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Effects of Processing on Availability of Total Plant Sterols, Steryl Ferulates and Steryl Glycosides from Wheat and Rye Bran

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Rye and wheat bran are excellent natural sources of plant sterols in the diet. Their content, however, may vary according to processing. Thermal (roasting and heating in a microwave oven), mechanical (milling and cryogenic grinding), and enzymatic treatments (hydrolysis with xylanase or β-glucanase or a mixture of these two enzymes) were performed, and their effect on sterol content, extractability of sterols and the characteristic steryl conjugates of cereals (steryl ferulates, steryl glycosides, and acylated steryl glycosides) were studied. Mechanical and enzymatic treatments increased the apparent sterol content, whereas aqueous processing without enzymes hindered the availability of total sterols, especially from rye bran. Changes were also seen in the amounts of steryl conjugates caused by the enzymatic treatments. On the basis of the results of this study, it can be speculated that a combination of fine particle size and enzymatic processing results in optimal sterol availability in cereal processing.

KEYWORDS: Rye; *Secale cereale* L. wheat; *Triticum aestivum* L.; bran; enzyme; hydrolysis; plant sterol; steryl ferulate; steryl glycoside; acylated steryl glycoside; processing

INTRODUCTION

In western diets, cereals and vegetable oils are the two most important sources of natural dietary plant sterols, the order of importance depending on the country (*1–3*). The contents of plant sterols as well as other bioactive components are highest in the germ, the outer layers of the cereal kernels (bran), and products derived of these materials (*4, 5*). Cereals have a somewhat different composition of sterols and steryl conjugates when compared to oilseeds and vegetables. As in most plant materials, in cereals, sitosterol and campesterol are the most common sterols. However, contents of their saturated counterparts, sitostanol and campestanol, are much higher in cereals than in vegetables. Furthermore, phenolic acid esters of sterols are found in significant quantities mainly in cereals. Cereals also contain substantial amounts of glycosylated sterols, which are found in other types of plant materials as well but are far less studied than free sterols and steryl fatty acid esters. Free sterol alcohols have a free hydroxyl group in C-3, which may form conjugates with fatty acids, phenolic acids, and sugars. Steryl ferulates (SFs) are the most common steryl phenolic acid esters (**Figure 1**) and the only ones found in wheat and rye. Steryl glycosides (SGs) are most often conjugates of single glucose, but dimers and oligomers also occur, as does binding to other carbohydrates. Steryl glycosides may further be esterified to fatty acids in the C-6-position of the carbohydrate, thus forming an acylated steryl glycoside (ASG).

Fiber-rich foods are very good sources of minerals and more than several bioactive components such as plant sterols, tocopherols and tocotrienols, folates, lignans, and phenolic acids. These so-called copassengers or cotravelers of dietary fiber add to the nutritionally beneficial attributes of fiber-rich foods like grains and vegetables and are an essential part of the healthy dietary fiber complex. The structural network formed by the dietary fiber matrix may, however, hinder the bioavailability of some components either by physically blocking the components inside the fiber complex or by various interactions (ionic binding, hydrogen binding, van der Vaal's forces, etc.) that may inhibit the compound from, for example, absorption or from interaction with other molecules in the gastrointestinal tract.

A number of studies have been performed to see whether processing affects the release of minor bioactive components from a food matrix by breaking these bonds or otherwise

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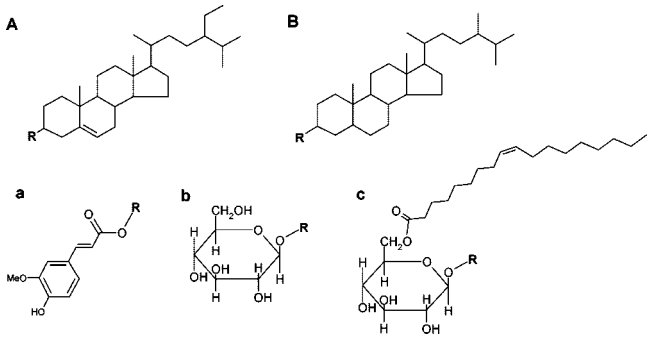

Figure 1. Structures of sitosteryl (**A**) and campestanyl (**B**) conjugates: ferulate (**a**), glycoside (**b**), and acylated glycoside (**c**).

interfering with the interactions. These treatments do not increase the total plant sterol content *per se*, but they may increase the amount available from the matrix (in this paper, referred to as the apparent sterol content). For example, Lane et al. (*6*) demonstrated that heating (especially using microwaves) increased the release of tocotrienols from rice bran. Further, Kim et al. (*7*) showed that the yield of steryl ferulates in rice germ oil could be increased by 0.5% from 2.8% to 3.3%, if the germ was roasted before extraction. On the other hand, no increase in the extractable tocotrienols from corn fiber or corn germ oil after thermal treatments was seen in the recent study by Moreau and Hicks (*8*).

Another way to increase the extractability of compounds from the surrounding matrix is to increase the surface area of particles. Cryogenic grinding is a very effective sample preparation method for biological tissues, which may be used for effective homogenization of the tissues, resulting in a fine particle size (*9*). Also regular milling increases the surface area of the particles, thus likely improving the availability of bioactive compounds. For example, in the case of flaxseed lignans, milling and crushing were shown to also enhance the bioavailability of lignans in the human digestive system (*10*). Another way to increase the release of minor compounds from the fiber-rich matrix could be to partially hydrolyze the fiber polymers, thus releasing the molecules possibly entrapped in the polymer network. To our knowledge, there are no studies so far that focus on the effects of processing (including the use of enzymes) on the availability of plant sterols from rye and wheat brans.

In the current study, we investigated processing as a means to improve the overall availability of plant sterols of rye and wheat bran. Bran samples were subjected to different thermal (heating in an oven or a microwave oven), mechanical (milling with a centrifugal mill and cryogenic grinding with liquid nitrogen), and enzymatic treatments (hydrolysis with xylanase or β-glucanase or their mixture). The effects of processing (i) on the apparent sterol content of rye or wheat bran, (ii) on the release of plant sterols using sequential extraction, and (iii) on the typical steryl conjugates of cereals (ASG, SG, and SF) were studied.

MATERIALS AND METHODS

Materials. Rye bran and wheat bran were obtained from Fazer Mill (Oy Karl Fazer Ab, Lahti, Finland). Particle sizes of the bran materials before and after milling were analyzed (at least 100 particles per sample) using a Leica DMLB microscope equipped with a Donpisha 3 CCD Color vision camera module, and the images were analyzed with Leica QWin software (Leica Microsystems, Heerbrugg, Switzerland). The enzymes used for the enzymatic processing were Multifect Xylanase (398100 nkat/mL; Genencor International B.V. Leiden, The Netherlands) and β-glucanase: Cereflo 200L (22000 nkat/mL; Novozymes A/S, Bagsvaerd, Denmark). All solvents were of HPLC grade and supplied by Rathburn (Walkerburn, Scotland) or J.T. Baker (Deventer, The Netherlands).

Thermal, Mechanical and Enzymatic Processing. In the thermal and mechanical processing, rye and wheat bran portions (10 g each) were treated in the following ways: roasting in open dishes at 120 °C for 1 h (Termaks TS 8056, Bergen, Norway), heating in a microwave oven for 3 × 2 min (630 W output, used at 90% power, Microwave MDS-81D, CEM Corporation, Matthews, NC), milling to a particle size < 0.5 mm (Cyclotec 1093 sample mill, Tecator, Sweden), and cryogenic grinding with liquid nitrogen on a Waring commercial laboratory blender. For the enzymatic processes, 2.5 g of rye or wheat bran was weighed, 20 g of distilled water was added, and the pH of the suspension was adjusted to 5.0 using 0.5 M hydrochloric acid. The weight of the suspension was adjusted to 25.0 g to produce a 10% (w/v) suspension. Enzyme solutions were prepared in distilled water and either xylanase or β-glucanase alone or their combination, or a corresponding volume of distilled water (for the controls without enzymes), were added to the samples. The dose was 2000 nkat/g of bran for each enzyme. Samples were incubated for 24 h at +40 °C in a water bath using magnetic stirring (250 rpm). At the end of incubation, bran suspensions were transferred quantitatively to tared centrifugation tubes, and enzymes in the samples were inactivated by incubation in a boiling water bath for 10 min. Samples were cooled, frozen, and freeze-dried. The dry weight (d.w.) of the samples was measured.

Each thermal, mechanical, and enzymatic treatment was performed four times on both rye and wheat bran, and a sample from each processed lot was analyzed for its total sterols and steryl conjugates.

Sequential Extraction. A subsample (2 g) was taken of each processed sample for extraction. Total lipids of each sample were extracted using a Soxtec Avanti 2050 extraction apparatus (Foss Tecator, Hillerød, Denmark). Free, unbound lipids were extracted at 170 °C for 90 min with heptane; the solvent was collected, and from the same sample, moderately bound lipids were extracted with acetone at 150 °C for 45 min. The heptane and acetone extracts were evaporated to dryness with a rotary evaporator at 50 °C and redissolved to 10 mL of heptane/diethyl ether (1:1, v/v). A subsample of the extract was taken for total sterol and steryl conjugate analysis. Further, the remaining tightly bound sterols were analyzed from the residue by direct hydrolysis (*11*).

Analysis of Total Sterols. The content of total plant sterols in the extracts and the residue was analyzed after acid and alkaline hydrolyses. The extract subsamples were evaporated to dryness, and 4 mL of ethanol was added to each sample (dried extracts and the residue) before hydrolysis. As acid hydrolysis may degrade some of the less-stable free sterols, milk was added to protect the sterols of the extracts as reported earlier (*5*). After hydrolysis, samples were derivatized to TMS-ethers and analyzed gas chromatographically with flame ionization detection (GC-FID) using dihydrocholesterol as an internal standard. Hydrolyses, derivatization, and GC analysis were performed as described by Piironen et al. (*11*). Total plant sterol contents of the untreated wheat and rye brans were also analyzed with the same method directly and after sequential extraction (*11*).

Analysis of Steryl Conjugates. Steryl ferulates, steryl glycosides, and acylated steryl glycosides in heptane and acetone extracts were analyzed by HPLC with a Hewlett-Packard 1090 II liquid chromatograph (Waldbronn, Germany) using a diol column (LiChrosorb Diol 5 μm, 100 × 3.0 mm; VDS Optilab, Berlin, Germany) and a gradient elution with a flow rate of 0.5 mL/min and an injection volume of 20–100 μL. The eluent composition and program were as follows: solvent A = heptane, B = isopropanol; 0–8 min 100% A, 8–10 min 100–99% A, 10–30 min 99% A, 30–40 min 99–75% A, 45–55 min 75% A. For chromatograms, see **Figure 2**. Column heating was set to 25 °C. Steryl glycosides and acylated steryl glycosides were quantified with an evaporative light-scattering detector (Cunow DDL21, Cunow Department DMS, France) using compressed air at 1 bar and a detector temperature of 50 °C. The standard curve for ASG and SG quantification was calculated as the negative logarithm of the detector response. The linear range ($R^2 > 0.99$) was 0.8–7.6 μg/injection for ASG and 0.4–4.8 μg/injection for SG. Steryl ferulates were quantified with a UV detector at 315 nm (linear range 0.2–6–2 μg/injection). Steryl

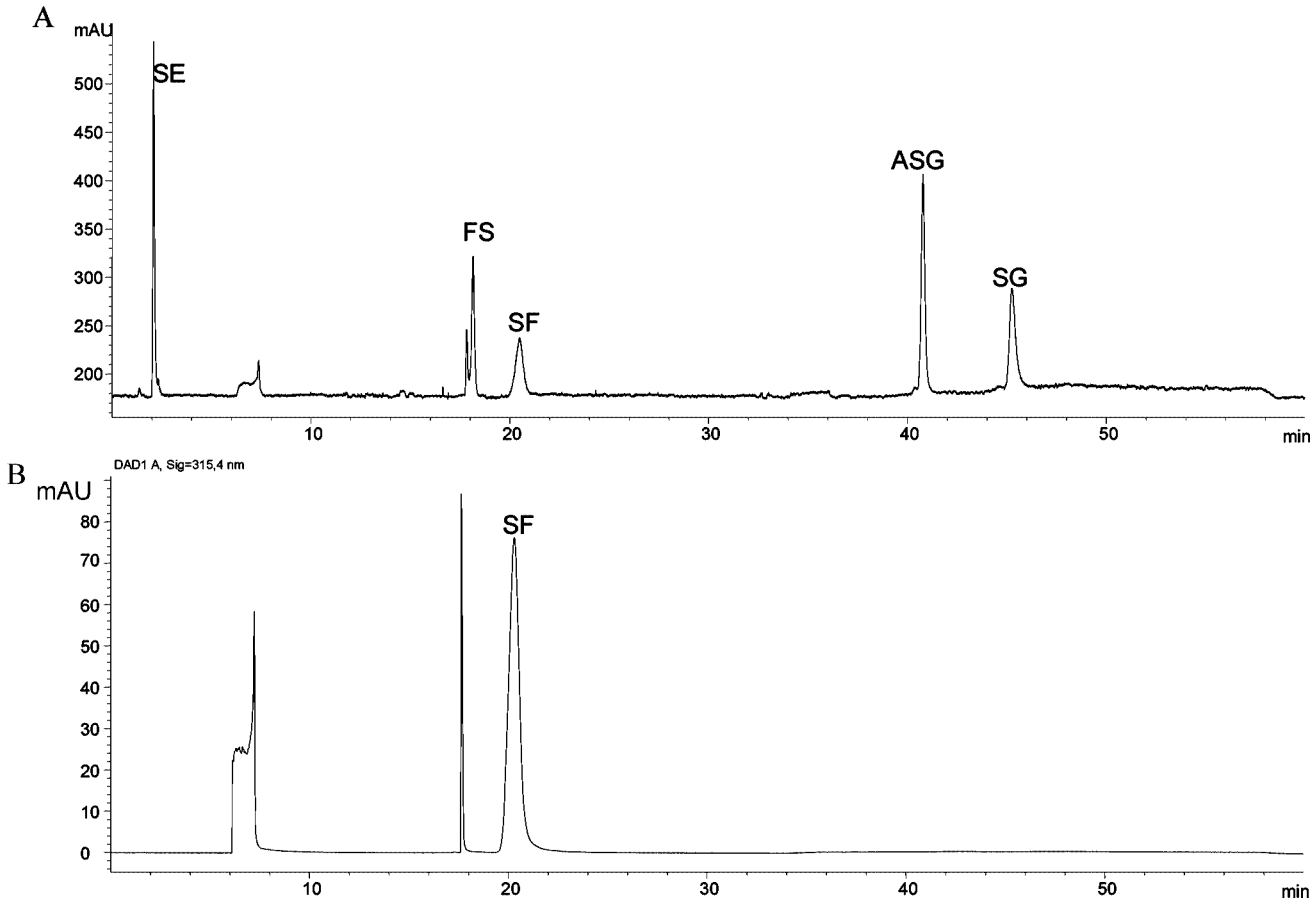

Figure 2. ELSD chromatogram of steryl esters (SE), free sterols (FS), steryl ferulates (SF), acylated steryl glycosides (ASG), and steryl glycosides (SG) (**A**) and a UV chromatogram of steryl ferulates (SF) at 315 nm (**B**).

glycoside (purity > 98%) and acylated steryl glycoside (purity > 98%) standards from Matreya, Inc. (Pleasant Gap, PA) and sitostanyl ferulate (synthesized by the method of Condo et al. (*12*), purity > 99%) were used as external standards for quantification.

Data Analysis. All statistical analyses were performed separately for rye and wheat. The total sterol contents were calculated as the sum of the heptane, acetone, and residue fractions. The results for each processing were summarized as the average (in mg/100 g on a dry weight basis) of four independent samples. The averages were compared separately for each type of processing (thermal, mechanical, or enzymatic) by an analysis of variance (ANOVA) and prestated contrasts. Similarities between the averages under comparison were summarized as end points of the 90% confidence intervals (corresponding to 95% certainty) for the corresponding contrasts (for details, see ref *13*). The effects of processing on the extractability of plant sterols and contents of steryl conjugates were studied using one-way ANOVA and Tukey's test for *post hoc* analysis. Statistical analyses were done using Statistix 8.0 (Analytical Software, Tallahassee, FL) and SPSS 13.0 software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effects of Processing on the Apparent Sterol Content. The thermal, mechanical, and enzymatic processing methods substantially affected the apparent sterol content (the amount of sterols available for analysis after processing) analyzed after sequential extraction (**Table 1**). The highest sterol content of rye bran was obtained after cryogenic grinding in liquid nitrogen (225.0 mg/100 g d.w.), whereas no effect was seen with the other mechanical processing, milling with a centrifugal mill, compared to the untreated starting material. The difference in the sterol content after these mechanical processes was 17.2 mg/100 g d.w. By analyzing the 90% confidence intervals for the contrast, we can conclude that the apparent sterol content of rye bran was at least 15.3 mg/100 g higher after cryogenic grinding than regular milling. Both of the size reduction treatments decreased the average particle size of rye bran significantly. The average length of particles was reduced from 263 to 37 and 39 μm by cryogenic grinding and milling, respectively, indicating that the particles were indeed smaller than those of the unprocessed bran and thus had a larger surface area. The average particle sizes after size reduction by milling and cryogenic grinding were, however, almost equal and do not therefore explain the difference in the amounts of sterols available for analysis.

Also, in wheat bran, mechanical processing affected the apparent sterol content the most when compared to the untreated bran, but for wheat bran samples, a greater effect was seen with conventional milling, which increased the total sterol content from 215.9 to 226.0 mg/100 g d.w., whereas cryogenic grinding did not have a significant effect on the sterol content. The difference between the sterol contents of wheat bran after mechanical processing was 12.3 mg/100 g ($p = 0.02$), and the data suggest that the sterol content of wheat bran after conventional milling is at least 2.0 mg/100 g, but at a maximum of 20.5 mg/100 g higher than after cryogenic grinding. The average particle length of wheat bran was 97 μm, which was reduced to 47 and 45 μm by milling and cryogenic grinding, respectively. Changes in the average particle size were smaller for wheat bran than for rye bran, which may partially explain the smaller difference caused by the mechanical processes.

Thermal treatments, on the other hand, did not significantly affect the apparent sterol contents. The difference between mean

Table 1. Total Plant Sterols (mg/100 g d.w.) in Rye and Wheat Brans after Processing[a]

					contrast value (SE)	*p* value
Rye	no processing 207.8 (8.5)					
process method, total sterols mean (*SD*)	milling 207.8 (*1.4*)	cryogenic grinding 225.0 (*1.5*)				
contrast coefficient	1	–1			–17.2	<0.001
	roasting 209.0 (*13.3*)	microwave heating 207.6 (*7.1*)				
	1	–1			1.5	0.85
	no enzyme 193.7 (*10.4*)	xylanase 202.1 (*11.7*)	β-glucanase 205.9 (*21.0*)	both enzymes 220.0 (*3.8*)		
effect of xylanase	1	1	–1	–1	−30.1 (13.6)	0.05
effect of β-glucanase	1	–1	1	–1	−22.5 (13.6)	0.12
interaction effect of enzymes	1	–1	–1	1	5.8 (13.6)	0.68
Wheat	no processing 215.9 (*8.4*)					
	milling 226.0 (*9.8*)	cryogenic grinding 213.8 (*4.2*)				
	1	–1			12.3	0.02
	roasting 209.3 (*3.8*)	microwave heating 203.2 (*4.8*)				
	1	–1			6.1	0.09
	no enzyme 195.1 (*5.3*)	xylanase 205.1 (*8.4*)	β-glucanase 208.0 (*9.9*)	both enzymes 209.6 (*4.9*)		
effect of xylanase	1	1	–1	–1	−15.0 (7.8)	0.08
effect of β-glucanase	1	–1	1	–1	−14.1 (7.8)	0.09
interaction effect of enzymes	1	–1	–1	1	−5.9 (7.8)	0.46

[a] The mean values are the averages ($n = 4$) of the sums of the totals sterols in three fractions (heptane, acetone, and residue) of each sample. The coefficients used in the statistical contrast (1/–1) are given below each processing.

sterol contents of rye bran after roasting and heating in a microwave oven was only 1.5 mg/100 g. The contrast of apparent sterol contents after thermal processing shows that there is no significant difference between the mean sterol contents ($p = 0.85$) and that the difference is at a maximum of 16.2 mg/100 g. Similarly, the thermal treatments of wheat bran did not increase the apparent sterol content, and compared to the untreated bran, the content of plant sterols rather decreased due to the thermal treatments. The difference between the mean sterol content of wheat bran after oven and microwave heating was 6.1 mg/100 g, which is somewhat greater than the difference seen in thermally treated rye bran. In neither rye bran nor wheat bran was the apparent sterol content increased with the thermal treatments (roasting and microwave heating). This is unlike the studies by Lane et al. (*6*) and Kim et al. (*7*) with rice germ, which stated that heating (especially microwave heating) cereal material would increase extractability and the apparent contents of bioactive components like sterols in resulting products by breaking bonds and interactions between the cereal matrix and the smaller compounds. On the other hand, baking has been shown to also decrease the apparent content of alkylresorcinols from rye bread (*14*). The decrease was due to the formation of new bonds between the alkyresorcinols and the surrounding starch matrix. In another study, thermal processing was shown to increase the available content of ferulic acid, but decrease the contents of tocopherols and tocotrienols from rye and wheat (*15*). The increase in the apparent content of free ferulic acid was accompanied with a decrease in the content of esterified ferulic acid, demonstrating that the thermal treatment caused a hydrolysis of the ester bond linking ferulic acid to the matrix. On the other hand, the decrease in tocopherol and tocotrienol contents was due to degradation rather than a decrease in the availability from the matrix. Sterols, however, do not form covalent bonds with the matrix and are more heat-stable than tocopherols and tocotrienols, which is likely the reason the thermal treatments did not significantly affect the apparent content of total sterols. Had the time of heating been longer or the temperature higher, the content of sterols could have decreased owing to the formation of oxidation products. However, sterols have been shown to be stable under regular baking conditions (*16*).

Enzymatic processing caused significant differences in the amounts of total plant sterols susceptible for analysis from the rye bran matrix. Treating the bran with water alone decreased the apparent sterol content from 207.8 mg/100 g to 193.7 mg/100 g d.w., which was recovered to the original levels of 202.1 and 205.9 mg/100 g d.w. after hydrolyzing the matrix with xylanase or β-glucanase, respectively. Furthermore, adding these enzymes as a mixture increased the apparent sterol content of rye bran even more to 220.0 mg/100 g d.w., but due to the high variation in the results with the single enzymes, the interaction (the effect of xylanase in the presence of β-glucanase) was not statistically significant ($p = 0.68$). The effect of xylanase alone, however, was significant ($p = 0.05$), which may be explained by the high content of arabinoxylan in the rye bran. When water is introduced to the sample (like the addition of distilled water without enzymes), arabinoxylan hydrates and forms a viscous structure, which may block the hydrophobic sterols inside. Once fiber is then hydrolyzed to smaller units, these hydrated structures are broken and sterols released from the matrix. The effect of β-glucanase was smaller and less significant ($p = 0.09$) than that of xylanases, as the content of β-glucan in rye is lower than the content of arabinoxylans, and thus the hydrolysis of β-glucan makes a smaller change. Andreasen et al. (*17*) demonstrated that commercial xylanase preparations increased the content of free ferulic acid from rye. However, this increase can rather be explained by liberation of the esterified ferulic acid from the arabinoxylan polymer than by the release of free ferulic acid that has been entrapped in the arabinoxylan network.

Aqueous treatment without enzymes also had a negative effect on the analytical susceptibility of total sterols from wheat bran, and even treatment with enzymes could not restore the level of sterols to that of the untreated bran. The aqueous treatment without enzymes decreased the apparent content of sterols to 195.1 mg/ 100 g d.w., and each of the two enzymes was able to restore some of this loss. However, the interaction effect of the combination of the two enzymes was not significant ($p =$

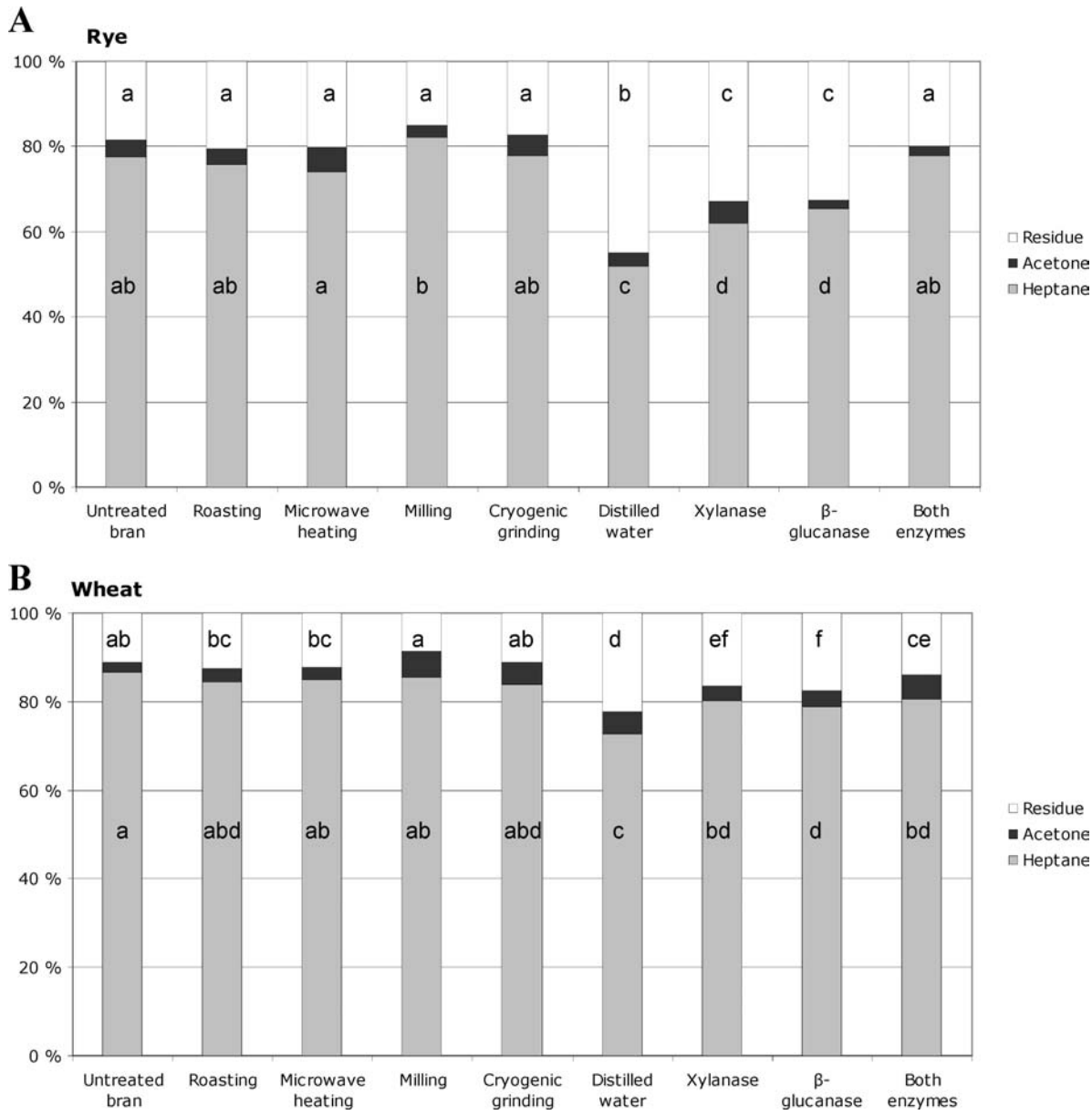

Figure 3. Proportion of fractions of sequential extraction (heptane and acetone) and the residue of the total plant sterols of rye bran (**A**) and wheat bran (**B**). Results within a fraction (heptane or residue) of each cereal bran with no statistically significant differences are given a common letter. Differences in the acetone fraction were statistically insignificant.

0.46). This is likely due to the fact that arabinoxylan and β-glucan contents in wheat are lower when compared to those of rye (*18*), and thus the change caused by their partial hydrolysis is not as great. Further, as wheat bran comprises over 20% cellulose, the use of cellulases could likely improve the release of sterols even more than the uses of β-glucanase and xylanase.

Effects of Processing on Extractability/ Release of Plant Sterols. To analyze not only the processing effects on the apparent content of sterols but also the ease with which they are released from the matrix (later on referred to as extractability), sterols were studied by sequential extraction. The three classes analyzed were (i) easily extractable lipids that were extracted with heptane, (ii) moderately bound lipids extracted with acetone, and (iii) tightly bound lipids released with acid and base hydrolyses from the residue. In untreated rye bran, 78% of the sterols were extracted with heptane (**Figure 3A**), suggesting that they are not bound in the matrix and are likely to be readily available in the gastrointestinal tract. Another 4% of sterols in the untreated rye bran was extracted with acetone, suggesting that the remaining 18% of sterols in the residue is tightly bound, and thus less available for use.

The extractability of sterols from rye bran was enhanced by milling, after which 82% of the sterols were in the easily extractable fraction. On the other hand, the aqueous treatment without enzymes significantly decreased the extractability of sterols, decreasing the portion of easily extractable sterols to 52%. Xylanase and β-glucanase treatments recovered part of this loss in extractability by increasing the portion of extractable sterols to 62% and 65%, respectively. The combination of these two enzymes returned the extractability of the sterols to the original level (78%) in the readily extractable fraction. There were no statistically significant differences in the amount of moderately bound sterols (acetone fraction), meaning that the aqueous treatment did not only modestly affect sterol extract-

Table 2. Steryl Conjugates in Heptane Fractions (mg/100 g d.w.)[a]

	rye						wheat					
	SF		SG		ASG		SF		SG		ASG	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
untreated bran	71.8	*14.2*	9.7	*1.6*	29.8	*5.7*	51.6	*11.8*	20.3	*1.9*	25.2	
Mechanical Processing												
milling	46.0*	*4.1*	8.3	*0.5*	17.4*	*1.0*	44.1	*4.9*	19.9	*4.1*	17.6	*5.1*
cryogenic grinding	66.1	*2.0*	10.2	*0.7*	24.4	*2.1*	46.6	*4.8*	15.6	*4.2*	35.5*	*4.3*
Thermal Processing												
roasting	64.4	*5.6*	8.8	*0.9*	23.1	*2.2*	35.0	*5.4*	19.5	*1.1*	33.3	*1.3*
microwave heating	71.9	*11.6*	4.3*	*1.4*	29.8	*3.1*	46.5	*4.7*	15.8	*0.4*	28.4	*1.2*
Enzymatic Processing												
no enzyme	88.5	*11.6*	2.7*	*0.4*	18.1*	*4.7*	35.9	*7.4*	34.6*	*5.6*	15.6	*9.7*
xylanase	71.8	*7.0*	2.4*	*0.6*	47.5*	*7.1*	49.6	*2.5*	8.2*	*1.2*	33.3	*3.2*
β-glucanase	75.0	*2.3*	7.8	*2.8*	46.1*	*8.9*	52.7	*3.8*	10.7*	*1.0*	29.1	*0.8*
both enzymes	90.9	*3.0*	5.9*	*0.8*	37.1	*3.1*	40.2	*12.0*	17.0	*3.2*	23.3	*3.1*

[a] Means with a statistically significant difference to their corresponding sample of untreated bran are marked with an asterisk.

ability but rather made sterols bind tightly to the matrix or formed new structures, which blocked the sterols inside.

The changes in sterol extractability were less severe in the processed wheat bran samples. The proportion of easily extractable sterols of the untreated bran was higher in wheat bran (87%) than it was for rye bran (78%), and changes after processing were less significant (**Figure 3B**). Unlike rye bran, milling did not enhance the sterol extractability of wheat bran. The aqueous treatments did decrease sterol extractability, like they did with rye bran, but the effect was not as great: the portion of easily extractable sterols in the sample with the addition of distilled water was 73%, which again was improved by xylanase and β-glucanase treatments to 80% and 79%, respectively.

The effects of enzymatic processing on the availability of sterols are even greater when the data of the apparent sterol contents and the extractability of them are combined. In aqueously treated rye bran without enzymes, the total sterol content was 193 mg/100 g, of which only 52% (100 mg/100 g) was readily available. When the rye bran matrix was hydrolyzed with the two enzymes, the total sterol content was increased to 220 mg/100 g, of which 78% (172 mg/100 g) was in the easily extractable fraction. Thus, hydrolysis with the enzyme mixture significantly increased the amount and availability of plant sterols from the rye bran. The effect was slightly smaller with wheat bran (content of the easily extractable sterols of untreated wheat bran, 187 mg/100 g, and after aqueous processing with and without enzymes, 169 and 142 mg/100 g, respectively) but could possibly be improved with a different selection of enzymes. These results show that the mere addition of water without enzymes may dramatically decrease the availability of sterols from cereal products. Enzymes may be used in baking and other processing to enhance various quality attributes by, for example, increasing the loaf volume of breads, resulting in a softer structure, and decreasing the rate of staling during storage (*19*, *20*). These results show that, in addition to the sensory properties, the use of enzymes may also positively affect the availability of sterols and other bioactive compounds from the final product.

Effects of Processing on Steryl Conjugates. The extracted heptane and acetone fractions were analyzed for their contents of the characteristic cereal steryl conjugates (SF, SG, and ASG). Only traces of these conjugates with no statistically significant differences were observed in the acetone fractions (data not shown). The heptane fractions, however, showed some variation in the contents of steryl conjugates (**Table 2**). Further, the effects of processing were somewhat different between the two cereals. The overall contents of these conjugates were slightly higher than would be expected according to our previous paper (*5*). The analysis was done with a different method, and the results are given as the mass of the total steryl conjugate molecule with the side chain, not calculated as free sterols. Thus, the values are not directly comparable and should, in this study, be studied rather as indicators of process-induced change compared to those values of the starting material. Like total sterols, the effects of mechanical and thermal processing were also minor with steryl conjugates, indicating that the conjugates generally act similarly in these treatments and no single conjugate is enhanced or suppressed more than the other.

Differences were again seen in the effects caused by aqueous treatments, with steryl glycosides being affected the most. There was a significant increase in the content of wheat steryl glycosides, but a decrease in rye steryl glycosides caused by the aqueous treatment without enzymes. These changes can be a result of some endogenous enzymes, which are activated by the addition of water. Further, adding the exogenous xylanase and β-glucanase significantly decreased the contents of steryl glycosides and increased the acylated steryl glycosides in both cereal brans. This phenomenon is similar to that reported by Moreau et al. (*21*) on tobacco cells that treatment with xylanase increased the content of acylated steryl glycosides at the expense of free sterols and other sterol conjugates (sterol fatty acid esters and steryl glycosides). For some reason, the effect was stronger with rye than with wheat bran, and a milder effect was seen when the two enzymes were added simultaneously. As the metabolism of these steryl conjugates (ASG, SG, and SF) is still poorly understood, more information is needed to estimate the significance of the changes in their total amounts and availability in human nutrition.

ABBREVIATIONS USED

ELSD, evaporative light scattering detector; HPLC, high-performance liquid chromatography; ASG, acylated steryl glycoside; SG, steryl glycoside; SF, steryl ferulate.

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