

Oat Bran Fermentation by Rye Sourdough

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ABSTRACT

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Hydration of oat bran including fermentation by rye sourdough was studied. Three types of oat bran suspensions were prepared (a control, one with whole meal rye flour added, and one with rye starter added). The suspensions were incubated for 1, 2, 3 and 4 hr. β -Glucan content and solubilities of protein and β -glucan were analyzed. Viscosity of the supernatants of oat bran suspensions was determined. Neither the rye sourdough nor the rye flour alone had a significant effect on the total β -

glucan content of oat bran suspensions. However, the addition of rye, either as whole meal rye flour or as sourdough starter, markedly increased the solubility of β -glucan and proteins and simultaneously decreased the viscosity of the water-soluble fraction of oat bran suspension. This suggests that a hydrolysis of β -glucan had occurred that could change the rheological properties of oat bran in baking and the physiological potential of oat bran in nutrition.

Bread rich in oat bran is a potential daily source of soluble fiber which in oat bran is mainly (1 \rightarrow 3)(1 \rightarrow 4)- β -D-glucan, referred to as β -glucan. However, when using bran in baking, the rigid fiber particles of bran disturb the formation of the gluten-protein matrix (Gan et al 1989). To reduce this disturbing effect, the bran particles can be softened by hydration, thus enabling enzymatic function. Oat bran itself should be enzymatically inactive because of the kiln-drying treatment performed in the milling process. Nevertheless, hydrating might risk activating micro-organisms and hydrolysis of β -glucan. In baking, micro-organisms such as bacilli and molds can be controlled by lowering the pH level (adding acids or by lactic acid fermentation). In oat bran baking, lactic acid fermentation would prevent harmful microbial growth and would also provide flavor to the bread. Sourdough starter could be used in oat bran hydration, especially in countries where sour rye bread is produced and bakeries keep their own rye sourdough starter on hand. However, the rye sourdough starter also contains enzymes such as the endogenous hydrolytic enzymes of rye flour. These could affect the properties of the oat polysaccharides and protein. In rye baking, rye flour components in the sourdough undergo considerable change involving swelling, solubilization, and partial hydrolysis, and the breakdown products have an important role as flavor components.

The aim of this study was twofold: 1) to determine the effect of oat bran fermentation by rye starter on the soluble β -glucan and protein contents in oat bran suspensions and on the viscosity properties of the solubles; and 2) to evaluate the potential for β -glucan hydrolysis by the rye flour and by rye sourdough.

MATERIALS AND METHODS

Commercial oat bran (Melia Ltd, Raisio) was used in this study. The oat bran was ground with a laboratory pin-mill KT-30 (Koneteollisuus Oy, Helsinki). The particle size distribution was obtained by sieving the milled oat bran (50 g) through sieves (1.6, 1, 0.71, 0.4, 0.2) for 10 min on a laboratory Bühler-Miag sieve (Braunschweig 1977). Most of the oat bran particles (85%) were retained on apertures of 1.6 to 0.4 mm. Amounts retained by sieves 1.0, 0.71, and 0.4 were 27.4, 26.8, and 30.5%, respectively. Only 1% of oat bran particles were >1.6 mm. The remaining two fractions retained by the 0.2-mm sieve and passed through the sieve were 8.5 and 6.7%, respectively.

The oat bran used for the suspensions contained 18.3% protein (standard Kjeldahl method, $N \times 6.25$), 37.6% starch (ICC 1994), 21.7% dietary fiber (Asp et al 1983), 9.2% β -glucan (Approved Method 32-23, AACC 2000), and 3.2% ash (Approved Method 08-02) on a dry basis. Commercial whole grain rye flour was used in this study. The enzyme activity of the rye flour was 0.7 U/kg for α -amylase and 62 U/kg for β -glucanase (McCleary and Sheehak 1987). A typical Finnish rye starter, which contained hetero- and homofermentative lactobacilli and *Candida milleri* yeast and which was based on the same rye flour, was used to initiate fermentation. Rye starter was prepared by fermenting 25% rye flour and 5% previous rye starter and 70% water at 30°C for 16 hr. The rye starter used for the incubation was at pH 3.60.

Incubations

Three types of suspensions (total amount of each was 160 g) were prepared: 1) oat bran + water (26 and 74%) as a control (OB); 2) oat bran + rye starter + water (25+5+70%) (OB+RS); and 3) oat bran + rye flour + water (25+2+73%) (OB+RF). Sodium-azide (0.02%, w/w, in water) was used in the OB and OB+RF suspension to inactivate micro-organisms (Nicholls and Chance 1974). The suspensions were incubated for 1, 2, 3, and 4 hr at 30°C. The pH level of the suspensions was measured during the incubations. After the incubation, the suspensions were centrifuged at $34,545 \times g$ for 10 min. The supernatants were decanted and centrifuged once more at $34,545 \times g$ for 10 min to sediment the remainder of the solid particles. All incubations were done in triplicate. Supernatants were freeze-dried for determination of protein content and molecular weight of β -glucan.

Analytical Measurements

Moisture content of suspensions and supernatants was measured according to a modified Approved Method 44-60 (AACC 2000). Modification was necessary because the viscous suspensions and supernatants formed a film that retarded drying. In the modified method, moisture dishes containing 5 g of quartz sand and a wooden toothpick were dried in a convection oven at 130°C for 1 hr, covered, cooled in a desiccator, and a tare weight was obtained. Approximately 1–2 g of the sample was precisely weighed into the dish and mixed thoroughly with the sand using the toothpick. The sample together with the sand and the toothpick was dried overnight at 70°C (\approx 18 hr), cooled in a desiccator, and weighed.

The β -glucan content in the fresh suspensions and supernatants was determined by the enzymatic method (Approved Method 32-23, AACC 2000) using the Megazyme kit (BBG 7/98, Megazyme, Bray Business Park, Bray, Co. Wicklow, Ireland). The samples (0.6 g of suspension and 1 g of supernatants [wb]) were preextracted with aqueous ethanol (50%) to remove free sugars.

The molecular weight of the β -glucan was analyzed by size-exclusion (SE) chromatography on serially connected columns (μ Hydrogel 2000, 500 and 250) using a refractive index detector containing a dual-angle laser light-scattering detector. The eluent (0.05M NaOH)

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TABLE I
Dry Matter, β -Glucan, and Protein Contents in Supernatants^{a,b}

	Incubation Period (hr)	OB	OB+RS	OB+RF
Dry matter (% wb)	1	1.83 ± 0.10a	2.73 ± 0.01a	2.56 ± 0.01a
	2	2.12 ± 0.01b	3.0 ± 0.1a	2.83 ± 0.01ab
	3	2.18 ± 0.05b	3.17 ± 0.04b	3.06 ± 0.01ab
	4	2.34 ± 0.11b	3.2 ± 0.2b	3.0 ± 0.2b
	Mean ^c	2.11a	2.99c	2.87b
Protein (% wb)	1	0.36 ± 0.01a	0.84 ± 0.03ab	0.75 ± 0.01ab
	2	0.47 ± 0.01b	0.96 ± 0.01c	0.79 ± 0.01b
	3	0.54 ± 0.01c	0.90 ± 0.02bc	0.75 ± 0.01ab
	4	0.68 ± 0.01d	0.83 ± 0.01a	0.73 ± 0.01a
	Mean ^c	0.52a	0.88c	0.76b
β -Glucan (% wb)	1	0.30 ± 0.04a	0.68 ± 0.03a	0.72 ± 0.01a
	2	0.37 ± 0.01ab	0.84 ± 0.03b	0.95 ± 0.01b
	3	0.43 ± 0.01bc	0.98 ± 0.06bc	1.07 ± 0.14b
	4	0.47 ± 0.05c	1.03 ± 0.07c	1.02 ± 0.08b
	Mean ^c	0.39a	0.88b	0.94c

^a Supernatants of suspensions oat bran (OB), oat bran + rye starter (OB+RS), oat bran + rye flour (OB+RF).

^b Values are means ± standard deviations of three incubations. Values followed by the same letter in the same column are not significantly different ($P < 0.05$). Degrees of freedom $4 \times 3 - 1 = 11$.

^c Mean values during 4 hr of incubation. Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Degrees of freedom $3 \times 4 - 1 = 11$.

was run at flow rate of 0.5 mL/min at 70°C. Calcofluor staining was used to detect β -glucan postcolumn online in the extracts (Suortti 1993).

The protein content of the freeze-dried supernatants was determined by the Kjeldahl method using $N \times 6.25$ as the conversion factor.

Rheological Measurements

The viscosity of oat supernatant was determined using a rheometer (RotoVisco RT 20, Haake, Germany) with a cone and plate attachment (35 mm diameter, 2° cone angle). Measurements were conducted immediately after centrifugation at 30°C, the same temperature as used for the incubations. The viscosity measurement was started by keeping the sample at 0.30/sec shear rate for 10 sec, then raising the shear rate to 400/sec and back in 120 sec, using 400 steps. Triplicate measurements were made per incubation.

The Haake software regression analysis was applied for the flow properties study. Flow properties were expressed using the power law equation (Ostwald de Waele model):

$$\eta = K\gamma^{n-1} \quad (1)$$

where η is apparent viscosity (Pa-sec), γ is shear rate (1/sec), K is the consistency index (Nsec ^{n} /m²), and the pseudoplasticity index n is a dimensionless constant that indicates deviation from Newtonian flow. The magnitudes of K and n were determined from linear regression analysis of $\log \eta$ versus $\log \gamma$. The magnitudes were calculated for $\gamma = 18.6$ /sec to 400/sec because the correlation coefficient for the applicability of the power law model was highest in this range ($r = 0.99$ to 0.98).

Statistical Analysis

The statistical analyses of the measured parameters were made using analysis of variance according to the III Sums of Squares statistical computing package (Statgraphics Plus for Windows 3.0, StatPoint Corporate Center, Englewood Cliffs, NJ). Tukey's honestly significant difference (HSD) multiple range test was used to determine the differences between group means at the 95.0% confidence level. All values are reported as mean ± standard deviation (SD).

RESULTS

Changes of pH Level During Incubation

The three types of oat bran suspensions were incubated for 4 hr at 30°C. Suspensions of oat bran (OB) and oat bran + rye flour (OB+RF) were stable at pH 6.2 throughout the incubation. Addition of 5% rye starter (pH 3.6) to the oat bran suspension caused

the pH level of oat bran + rye starter (OB+RS) suspension to decrease initially from 6.2 to 5.2. There was a slight initial rise in pH to 5.4 during the first 40 min of the fermentation followed by a decrease to pH 4.9 (SD ± 0.12) during the rest of the incubation.

Composition of Supernatants

The incubation treatments had a significant effect on the composition of the centrifuged supernatants (Table I). The presence of the rye flour, and the rye starter in particular, affected dry matter, β -glucan, and protein contents.

The dry matter content of the supernatants significantly increased during the first 2 hr of incubation and more in the OB+RS and OB+RF supernatants than in the OB supernatant. Protein solubility increased with time in the OB suspension during 4 hr of incubation, nearly doubling the protein content (Table I). Compared with OB, the presence of rye doubled the protein solubility.

The total β -glucan (db) of all suspensions remained unchanged during 4 hr of incubation: OB 8.3 ± 0.4; OB+RS 7.6 ± 0.6; OB+RF 8.0 ± 1.0%. However, the solubility of β -glucan in all the suspensions increased significantly during the first 3 hr of incubation but leveled off thereafter (Fig. 1). The solubility of β -glucan in the OB+RS and OB+RF suspensions was double that in the OB suspension.

Molecular weight determinations of soluble β -glucan showed that in the OB supernatant, there was an even distribution of amounts of β -glucan in the three selected ranges of weight-average molecular weight (M_w) (Fig. 2). The high M_w (>10⁶) fraction accounted for 32% (0.096%, wb) of soluble β -glucan in the OB supernatant. In contrast, the high M_w fraction made up only 5 and 2% of soluble β -glucan in OB+RS (0.034%, wb) and OB+RF (0.014%, wb) supernatants, respectively. The presence of the rye component, whether from rye flour or rye starter, led to a reduction in the M_w of β -glucan, and this tendency was dependent on time. However, the somewhat higher percentage of high M_w (>10⁶) fraction of β -glucan in the OB+RS supernatant than in the OB+RF supernatant indicated a lower degree of β -glucan hydrolysis by rye starter compared with rye flour.

Rheological Parameters

The viscosity parameters of the supernatants depended greatly on the presence of rye. The OB supernatant was more viscous and pseudoplastic than OB+RS and OB+RF supernatants. With incubation time pseudoplasticity, the consistency index K and viscosity of OB supernatant increased. However, in the OB+RS and OB+RF supernatants, incubation time had no significant effect on flow properties. The n and K values of supernatants with rye were 0.7 and 0.4 Nsec ^{n} /m², respectively.

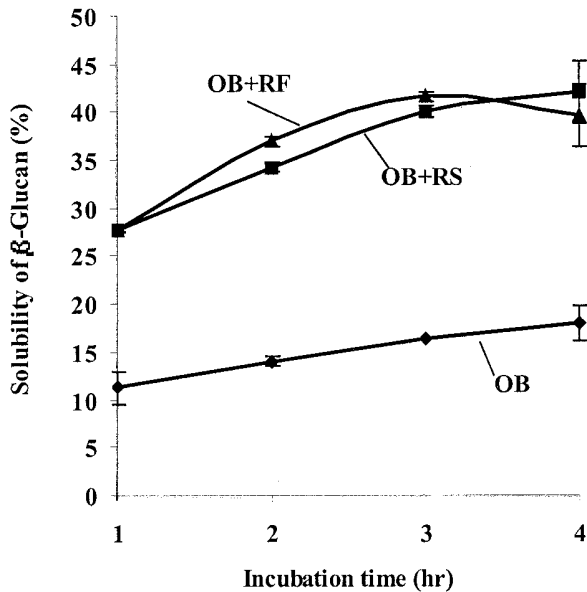


Fig. 1. Effect of incubation time and treatment on β -glucan solubility expressed as % of total β -glucan in oat bran suspensions (OB) and oat bran suspensions containing 2% rye flour (OB+RF) or 5% rye starter (OB+RS).

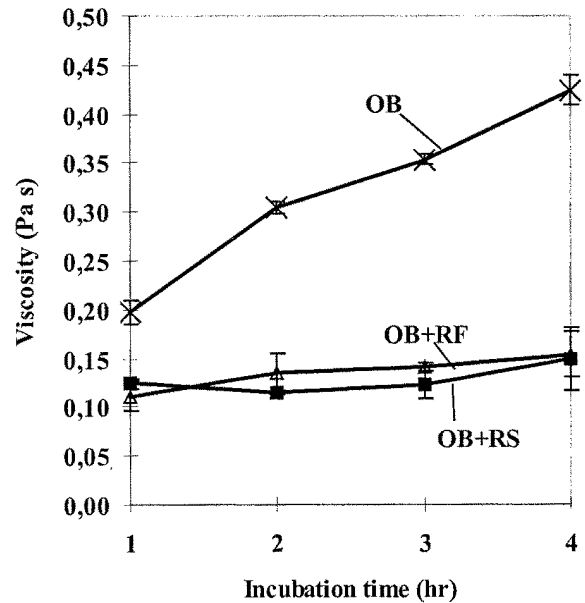


Fig. 3. Apparent viscosity of supernatants at shear rate 50/sec after incubations of suspensions for 1, 2, 3, and 4 hr.

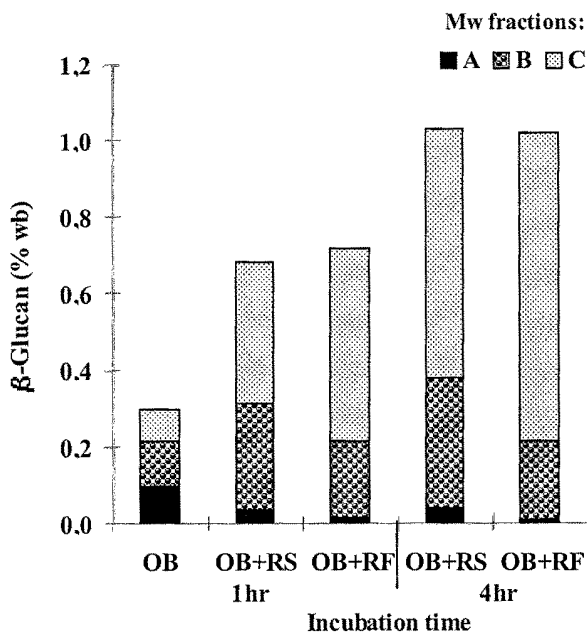


Fig. 2. Percentage concentrations of three different molecular weight fractions of soluble β -glucans in supernatant from treated oat bran suspensions. A ($M_w > 10^6$), B ($10^6 > M_w > 0.25 \times 10^6$), C ($M_w < 0.25 \times 10^6$).

The n value of oat bran supernatant decreased from 0.4 to 0.3, and K increased from 1.8 to 6.5 Nsec^n/m^2 . Increase in differences between apparent viscosity of supernatants within the time (Fig. 3) were in agreement with pseudoplasticity properties (power law indexes). The viscosity (at 50/sec) of the OB supernatant was much higher than the viscosity of the OB+RF and OB+RS supernatants after 1 hr of incubation, and the difference increased with time to 3.5-fold.

DISCUSSION

Success in oat bran baking demands improvements in the sensory properties of the products, which may require additional ingredients such as other flour or sourdough that could lead to the hydroly-

ysis of the β -glucan. In the present study, the potential for use of rye sourdough in oat bran baking was studied. The amount of rye starter (5%) used in this study is traditionally added in the sourdough process. Results showed that incubation of oat bran suspensions with small amounts of rye increased the solubility of oat bran components, particularly β -glucan, as indicated by the higher dry matter content of the OB+RS and OB+RF supernatants. Simultaneous with the marked increase in solubility, the proportion of low molecular weight β -glucan also increased. However, the total β -glucan content of the suspension during the incubation was not affected significantly by rye. Thus, enzymes of rye flour or rye starter released polymeric β -glucan from the oat bran cell walls without hydrolyzing the released material to low molecular mass oligosaccharides that would not precipitate in 50% ethanol. Previously, Bamforth (1982) showed such activity for germinated barley. Hrmova et al (2000) suggested that the enzymes responsible could be endocellulases that require 8–10 adjacent (1,4)-linked β -glucosyl residues. These enzymes would release only relatively large molecular weight fragments based on the known structure of β -glucan (Wood et al 1994).

The viscosity of random coil polymers increases rapidly with concentration and molecular weight as the result of coil overlap and entanglement. Doublier and Wood (1995) showed that aqueous solutions of high molecular weight oat β -glucan developed pseudoplastic behavior at a concentration above $\approx 0.3\%$. Although the β -glucan concentrations were higher than this in the oat bran supernatants from the rye incubation and rye fermentation, the molecular weight distribution of β -glucan was shifted to a lower value, hence, the lower apparent viscosities and less pseudoplastic behavior when compared with the oat bran supernatant without rye addition.

Marklinder (1996) suggested that most β -glucan degradation in fermented barley was caused by an endogenous β -glucanase originating from the barley flour. Using sodium azide to prevent microbial activity in oat bran incubations, showed β -glucan depolymerization in oat bran fermentation was mainly caused by endogenous enzymes of rye flour and not by growth of micro-organisms. In addition, in the OB+RS supernatant, soluble β -glucan had a higher molecular weight than in the OB+RF supernatant. Possibly, the low pH level of the suspension with rye starter reduced the activity of rye endo- β -glucanase.

CONCLUSIONS

Oat bran fermentation by rye sourdough increased the solubility of two oat bran components twofold (protein and β -glucan) and

increased the dry matter of the water-soluble fraction. Neither the rye sourdough nor the rye flour alone had a significant effect on the total β -glucan content of oat bran suspensions. However, they reduced the molecular weight of soluble β -glucan and it is probable that the β -glucan hydrolysis was caused mainly by endogenous rye enzymes. The hydrolysis of β -glucan was also manifested by the decreased viscosity of the water-soluble fraction of the oat bran suspension.

The rye starter in the oat baking did not lower β -glucan content, therefore application of rye sourdough satisfies the requirements for the β -glucan content. However, the reduced molecular weight of β -glucan and lower viscosity may also affect the rheological properties of oat bran in food systems and may also influence the physiological potential of oat bran in nutrition.

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