermuth, J.; Fisette, L.; Nathans, R. S.; Crowley, J. J.; Tufts, J. C.; Zimmerman, J.; Mackin, W.; Adams, D. S. PGG-glucan, a soluble β-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF*k*B-like factor in human PMN: evidence for a glycosphingolipid β-(1,3)-glucan receptor. *Immunopharmacology* **1999**, *41*, 89−107.
(11) Bohn, J. A.; BeMiller, J. N. (1,3)-β-D-glucan as biological response modifiers: a review of structure−functional activity relationship. *Carbohydr. Polym.* **1995**, *28*, 3−14.
(12) Braaten, J. T.; Wood, P. J.; Scott, F. W.; Wolynetz, M. S.; Lowe, M. K.; Bradley-White, P.; Collins, M. W. Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* **1994**, *48*, 465−474.
(13) Nicolosi, R.; Bell, S. J.; Bistrian, B. R.; Greenberg, I.; Forse, R. A.; Blackburn, G. L. Plasma lipid changes after supplementation with β-glucan fiber from yeast. *Am. J. Clin. Nutr.* **1999**, *70*, 208−212.
(14) Estrada, A.; Yun, C. H.; van Kessel, A.; Li, B.; Hauta, S.; Laarveld, B. Immunomodulatory activities of oat β-glucan *in vitro* and *in vivo*. *Microbiol. Immunol.* **1997**, *41*, 991−998.
(15) Smith, M. M.; McCully, M. E. A critical evaluation of the specificity of aniline blue induced fluorescence. *Protoplasma* **1978**, *95*, 229−254.
(16) Evans, N. A.; Hoyne, P. A. A fluorochrome from aniline blue: structure, synthesis and fluorescence properties. *Aust. J. Chem.* **1982**, *35*, 2571−2575.
(17) Evans, N. A.; Hoyne, P. A.; Stone, B. A. Characteristics and specificity of the interaction of a fluorochrome from aniline blue (sirofluor) with polysaccharides. *Carbohydr. Polym.* **1984**, *4*, 215−230.
(18) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.
(19) Shedletzky, E.; Unger, C.; Delmer, D. P. A microtiter-based fluorescence assay for (1,3)-β-glucan synthases. *Anal. Biochem.* **1997**, *249*, 88−93.
(20) Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−252.
(21) Nakanishi, I.; Kimura, K.; Kusui, S.; Yamazaki, E. Complex formation of gel-forming bacterial (1→3)-β-D-glucans (curdlan-type polysaccharides) with dyes in aqueous solution. *Carbohydr. Res.* **1974**, *32*, 47−52.
(22) Wood, P. J.; Fulcher, R. G. Specific interaction of aniline blue with (1→3)-β-D-glucan. *Carbohydr. Polym.* **1984**, *4*, 49−72.
(23) Anantharam, K.; Finot, P. A. Nutritional aspects of food proteins in relation to technology. *Food Rev. Int.* **2003**, *9*, 629−655.
(24) Kitamura, S.; Hori, T.; Kurita, K.; Takeo, K.; Hara, C.; Itoh, W.; Tabata, K.; Elgsaeter, A.; Stokke, B. T. An antitumor, branched (1,3)-β-D-glucan from a water extract of fruiting bodies of *Crytoporus volvatus*. *Carbohydr. Res.* **1994**, *263*, 111−121.
(25) Ohno, N.; Furukawa, M.; Miura, N. N.; Adachi, Y.; Motoi, M.; Yadomae, T. Antitumor β glucan from the cultured fruit body of *Agaricus blazei*. *Biol. Pharm. Bull.* **2001**, *24*, 820−828.
(26) Lee, J. N.; Lee, D. Y.; Ji, I. H.; Kim, G. E.; Kim, H. N.; Sohn, J.; Kim, S.; Kim, C. W. Purification of soluble β-glucan with immune-enhancing activity from the cell wall of yeast. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 837−841.
(27) Yan, J.; Vetvicka, V.; Xia, Y.; Coxon, A.; Carroll, M. C.; Mayadas, T. N.; Ross, G. D. β-glucan, a "specific" biologic response modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). *J. Immunol.* **1999**, *163*, 3045−3052.
(28) Willment, J. A.; Gordon, S.; Brown, G. D. Characterization of the human β-glucan receptor and its alternatively spliced isoforms. *J. Biol. Chem.* **2001**, *276*, 43818−43823.

Received for review November 29, 2003. Revised manuscript received March 4, 2004. Accepted March 25, 2004. This work was supported by National Science Council of Taiwan, R.O.C., under Health Food Research and Development Grant NSC 89-2312-B-039-002.

JF0354085