Potential for β-Glucan Enrichment in Brans Derived from Oat (*Avena sativa* L.) Cultivars of Different (1→3),(1→4)-β-D-Glucan Concentrations[1]

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ABSTRACT

Eleven cultivars of oats were fractionated into coarse and fines fractions by a simple milling procedure. There were significant differences between cultivars in the (1→3),(1→4)-β-D-glucan (β-glucan) concentration of the whole groat (3.91–6.82%) and in the bran (5.81–8.89%). The average bran yield of 53.3% produced an average β-glucan concentration in the bran that was one and a half times the concentration of the groat. Although there was a weakly significant correlation between groat β-glucan and bran β-glucan ($r^2 = 0.823$), there was sufficient variation in the ratio of bran to groat β-glucan (1.28–1.60) to observe significant changes in the ranking of cultivars for bran β-glucan after milling. The average starch concentration of the groat (59.1%) dropped to 49.3% in the bran and was raised to 69.7% in the fines fraction. Starch was less variable than β-glucan with a between-cultivar coefficient of variation of 4.9% in the groat and 6.9% in the bran compared with 15.9 and 13.8%, respectively, for β-glucan.

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In recent years, a considerable market for oat bran has developed. This commercial growth is based on the nutrition conscious (Farr 1987) consumer's awareness of the potential benefits from increased dietary fiber intake (Anderson 1980, Jenkins 1980) and the particular claim that oat bran may lower serum cholesterol (Anderson 1986). This property has been attributed to the soluble fiber (primarily β-glucan) concentration of oat bran (Chen et al 1981, Klopfenstein and Hoseney 1987, Jennings et al 1988).

Commercial development has outpaced our knowledge of β-glucan concentration of oat cultivars and the brans produced from them. Indeed, what is meant by "oat bran" has been unclear because there has been no traditional milling process, as there has for wheat, to establish an acceptable standard. The definition cannot, and should not, be simply morphologically based (e.g., bran is aleurone plus outer pericarp and seed coat) although exclusions can be made (e.g., bran does not contain hull).

To address this, the AACC organized a committee to establish a definition of oat bran (Anon. 1989). A problem encountered by that committee was a lack of published analytical values. This inadequacy had already been recognized, and a number of groups (Åman and Graham 1987, Welch and Lloyd 1989) have undertaken surveys of cultivar and environmental effects on β-glucan concentration of oats. However, the product of major current interest is not the whole groat but the bran, a milling fraction obtained by separation of the coarser bran from fine particles. Thus, for the present, in addition to total β-glucan in the groat, the potential for β-glucan enrichment by milling requires consideration. As surveys and breeding programs progress, cultivars with levels of β-glucan equal to or greater than today's best commercially available brans (7–10%) may appear. For example, barley cultivars normally contain β-glucan in amounts similar to oats but cultivars with as much as 14–16% have been reported (Åman and Graham 1987, Newman et al 1989).

This paper describes a simple, small-scale milling process to produce coarse and fine fractions from a selection of oat cultivars and reports their concentrations of β-glucan and starch.

MATERIALS AND METHODS

Starch Analysis

Starch was analyzed by the method of Batey (1982) as modified by Wood et al (1991), but using 100 μl of heat-stable α-amylase from *Bacillus subtilis* (2 mg/ml; Calbiochem-Behring Corp., La Jolla, CA; catalogue 171568) and 500 μl of amyloglucosidase (50 mg/ml; Agidex, BDH, Toronto, ON). Corn starch (Fisher Scientific, Ottawa, ON) was analyzed ($n = 3$) at 101.6 ± 0.14% starch. The assay detected 1.3% anhydroglucose in lichenan (Sigma Chemical Co., St. Louis, MO), 2.1% anhydroglucose in purified (Wood et al 1991) oat β-glucan, and 2.0% in laminaran (Sigma).

Enzyme Analysis of β-Glucan

Samples were analyzed for β-glucan by the method of McCleary and Glennie-Holmes (1985) using kits marketed by Biocon (USA) Inc. (Lexington, KY). In this and the starch assay, the glucose analysis was automated using a Technicon System II Autoanalyser with the reagents from the Biocon kit (Wood et al 1991). The barley flour provided as a standard containing 4.4% β-glucan was analyzed ($n = 3$) at 4.45 ± 0.06% β-glucan.

Preparation of Oat Bran and Fines

Whole grain oats, obtained from V. D. Burrows (Plant Research Centre, Agriculture Canada, Ottawa), were dehulled using an experimental impact dehuller obtained from the Quaker Oats Co., Peterborough, Ont. Remaining hulled seeds were removed from the dehulled or hulless cultivars (Tibor and NO-1) by hand sorting. Groats were adjusted to 8.3–8.9% moisture (mean 8.61 ± 0.15%) in a Forma Scientific Incubator (model 3029, Mallinckrodt Inc., Marietta, OH) and milled at setting "0" in a falling number mill (model KT-30, Falling Number AB, Stockholm, Sweden). Duplicate 10-g portions of the flours obtained were fractionated into coarse (bran) and fines fractions in an AM Sonic Sifter (model L3P, ATM Corp. Sonic Sifter Division, Milwaukee, WI) fitted with a 40-mesh (U.S. standard; 425-μm) screen. Samples were sifted for 5 min at setting 5. Coarse and fines fractions were weighed, and portions were analyzed for β-glucan and starch.

RESULTS

Milling of Samples

Because moisture concentration influences milling and sieving characteristics (Hoseney 1986), it was controlled to between 8.3 and 8.9%. Hulls do not contain β-glucan (Wood 1987); therefore, after the dehulling procedure any hulled seeds remaining were removed to avoid shifts in analytical results arising from different levels of hull contamination. The amounts of hulled seed (Table I) ranged from 0 to 8.9%. On the basis of a 25% hull content and milling extraction of 50% bran, the apparent β-glucan concentration in the groat might have been reduced to a maximum extent (in cultivar Marion) of 0.95 of the hull-free value.

On the falling number mill, setting 0 gave the finest grind, and approximately 50% of the resultant flour was retained on a 40-mesh screen. This was judged a reasonable cut for assessing

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the potential of each cultivar for β-glucan enrichment, and so setting 0 was routinely used. Although alternative fractionation methods produce brans of higher β-glucan concentration (Wood et al 1989, and *unpublished*), these products may not accurately reflect potential for commercial milling, which aims normally for 35–40% bran yield (M. Lenz, *personal communication*). Table II shows the replicate bran yields for each cultivar. With the exception of Woodstock, these were in good agreement, with an average bran yield of 53 ± 4% (range 48–58%). In each milling, the total recovery of bran and fines was 100% of the unsieved flour.

Effect of Milling on β-Glucan Concentration

β-Glucan analyses of bran and fines fractions from replicate milling were in good agreement (Table II), with an average coefficient of variation (3.4%) from separate millings similar to that from pooled values (3.9%); that is, most variation arose from analytical replication. The β-glucan concentration of groats before milling ranged from 3.91 to 6.82% (Table II), just overlapping with brans with the lowest β-glucan (range 5.81–8.89%). This illustrates the problem of assigning analytical limits in a definition of oat "bran." The mean β-glucan concentration of all the brans was 7.4%, with an average enrichment factor of 1.5. The range of β-glucan concentration (1.60–2.32%) of the fines fraction was narrow; indeed seven of the 11 samples containing between 1.6 and 1.8% were not statistically different.

Table III summarizes the data for β-glucan concentration and potential for β-glucan enrichment of each cultivar. Significant differences in β-glucan concentration ($P \leq 0.05$) were found between cultivars for both the whole groats and the brans. Marion and Capital were highest in β-glucan and produced the highest β-glucan brans. Because of the higher enrichment factor, Capital's significantly lower β-glucan concentration in the groat becomes a higher β-glucan in the bran although the difference between the two brans was not significant. Similarly, the fifth and sixth ranked groats, Ogle and Hinoat, significantly lower in β-glucan than the third ranked groat, Woodstock, became sixth and fourth ranked brans, respectively, and these brans were not significantly different from that of Woodstock (dropped to fifth ranked bran). Hinoat bran was not significantly different from the third ranked bran of Sentinel. This resulted from the high enrichment factor (1.6) observed with Hinoat. On the other hand, different enrichment factors for the identical Tibor and NO-1 groats (4.73 and 4.71% β-glucan, respectively) gave significantly different brans (6.66 and 7.17%).

Starch concentration was decreased in the bran (mean 49.3%) and increased in the fines (mean 69.7%) compared with the whole groat (mean 59.1%). The change in starch concentration (mean ratio of bran/groat 0.85 ± 0.05) was less than the change in β-glucan and did not vary greatly between cultivars. Again, Hinoat

TABLE I
Percentage of Whole Seed and Moisture Content of Seed Used in Milling

Cultivar	% Whole Seed	% Moisture
Marion	8.91	8.58
Capital	2.41	8.70
Woodstock	2.32	8.56
Sentinel	3.59	8.47
Ogle	0.73	8.77
Hinoat	6.89	8.57
Tibor[b]	4.65	8.86
NO-1[b]	0	8.52
Donald	5.16	8.75
OA 516-2[c]	4.74	8.55
Harmon	2.01	8.33

[a] Remaining whole seed was removed by hand prior to milling.
[b] Hulless.
[c] Dormoat.

TABLE II
Bran Yields and β-Glucan Concentration of Fractions Obtained from Duplicate Millings of 11 Oat Cultivars

Cultivar	Whole Groat β-Glucan[a] (% dwb)	Milling Replicate	Bran Yield (%)	β-Glucan[a] (% dwb) Bran	β-Glucan[a] (% dwb) Fines
Marion	6.82 ± 0.30[b]	1	57.9	8.63 ± 0.20	2.22 ± 0.08
		2	58.1	8.87 ± 0.16	2.42 ± 0.04
Capital	5.98 ± 0.46[b]	1	57.2	8.83 ± 0.25	1.88 ± 0.02
		2	57.4	8.95 ± 0.05	1.67 ± 0.06
Woodstock	5.50 ± 0.24[b]	1	64.4	7.85 ± 0.32	2.09 ± 0.03
		2	50.5	7.81 ± 0.55	2.01 ± 0.05
Sentinel	5.35 ± 0.23[b]	1	54.5	7.84 ± 0.17	1.57 ± 0.06
		2	55.2	8.31 ± 0.40	1.63 ± 0.09
Ogle	5.05 ± 0.21[b]	1	50.9	7.68 ± 0.37	1.76 ± 0.02
		2	49.5	7.68 ± 0.43	1.71 ± 0.03
Hinoat	5.03 ± 0.12	1	47.6	7.91 ± 0.22	2.04 ± 0.16
		2	49.1	8.15 ± 0.29	1.98 ± 0.04
Tibor	4.73 ± 0.43[b]	1	53.1	6.78 ± 0.16	1.66 ± 0.01
		2	48.5	6.52 ± 0.29	1.73 ± 0.13
NO-1	4.71 ± 0.13[b]	1	55.3	7.40 ± 0.11	2.06 ± 0.01
		2	55.4	6.93 ± 0.21	2.14 ± 0.07
Donald	4.56 ± 0.13	1	53.8	6.27 ± 0.03	1.65 ± 0.02
		2	52.1	6.29 ± 0.31	1.75 ± 0.10
OA 516-2	4.30 ± 0.53[b]	1	54.1	6.39 ± 0.32	1.65 ± 0.03
		2	51.3	6.55 ± 0.15	1.67 ± 0.12
Harmon	3.91 ± 0.16	1	47.0	5.66 ± 0.30	1.54 ± 0.07
		2	49.5	5.97 ± 0.23	1.84 ± 0.02

[a] β-glucan concentration ± standard deviation ($n = 3$).
[b] $n = 6$.

TABLE III
Summary of Analytical Data from Milling of 11 Oat Cultivars[a]

Cultivar	Whole Groat β-Glucan	Whole Groat Starch	Bran β-Glucan[b]	Bran Starch[c]	Fines β-Glucan[c]	Fines Starch[b]	EF[d]	Ranking[e] Whole Groat	Ranking[e] Bran
Marion	6.82 a	55.8 f	8.75 a	48.7 bc	2.32 a	67.5 def	1.28	1	2
Capital	5.98 b	60.8 bc	8.89 a	52.6 a	1.78 c	71.9 ab	1.49	2	1
Woodstock	5.50 c	61.7 b	7.83 bc	51.9 ab	2.05 b	70.9 bc	1.42	3	5
Sentinel	5.35 cd	57.7 e	8.08 b	46.7 c	1.60 c	71.2 bc	1.51	4	3
Ogle	5.05 de	60.4 c	7.68 c	50.9 ab	1.73 c	71.0 bc	1.52	5	6
Hinoat	5.03 de	54.9 f	8.03 bc	41.1 d	2.01 b	66.2 ef	1.60	6	4
Tibor	4.73 ef	59.2 d	6.66 e	50.0 abc	1.69 c	70.1 bc	1.41	7	8
NO-1	4.71 ef	55.1 f	7.17 d	47.1 c	2.10 b	65.1 f	1.52	8	7
Donald	4.56 f	63.6 a	6.28 f	52.5 a	1.70 c	73.7 a	1.38	9	10
OA 516-2	4.30 fg	60.3 c	6.47 ef	50.7 ab	1.65 c	69.9 bcd	1.51	10	9
Harmon	3.91 g	59.8 cd	5.81 g	49.5 abc	1.70 c	68.6 cde	1.49	11	11
Overall mean[f]	5.09 ± 0.81	59.1 ± 2.9	7.43 ± 1.03	49.3 ± 3.4	1.85 ± 0.23	69.7 ± 2.6	1.47 ± 0.09	...	...

[a] Numbers in column not followed by the same letter are significantly different ($P \leq 0.05$).
[b] Cultivar-by-milling interaction pooled with error because it was nonsignificant.
[c] Significant cultivar-by-milling term used as error term.
[d] Enrichment factor (EF) is the ratio of β-glucan concentration in bran to β-glucan concentration in groat.
[e] Ranking is based on the β-glucan concentration of groat and bran.
[f] Overall mean ± standard deviation of the cultivar means.

had the greatest change with a bran/groat ratio of 0.75. The cultivar variation in starch concentration of brans (coefficient of variation [CV] 6.9%) and whole groats (CV 4.9%) was less than the variation in β-glucan of brans (CV 13.8%) or whole groat (CV 15.9%).

Materials balance data showed an average 95 ± 5% recovery of the original groat β-glucan and 100 ± 1% of groat starch in the bran and fines fractions.

DISCUSSION

The β-glucan concentration (3.91–6.82%) of the groats from the 11 North American cultivars chosen for this study is similar to that reported by Welch and Lloyd (1989) for a much wider selection of mainly European cultivars (3.2–6.3%). Analysis of Swedish cultivars indicated lower amounts and range (≈2.2–4.0%) but these data were based on whole grain oats (Åman and Graham 1987). The 11 cultivars selected for this study provided a good range for determining the effects of milling to produce bran. The study was not intended to establish characteristic cultivar levels, although other data (S. Miller, *personal communication*) indicate that, for example, Marion is normally high in β-glucan. The effect of environment on β-glucan levels or milling characteristics and enrichment factors remains unknown.

Specific histochemical techniques (Wood and Fulcher 1978, Wood et al 1983) have allowed qualitative comparison of the distribution of β-glucan in oat cultivars, and developments in computer-assisted microspectrofluorimetry (Miller and Fulcher 1989) may permit quantitative assessments. The histochemical evidence showed that, like barley β-glucan (Fincher 1975), oat β-glucan was located mainly in the endosperm cell walls with lesser amounts in the aleurone walls. An oat bran consisting solely of aleurone and seed coat would probably be relatively low in β-glucan. The thickest oat endosperm cell wall is at the aleurone-endosperm junction, and resistance of this region to milling attrition results in a coarser particle that forms part of the β-glucan rich bran. This fact was known many years before histochemical explanations could be offered (Acker et al 1955). Although other physical properties of the seed, such as cell size, might influence milling characteristics, microscopic examination (Fulcher and Wood 1983, Fulcher 1986) reveals endospermic cell wall thickness is probably the basis for β-glucan enrichment in oat bran, that this is variable throughout the endosperm, and that both cell wall thickness and the degree to which this varies throughout the endosperm differ among cultivars.

The β-glucan analyses presented here are the first to quantitatively demonstrate what was indicated by histochemistry, namely that different oat cultivars may show different abilities to produce β-glucan rich oat bran. The value of a particular cultivar as a source of β-glucan in the bran is clearly not solely dependent on the concentration of β-glucan in the groat. This is an indicator, since there is a linear correlation between groat and bran β-glucan ($r^2 = 0.823$), but this was only statistically significant at a level of $P < 0.1$. Although low bran yield might be associated with high enrichment, and vice versa (e.g., Hinoat and Marion), there was no statistically significant correlation ($r^2 = 0.248$). It is, however, possible to increase the β-glucan concentration of the brans by alternative milling procedures designed to improve the fractionation of coarse from fine particles. Associated with this is a decrease in bran yield (Wood et al 1989).

The choice of ≈50% for bran yield (percentage of whole groat) coincides with the value in the definition suggested by the AACC Committee on Oat Bran. With the cultivars chosen, all brans achieved β-glucan concentrations well above the minimum of 5.5% suggested by the AACC committee, even with bran yields somewhat higher than the suggested 50%. Three cultivars started at or above 5.5% β-glucan in the whole groat. It is probable that these cultivars would be distinguishable from bran on the basis of a total dietary fiber analysis.

The milling method used a small sample size (10 g), which is appropriate for cultivar survey in breeding programs. However, since we did not further grind the samples because of possible material loss, this may have increased sampling error. Standard deviations were somewhat higher than usual in this analysis.

Although oat brans have become an item of commerce, incorporated into ready-to-eat cereal foods consumed by the general population, the basis for interest in this ingredient is its potential value in dietary management of diabetes and hypercholesterolemia (Anderson 1980, Anderson and Tietyen-Clark 1986). The active ingredient is believed to be the β-glucan (Chen et al 1981, Jennings et al 1988), and recent studies with a purified β-glucan extract appear to confirm this (Wood et al 1989, 1990). It is not known what level of β-glucan in the oat product consumed might be appropriate, nor what would be a minimum effective amount, although the maintenance diet suggested by Anderson and Tietyen-Clark (1986) includes 50 g of oat bran per day, and 30 g/day is effective in metabolic ward studies (Anderson et al 1989). Commercial oat brans typically contain 7–10% β-glucan, and levels close to this can be achieved in cultivar Marion without recourse to fractionation. This is encouraging because it indicates whole groat rolled oats (normally ≈4% β-glucan commercially) could be produced with levels of β-glucan that are physiologically effective without excessively large intake (e.g., 140 g/day, deGroot et al 1963). To many, whole rolled oats are more palatable than bran. It is additionally encouraging to see that modest bran cuts of 50% in all cases produced brans over 5.5% β-glucan and in two cases close to 9%.

Starch is generally evenly distributed throughout the endosperm but is not found in the aleurone or germ and tends to be lower in the immediate subaleurone layer (Fulcher 1986). The changes observed in starch concentration were greatest in Hinoat, which reflects the morphological distinctness of this high-protein cultivar whose outer endosperm cells are markedly lower in starch and enriched in protein bodies.

In conclusion, the β-glucan concentration of oat groats shows considerable variability. A simple milling and sieving procedure reveals, in addition, variability in the potential for β-glucan enrichment in the bran. Understanding the factors controlling this variability will require further study, but they are probably partly genetic in origin. Whatever the basis, breeding or purchasing oats for β-glucan concentration and bran production requires consideration of both sources of variation.

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