

# Molecular Weight Distribution of $\beta$ -Glucan in Oat-Based Foods

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ABSTRACT

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Oats, different oat fractions as well as experimental and commercial oat-based foods, were extracted with hot water containing thermostable  $\alpha$ -amylase. Average molecular weight and molecular weight distributions of  $\beta$ -glucan in extracts were analyzed with a calibrated high-performance size-exclusion chromatography system with Calcofluor detection, specific for the  $\beta$ -glucan. Oats, rolled oats, oat bran, and oat bran concentrates all had high Calcofluor average molecular weights ( $206 \times 10^4$  to  $230 \times 10^4$  g/mol) and essentially monomodal distributions. Of the oat-containing experimental foods, extruded flakes, macaroni, and muffins all had high average molecular weights. Pasteurized apple juice, fresh pasta, and teacake, on the other hand, contained degraded  $\beta$ -glucan. Calcofluor aver-

age molecular weights varied from  $24 \times 10^4$  to  $167 \times 10^4$  g/mol in different types of oat bran-based breads baked with almost the same ingredients. Large particle size of the bran and short fermentation time limited the  $\beta$ -glucan degradation during baking. The polymodal distributions of  $\beta$ -glucan in these breads indicated that this degradation was enzymatic in nature. Commercial oat foods also showed large variation in Calcofluor average molecular weight (from  $19 \times 10^4$  g/mol for pancake batter to  $201 \times 10^4$  g/mol for porridge). Boiling porridge or frying pancakes did not result in any  $\beta$ -glucan degradation. These large differences in molecular weight distribution for  $\beta$ -glucan in different oat products are very likely to be of nutritional importance.

Dehulled oats (groats) have a high content (3–7%) of linear mixed linkage (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan (referred to as  $\beta$ -glucan) (Wood and Beer 1998). Oat bran is produced by grinding the groats or rolled oats and separating the resulting flour by sieving, bolting, or other suitable means into a fraction enriched in the outer parts of the groats, especially the subaleurone layer with thick and  $\beta$ -glucan-rich cell walls. On a dry matter basis, oat bran should contain at least 5.5%  $\beta$ -glucan and 16% total dietary fiber (Anonymous 1989). Recently, commercial oat brans from different countries were analyzed. Their  $\beta$ -glucan content varied widely (4.7–8.3%) while  $\beta$ -glucan solubility was more stable (32–40%) (Luharoo et al 1998). Highly significant correlations were found between the contents of both total and extractable  $\beta$ -glucan and viscosity after dispersion of the brans in water. Oat fractions with higher amounts of  $\beta$ -glucan (up to 17.5%) can be obtained by dry fractionation of nondefatted oat bran (Mälkki 2001). Defatting with organic solvents to avoid clogging and to improve material flow has made it possible to isolate small amounts of dry fractions with even higher  $\beta$ -glucan contents (up to 27%).

The hypocholesterolemic effects of fiber-rich oat products are well documented (deGroot et al 1963; Ripsin et al 1992), and elevated low-density lipoprotein-cholesterol levels are associated with an increased risk for coronary heart disease (Grundy 1997). This lipid-lowering effect of foods such as oat bran, rolled oats, or oatmeal was recognized by the U.S. Food and Drug Administration in 1997 and a food-specific health claim was approved. Viscous oat  $\beta$ -glucan was identified as a major cholesterol-reducing component in oats. It is supposed to bind bile acids and increase their excretion within the feces, but the importance of structure or molecular weight distribution of the  $\beta$ -glucan was not addressed. Oat  $\beta$ -glucan also attenuates the glycemic responses (Braaten et al 1991). However, action mechanism in the human gastrointestinal tract remains uncertain, although blood glucose and insulin responses are viscosity-related (Wood et al 1994). Among other things, the viscosity in the gut will depend on solubility (concentration), structure, and molecular weight distribution of the  $\beta$ -glucan. Recently, a product-specific health claim was approved in Sweden for a yogurt with müsli which evens out the glucose level after a meal. Cereal  $\beta$ -

glucan also has a molecular-weight-dependent stimulating effect on the production of tumor necrosis factor (Roubroeks et al 2000). It is easily fermented and has a prebiotic effect (Graham and Åman 1991).

Molecular weight distribution of  $\beta$ -glucan in extracts can be determined by size-exclusion chromatography with online post-column detection with Calcofluor (Wood et al 1991; Suortti 1993). Recently, an improved calibration and calculation of average molecular weight and molecular weight distribution ameliorated the method (Rimsten et al 2003). Different methods of extraction were used in that study, giving variable extraction yields, but molecular weight distribution was generally independent of extraction method or yield. However, three consecutive extractions with hot water and added thermostable  $\alpha$ -amylase have revealed the presence of a small fraction of  $\beta$ -glucan with lower peak molecular weight in the third extract (Beer et al 1997).

In this study, we used the improved method to analyze the molecular weight distribution of  $\beta$ -glucan in oats, rolled oats, oat bran, and oat bran concentrate. Experimental and commercial oat-based foods were also analyzed to reveal effects of processing on the distribution as well as the influence of oat bran particle size and fermentation time in baking experiments with yeast leavened bread.

## MATERIALS AND METHODS

### Oat Raw Materials

Oats, rolled oats, and oat bran are commercial samples obtained from Cerealia R&D AB (Järna, Sweden). Low-fat oat bran concentrates (OBC), with normal (<15%), 15–17%, and 21–23%  $\beta$ -glucan content were gifts from Swedish Oat Fibre (Bua, Sweden).

### Experimental Foods

Experimental foods were prepared with OBC (normal  $\beta$ -glucan) and, in some cases, rolled oats (5.0%  $\beta$ -glucan) or oat bran (8.3%  $\beta$ -glucan) on a pilot scale at Cerealia R&D AB. Apple juice with 2.76% OBC was pasteurized at 94°C for 30 sec. Flakes with 24% OBC were produced by extrusion at 60 bars and 115°C, drying at 70°C, and roasting at 300°C. Fresh pasta with 7% OBC was prepared and boiled for 3 min before analysis. Macaroni with 10.2% OBC was processed at 100 bars and 40°C, and thereafter dried at 80, 90, 80, and 30°C. The macaroni was boiled for 9 min before analysis. Muffins with 4.2% OBC and 9.0% oat bran were prepared at 220°C for 8 min. Yeast-leavened soft bread with 4.5% OBC and 5.3% rolled oats was fermented twice for 35 min each time and baked at 220°C for 8 min. All products were kept frozen at  $-20^\circ\text{C}$  until analyzed.

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## Baking Experiments with Oat Bran Breads

As outlined in Table I, three different types of oat bran breads (normal, prefermented, and with sourdough) were prepared. The breads contained oat bran with 8.3%  $\beta$ -glucan (Cerealia AB) or finely disintegrated oat bran that was milled in a hammer mill with a 1.0-mm screen (President, Poul Diness, Hølbeck, Denmark). Fermentation times were 10 or 40 min (short and long, respectively) two times each at 28°C. Small bread rolls (70 g) were formed and baked at 200°C for 10 min. The ingredients in the normal oat bran breads were 29.3% oat bran, 19.5% white wheat flour, 4.9% dry yeast, 0.9% sugar, 0.7% syrup, 0.5% salt, and 43.4% water. The same ingredients were used in the prefermented oat bran bread. However, in this case, the oat bran, dry yeast, and water were mixed and prefermented for 2 hr at 38°C before mixing with the rest of the ingredients. The ingredients in the oat bran breads with sour dough were 25.8% oat bran, 19.5% white wheat flour, 4.3% wheat gluten, 1.3% dry yeast, 0.9% sugar, 0.7% syrup, 0.5% salt, and 36.9% water. The sour dough premix was prepared by mixing 30% oat bran, 60% water, and 10% sour dough starter (a mixture of 50:50 rye flour and water), fermenting for three days at 28°C, and thereafter mixing it with the rest of the ingredients at a 10% level. All breads were baked two times and kept frozen at -20°C until analyzed.

## Commercial Oat Foods

Some commercial oat foods were bought in local stores: porridge prepared according to the recipe on a package of oat flakes

(Axa havregryn, Järna, Sweden); yeast-leavened oat bread (Må Bättre Havre, Fazer, Lidköping, Sweden); crisp bread (Havreknäcke, Wasabröd, Filipstad, Sweden); extruded oats (Havrefras, Quaker Oats, Chicago, IL); a fermented yogurt-like oat product (Yosa, Bioferme AB, Piispanristi, Finland); pancake batter (Oatly, Ceba Food, Malmö, Sweden); fried pancakes of the batter; fermented oat soup with black current flavor (Proviva, Skånemejerier, Malmö, Sweden); and a yogurt with müsli with an approved product-specific health claim (Primaliv, Skånemejerier, Malmö, Sweden). All products were kept frozen at -20°C until analyzed.

## General Analysis

All samples containing >10% moisture were freeze-dried. Before analysis, samples were ground in a cyclone sample mill (Foss Tecator AB, Höganäs, Sweden) to pass a 0.5-mm screen. Total  $\beta$ -glucan content was determined enzymatically (Åman and Graham 1987). All analysis were run in duplicate, and the results are reported on a dry matter basis determined by drying samples for 6 hr at 105°C.

## Extraction and Analysis of $\beta$ -Glucan MW Distribution

Enzymes in samples were inactivated by boiling in 50% ethanol for 15 min.  $\beta$ -Glucan in products (200 mg) were extracted with hot deionized water (20 mL) with added  $\text{CaCl}_2$  (0.28 mg/mL of  $\text{H}_2\text{O}$ ) and thermostable  $\alpha$ -amylase (50  $\mu\text{L}$ , EC 3.2.1.1, 3,000 U/mL, Megazyme, Wicklow, Ireland) (Rimsten et al 2003). The mixture was immediately placed in a boiling water bath for 90 min with

TABLE I  
Baking Experiments of Oat Bran-Based Breads

Sample and Time of Fermentation	$\beta$ -Glucan Content (% of dry matter)	Calcofluor Average MW ( $\times 10^{-4}$ g/mol)	Distribution <sup>a</sup>		
			10%	50%	90%
Normal					
Bran 10 min	7.0	159	14	134	339
Bran 40 min	7.0	138	8.8	102	323
FD bran <sup>b</sup> 10 min	7.0	84	8.4	50	214
FD bran 40 min	7.0	48	5.0	20	132
Prefermented					
Bran 10 min	7.0	167	21	141	346
Bran 40 min	7.0	139	13	106	311
FD bran 10 min	7.0	31	4.6	14	68
FD bran 40 min	7.0	24	4.1	12	46
Sourdough					
Bran 10 min	5.5	135	6.2	104	311
Bran 40 min	5.5	121	5.3	72	310
FD bran 10 min	5.5	76	6.8	40	202
FD bran 40 min	5.5	44	4.7	18	119

<sup>a</sup> Percentiles describing molecular weight ( $\times 10^{-4}$ ) at which 10, 50, and 90% of the distribution fall below that value.

<sup>b</sup> Finely disintegrated bran.

TABLE II  
Oat Raw Materials and Experimental Foods

Sample	$\beta$ -Glucan Content (% of dry matter)	Calcofluor Average MW ( $\times 10^{-4}$ g/mol)	Distribution <sup>a</sup>		
			10%	50%	90%
Raw materials					
Oats	3.4	225	71	213	387
Rolled oats	5.0	230	60	221	407
Oat bran	8.3	215	54	207	379
OBC <sup>b</sup> normal	13.6	206	47	200	364
OBC 15–17 %	16.4	216	42	210	382
OBC 21–23 %	20.0	206	39	199	369
Experimental foods					
Apple juice	2.8	58	14	53	108
Extruded flakes	3.1	189	40	178	347
Fresh pasta	1.4	57	4.2	23	164
Macaroni	1.6	188	29	177	354
Muffin	1.9	192	44	182	347
Teacake	1.5	45	2.8	11	148

<sup>a</sup> Percentiles describing molecular weight ( $\times 10^{-4}$ ) at which 10, 50, and 90% of the distribution fall below that value.

<sup>b</sup> Low-fat oat bran concentrates with normal (<15%), 15–17%, and 21–23%  $\beta$ -glucan content.

occasional stirring. After cooling to room temperature, the tubes were centrifuged ( $1,500 \times g$  for 15 min) and supernatants were filtered ( $0.45 \mu\text{m}$ ) before injecting into the high-performance size-exclusion chromatography system with fluorescence detection (HPSEC-FD). This system was calibrated using  $\beta$ -glucan fractions with narrow molecular weight ranges. Calcofluor average molecular weight, including only molecules large enough to be detected with Calcofluor, and percentiles describing the molecular weight at which 10, 50, and 90% of the distribution fall below were calculated. This method has repeatability at  $<5\%$  for molecular weight analysis. Results are means of two analyses.

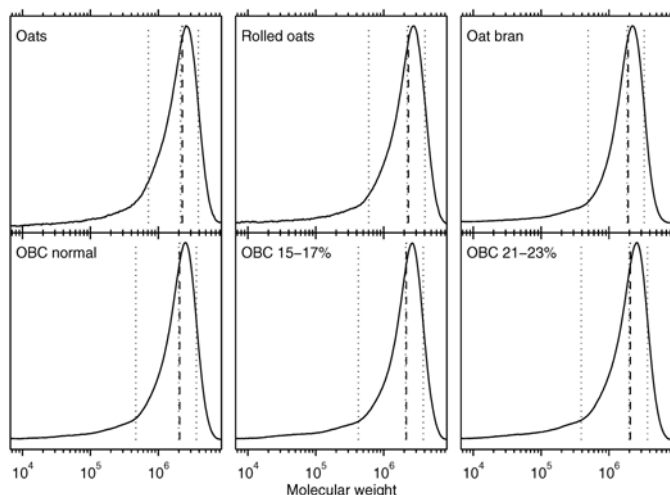
## RESULTS AND DISCUSSION

All products were pretreated with 50% hot ethanol to inactivate any remaining  $\beta$ -glucanase in the samples (Rimsten et al 2003). Soluble  $\beta$ -glucan was extracted with hot water during starch hydrolysis with a thermostable  $\alpha$ -amylase. This method extracts a significant amount of the  $\beta$ -glucan in cereal samples (7–75%) with no apparent degradation of the molecular weight. Molecular weight distribution was determined by HPLC-SEC with Calcofluor detection. This detection method is selective for the  $\beta$ -glucan in extracts and independent of molecular weight. It will, however, exclude  $\beta$ -

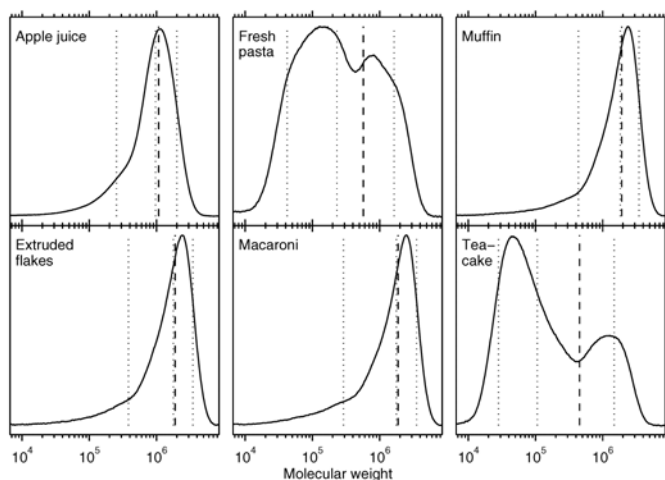
glucan of lower molecular weight ( $<10,000$ ) (Munck 1989). Therefore, MW is given as Calcofluor average molecular weight (g/mol), which is the weight average molecular weight for only the higher molecular weight  $\beta$ -glucan that is detected by Calcofluor.

### Raw Materials

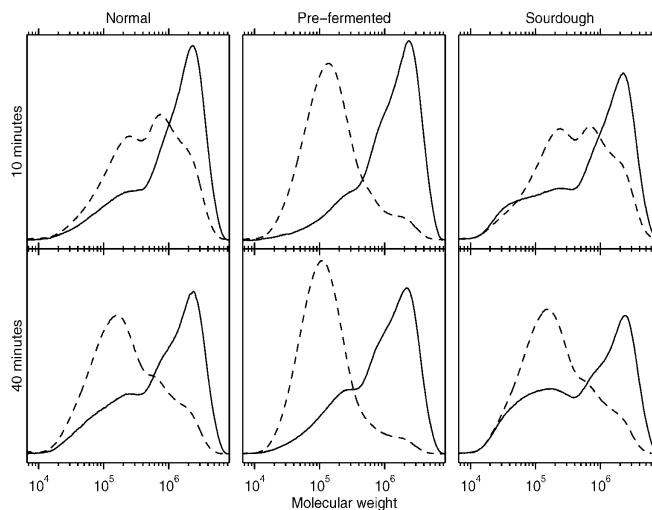
Oats, rolled oats, oat bran, and different oat bran concentrates were studied and  $\beta$ -glucan content in the samples was 3.4–20.0% (Table II). All extracted  $\beta$ -glucans showed a monomodal distribution with similar molecular weight distribution (Fig. 1). The Calcofluor average molecular weight varied by only  $206 \times 10^4$  to  $230 \times 10^4$  g/mol in the samples; the calculated percentiles (10, 50, and 90%) also showed relatively small variations. These results show



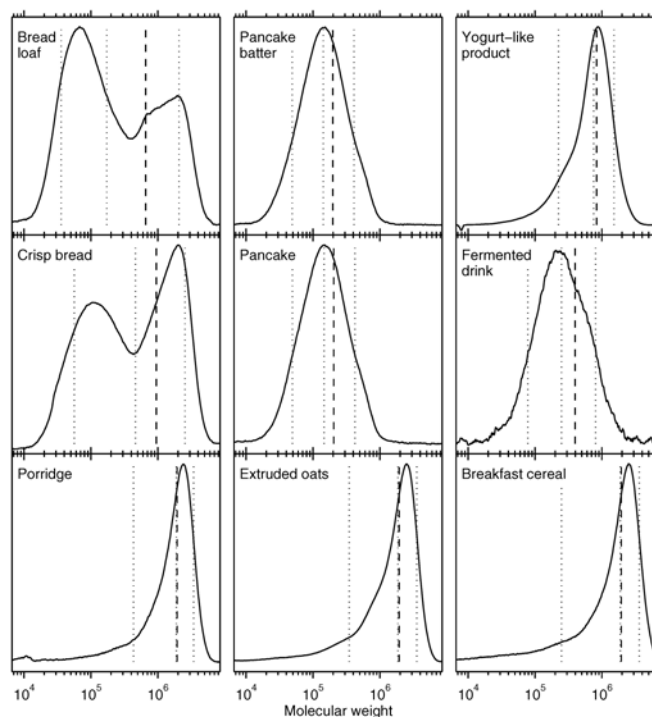
**Fig. 1.** Molecular weight (g/mol) distribution of  $\beta$ -glucan in oat raw materials. Dotted lines represent 10, 50, and 90% percentiles and dashed line represents Calcofluor average molecular weight.



**Fig. 2.** Molecular weight (g/mol) distribution of  $\beta$ -glucan in experimental foods with oats. Dotted lines represent 10, 50, and 90% percentiles and dashed line represents Calcofluor average molecular weight.



**Fig. 3.** Molecular weight (g/mol) distribution of  $\beta$ -glucan in oat bran breads (normal, pre-fermented oat bran, and with sourdough). Breads were fermented 2 $\times$  at 10 min or 2 $\times$  at 40 min. Solid lines represent breads baked with normal oat bran and dashed lines represent breads baked with finely disintegrated oat bran.



**Fig. 4.** Molecular weight (g/mol) distribution of  $\beta$ -glucan in commercial foods with oats. Dotted lines represent 10, 50, and 90% percentiles and dashed line represents Calcofluor average molecular weight.

that the molecular weight distribution of solubilized  $\beta$ -glucan in oats is retained in rolled oats, oat bran, and different types of OBC. Consequently, dry processing such as milling, sieving, and rolling, as well as extraction with aqueous ethanol, will not significantly degrade the  $\beta$ -glucan in oats. In a previous study, enzyme-inactivated samples were extracted with a carbonate buffer at 60°C (Wood et al 1991). In that study, no major differences were found in peak molecular weight between cultivars or between raw oats, heat-treated oats, rolled oats, and oat bran.

### Experimental Foods

In the experimental foods,  $\beta$ -glucan content varied between 1.4 and 3.1% (Table II). The products were made with added rolled oats, oat bran, or OBC normal as described above.  $\beta$ -Glucan from the extruded flakes, macaroni, and muffins all showed essentially monomodal molecular weight distributions (Fig. 2) and retained Calcofluor average molecular weights between  $188 \times 10^4$  and  $192 \times 10^4$  g/mol compared with  $\beta$ -glucan from the raw materials.  $\beta$ -Glucan extracted from the apple juice also showed a monomodal distribution but had a much lower average molecular weight. It is suggested that the acid in the juice randomly hydrolyzed glucosidic linkages. The fresh pasta and teacake also had low average molecular weight  $\beta$ -glucans but with bi- or polymodal distributions. In bread and fresh pasta, bi- or polymodal distributions and low molecular weights indicated that an extensive nonrandom enzymatic hydrolysis had occurred.  $\beta$ -Glucan degradation was reported in extruded ready-to-eat cereals (Wood et al 1991) and during breadmaking (Sundberg et al 1996).

### Baking Experiments with Oat Bran Breads

Experiments with yeast-leavened breads were conducted to find factors of importance for the detected degradation of  $\beta$ -glucan during breadmaking. Two types of oat bran (normal bran with relatively large particles and finely disintegrated bran) and two different fermentation times were used in the experiments. Stack sieving of coarse bran (20.1% >1,110  $\mu$ m; 71.9% >460  $\mu$ m; 3.6% >150  $\mu$ m; 3.5% >75  $\mu$ m; 0.4% >0  $\mu$ m) and finely disintegrated bran (0.8% >460  $\mu$ m; 18.1% >150  $\mu$ m; 73.2% >75  $\mu$ m; 7.0% >0  $\mu$ m) revealed large differences in particle size. Oat bran was added in the flour mix or as prefermented or sourdough breads (Table I). Two or three populations of  $\beta$ -glucan were found in all breads (Fig. 3). When the  $\beta$ -glucan was more degraded, all populations seemed to move to lower molecular weights.

These results clearly demonstrate an enzymatic hydrolysis with enzymes present in the flour mix or in the added yeast. It is not likely that this activity came from the oat bran because it had been heat-treated in such a way that this enzyme should be completely inactivated. However, the wheat flour most likely contained active  $\beta$ -glucanases. For all oat bran breads, a notable degradation of the  $\beta$ -glucan was observed, the Calcofluor average molecular weight decreased from  $215 \times 10^4$  g/mol in oat bran to  $24$ – $167 \times 10^4$  g/mol in breads. A large effect was seen for particle size with an

extensive degradation when the finely disintegrated bran was used. A notable effect was also seen for fermentation time, however this effect was less pronounced than for particle size. Some differences could also be seen between different ways of adding the bran to the dough. For bran not disintegrated, addition as sourdough seemed to give a high degradation of  $\beta$ -glucan. For finely disintegrated bran, addition of prefermented bran seemed to result in a very high degradation. These results show that baking yeast-leavened bread results in an enzymatic degradation of  $\beta$ -glucan in oat bran and that particle size and time of fermentation are important factors for this degradation.

### Commercial Oat Foods

Of the commercial oat foods studied, porridge made of rolled oats, a breakfast cereal product, and an extruded oat product showed monomodal distributions and high Calcofluor average molecular weights ( $193 \times 10^4$  to  $201 \times 10^4$  g/mol) (Fig. 4 and Table III). In these products, it is very likely that no  $\beta$ -glucanase activity was present or that any endogenous enzymes present had only a very limited time for hydrolysis. The crisp bread (bimodal distribution) and the yogurt-like product (monomodal distribution) contained medium-degraded  $\beta$ -glucans, while the bread loaf, pancake batter, fried pancakes, and fermented oat soup had highly degraded  $\beta$ -glucan. It is obvious that commercial products on the market contain soluble  $\beta$ -glucan with very different average molecular weight and molecular weight distribution, which will have a large effect on physicochemical properties and nutritional effects. It is also notable that heat treatment such as boiling porridge and frying pancakes did not degrade the  $\beta$ -glucan.

## CONCLUSIONS

Oat raw materials like groats, bran, and OBC were produced by essentially dry processing and contain intact  $\beta$ -glucan with high average molecular weight. Processing such as baking, including a fermentation step, fresh pasta preparation, and production of fermented soup and pancake batter, all seem to result in extensive degradation of the oat  $\beta$ -glucan. It is evident that heat treatment is relatively lenient for the  $\beta$ -glucan, while prolonged treatment at lower temperatures may result in extensive enzymatic degradation. Large oat bran particles and short fermentation time are important factors for reduction of  $\beta$ -glucan degradation during baking of yeast-leavened bread. The solubility and molecular weight distribution of  $\beta$ -glucan in a product is probably of nutritional importance. For example, a high molecular weight is desirable in products that lower serum cholesterol and blood sugar; a molecular weight of  $\approx 20 \times 10^3$  is desirable for tumor necrosis factor stimulation.

## ACKNOWLEDGMENTS

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TABLE III  
Commercial Oat-Based Foods

Sample	$\beta$ -Glucan Content (% of dry matter)	Calcofluor Average MW ( $\times 10^4$ g/mol)	Distribution <sup>a</sup>		
			10%	50%	90%
Bread loaf	1.1	63	3.4	15	201
Crisp bread	2.5	95	5.6	46	254
Porridge	4.9	201	47	195	356
Pancake batter	0.99	19	4.9	14	41
Pancake	0.97	20	4.9	15	43
Extruded oats	3.2	193	35	186	355
Yogurt-like product	2.8	83	22	75	152
Fermented drink	0.24	39	7.9	25	79
Breakfast cereal	16.6	194	24	188	362

<sup>a</sup> Percentiles describing molecular weight ( $\times 10^4$ ) at which 10, 50, and 90% of the distribution fall below that value.

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