EXTRACTION OF HIGH-VISCOSITY GUMS FROM OATS1

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ABSTRACT

Gum extracted by alkali from oats (Avena sativa L.) contained 70–87% β -glucan. Flour particle size, and temperature, pH, and ionic strength of the extraction media, affected β -glucan yields and its distribution between three consecutive extracts. Oat flour contained significant endo β -1,3-glucanase, endo β -1,4-glucanase, and mixed linkage endo β -1,3-1,4-glucanase activities, which survived the alkaline extraction procedure. Enzyme deactivation of flour slightly decreased the yield of β -glucan, but

increased the viscosity of each extract. Viscosities considerably greater than previously reported for cereal β -glucan, and greater than for many industrially important gums, have been obtained. High-viscosity samples had a high limiting viscosity number (17–18 dl/g) in both dimethylsulfoxide and 7M urea. Two cultivars of barley (Hordeum vulgare L.) contained somewhat less β -glucan of lower viscosity than did most oat cultivars tested.

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In recent years, a number of laboratories have studied new sources and uses of vegetable protein (1). This has been in response to both a real and an anticipated demand for processed vegetable protein in the food industry. Oats, particularly high-protein varieties, possess a number of qualities that make them potentially desirable protein sources (1), and the work described in this article forms part of a program to evaluate the potential use of the high-protein variety Hinoat.

A major problem in any wet-milling process for oats is the development of high viscosity in aqueous extracts. The present study was done to characterize the "gum" (mostly β -glucan) responsible for viscosity development to determine its potential use and discover means to avoid problems associated with its presence. In a previous article dealing with methods for analysis of cereal β -glucans, the alkaline extraction procedure we (2) described was considered to have commercial potential. This article describes a number of factors affecting alkaline extraction and presents data on viscosity of extracts.

MATERIALS AND METHODS

General

Except as otherwise noted, preparation of oat flour; successive extractions with alkali to give extracts 1, 2, and 3; estimation of β -glucan by subtraction of starch content from colorimetrically determined glucan; and acid hydrolysis and paper chromatography were as previously described (2). Flour was obtained from three different batches of the cultivar Hinoat (Hinoat 1973a, Hinoat 1973b, and Hinoat 1975) and from one batch of the cultivar Rodney.

Dimethylsulfoxide (DMSO) was purified by distillation under reduced pressure from calcium oxide and stored over anhydrous calcium chloride. Kinematic viscosities were determined at 20°C in appropriate sizes of Cannon Manning semimicro viscometers (Cannon Instrument Company, State College, PA). Dilution studies, particularly determination of limiting viscosity numbers,

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were made in Cannon Ubbelohde semimicro viscometers, which allow dilutions within the viscometer itself. Solutions for viscosity measurements were prepared by magnetically stirring samples of gum (ground to pass a 40-mesh screen) at 55-60°C in water, 7M urea, or DMSO. Samples to be dissolved in aqueous systems were first "wetted" with a little ethanol; particular care was taken during the initial stages of dissolution that no buildup of gel-like particles occurred. After appropriate dilution, samples were filtered through a coarse glass filter to remove traces of insoluble debris. Thereafter, all apparatus used was cleaned with chromic acid and rinsed with filtered solvent.

Effect of Particle Size, Temperature, pH, Ionic Strength, Defatting, and Enzyme Deactivation on Extraction Yields

Hinoat (1975) flour prepared in a Retsch mill from dehulled seed was used. Duplicate extractions were made according to the shortened analytic procedure (2), but each consecutive extract was analyzed separately. Different particle sizes were obtained using 3.0-, 0.75-, 0.5-mm collars in the Retsch mill. To study the other variables, the 0.5-mm collar was used, and a starch analysis was included. Sodium carbonate-sodium bicarbonate buffer, pH 10, ionic strength (I) 0.2, was used for extraction except when pH and I were under study. Temperature control was achieved using a thermostatically controlled water bath (±3°C). Except when temperature was the variable under study, the temperature of extraction was 45°C.

To study the effect of defatting, flour was stirred overnight with methanol/chloroform/water (2:1:0.8) to give the defatted residue (87% yield db) following solvent exchange and air drying. Enzyme deactivation was performed by refluxing twice for 2 hr in 70% ethanol to give deactivated flour (85% yield db) following solvent exchange and air drying.

Analysis of Hinoat (1973a) and Rodney Flour

Samples of Hinoat (1973a) and Rodney flour were extracted in duplicate as previously described (2). Kinematic viscosity determinations were made on duplicate solutions from each extract, and limiting viscosity numbers were determined from a single solution of each extract diluted appropriately.

Replicate Extractions of Untreated Flour and Enzyme-Deactivated Flour

Six batches each of untreated and enzyme-deactivated Hinoat (1973b) flour were prepared and extracted as described. After precipitation at 50% 2-propanol (IPA), precipitates were resuspended immediately in 100% IPA to minimize possible contact with enzyme in an aqueous environment.

Endo-β-Glucanase Activity

Extraction. Extraction was done essentially by the method that Manners and Wilson (3) described. Oat flour was stirred with 4 vol of 0.2M acetate buffer, pH 5.0, for 3 hr at 4°C. The mixture was clarified by centrifugation and filtration, and the supernatant was dialyzed against 0.02M acetate buffer, pH 5.0, overnight. The dialysate was brought to 80% saturation with respect to $(NH_4)_2SO_4$, and the precipitate was collected by centrifugation, dissolved in 0.01M acetate buffer, dialyzed for 24 hr against the same buffer, dialyzed for 72 hr against distilled H_2O at $4^{\circ}C$, filtered, and freeze-dried.

Measurement of Enzyme Activity. Endo- β -glucanase activities were measured viscometrically, essentially as Manners and Marshall (4) described. Oat gum (provided by R. Hyldon, Quaker Oat Company, Barrington, IL); carboxymethyl-pachyman, prepared as described by Clarke and Stone (5); and hydroxyethylcellulose (Natrosol 250-M, Hercules Inc., Wilmington, DE) were used as substrates. A filtered solution of the enzyme without substrate showed no viscosity change over 5 hr. Essentially linear relationships between the reciprocal of the specific viscosity $(1/\eta_{sp})$ and time, and d/dt $(1/\eta_{sp})$ and amount of enzyme used, were observed. Activities obtained using oat gum as substrate are referred to as "gumase," or endo β -1,3; 1,4-glucanase activities.

Effect of Alkali on Oat Gumase Activity. Enzyme (20 mg) was dissolved in water (2 ml) and adjusted to pH 10 with sodium carbonate. After 0.5 hr at 45°C, the pH was adjusted to 5.3 with acetic acid and the solution was diluted with 0.2M acetate buffer, pH 5.3, to 10 ml. Following filtration, aliquots were tested for activity.

Enzyme Activity in an Alkaline Extract From Oat Flour

Oat flour was treated at pH 10 as for gum extraction, but following removal of starch, bran, and protein, the crude gum liquor was dialyzed for 48 hr against distilled water at 5°C and the dialysate was freeze-dried. Enzyme activity was measured in this product as previously described. A control containing no substrate showed no viscosity change over the period of assay.

Extraction of Barley β -Glucan

Barley gum extracts were prepared as previously described for oats (2).

RESULTS AND DISCUSSION

No difference in yields of gum were noted for samples extracted with a 10:1 and 20:1 liquid/solids ratio.

Flour particle size affected efficiency of extraction as indicated by increased total yield and yields of extracts 1 and 2, and decreased yield of extract 3 from the more finely ground flour (Table I).

Efficiency of extraction was lower at low temperatures (Table II). At 63°C, when starch gelatinization commenced and increasing starch contamination of extracts was evident, the ratio of extract 1 relative to extracts 2 and 3 decreased somewhat, but increased rapidly again at 80°C. Although overall yield of β -

TABLE I

Effect of Particle Size on Yield of Glucan Extracted From Oats (Hinoat, 1975)

Average of Duplicate Extractions

Flour Particle Size (Size of Collar Mesh)	Glucan Content (% db) of Flour						
	Extract 1	Extract 2	Extract 3	Total			
3.0 mm	1.80	0.97	0.54	3.32			
0.75 mm	2.08	1.02	0.46	3.56			
0.5 mm	2.45	1.22	0.39	4.07			

glucan increased throughout this temperature range, 45°C was the optimum extraction temperature to avoid starch gelatinization and contamination of extracts.

I and pH (Fig. 1 and 2) are important variables to be considered in extraction. Further increase in I at pH 10 by addition of 1M sodium chloride (total I, 1.2) brought no essential changes in yields. At low I, buffering power was inadequate to deal with extracted material, and pH dropped during extraction. Thus, at 0.05 I the pH of the buffer changed during the 0.5-hr extraction from 10 to 8.7, whereas at 0.2 I the change was to 9.6 and at 0.4 I to 9.8. Buffer of pH 9 (I, 0.05) dropped to pH 8.0 during extraction.

Oats contain an oil comparable in quality to corn oil (1), but unlike corn, the oil is distributed throughout the kernel. Nevertheless, solvent extraction might be commercially feasible. For this reason, and to determine if lipids interfered with extraction efficiency, a sample of flour was defatted before alkaline extraction. Defatting was shown to have little effect on β -glucan extraction (Table III), (compare with 45°C extraction, Table II), but starch contamination increased slightly. Included in Table III are the results of enzyme deactivation on β -glucan extraction. The results confirm that enzyme deactivation has little effect on β -glucan yield.

TABLE II Effect of Temperature on Yield of β -Glucan Extracted From Oats (Hinoat, 1975) Average of Duplicate Extractions

Temp.	Extract	Glucan Yield (% db of Flour)	Starch Content (% db of Glucan)	β-Glucan Yield (% db of Flour)
5° C	1	1.42	0.95	1.40
3 C	2	1.37	1.14	1.35
	1 2 3	0.69	4.78	0.65
Total		3.47	1.78	3.41
25° C	1	2.09	0.45	2.08
	2 3	1.21	2.17	1.18
	3	0.40	3.73	0.40
Total		3.71	1.37	3.66
45° C	1	2.36	0.52	2.35
	2 3	1.10	0.69	1.09
	3	0.46	1.96	0.45
Total		3.92	0.72	3.89
63° C	1	2.25	2.41	2.20
	2 3	1.47	3.31	1.41
	3	0.55	10.03	0.50
Total		4.27	3.72	4.11
80° C	1	3.13	4.40	3.00
	2 3	1.53	7.95	1.40
	3	0.32	41.48	0.19
Total		5.03	7.90	4.59

In the initial phase of these studies, it was desirable to compare the viscosities of extracts obtained in our laboratories with some of those reported in the literature, which were measured mostly at 0.5% or 1% (w/v) in water (Table IV). For high viscosity extracts, however, Fig. 3 shows that viscosity measurements at concentrations greater than 0.5% w/v are difficult because of the extremely rapid rise in viscosity with concentration. Nevertheless, at 0.5% w/v, viscosities considerably in excess of those previously reported can be obtained from alkaline extracts of oat flour. In previous reports (12,13), poor reproducibility of viscosity measurements of barley β -glucan has been attributed to poor dispersion of samples. In this study, we found DMSO preferable to water as a solvent because of improved dispersibility of sample and improved stability of viscosity. (Solutions have been stored at 5°C for more than eight months without loss in viscosity.) This latter feature was important in dilution studies. A loss of viscosity with time in aqueous solutions, which was variable, was attributed to residual β -glucanase activity in gum extracts.

Although viscosities of gum extracts at 0.5% w/v in DMSO allowed some

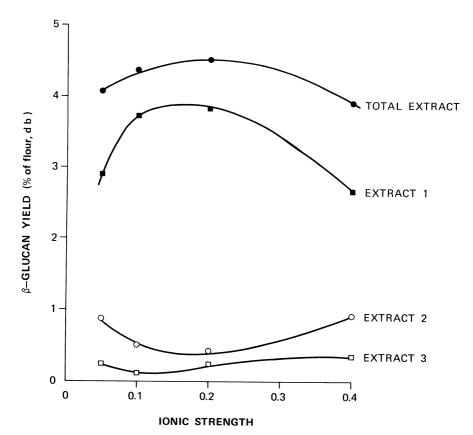


Fig. 1. Effect of ionic strength on yield of β -glucan extracted at pH 9.0 from oats (Hinoat, 1975). Average of duplicate extractions at each ionic strength.

distinction between samples (eg, between extracts 1 and 2) the results at this concentration were irreproducible, presumably because of the extreme concentration dependence of viscosity and the attendant difficulties in preparing solutions accurately. Thus, Hinoat (1973a) showed values for extract 2 between 422 and 1,203 centistokes (cSt) (mean, 1,000 cSt). Flour from enzymedeactivated Hinoat (1973b) gave the highest aqueous viscosity value at 0.5% w/v, about 2,000 cSt. At 0.2% w/v, however, viscosities (in DMSO) showed reasonable reproducibility. Samples dissolved with stirring and heating within 1 hr, but normally 2–4 hr, were used. The duration of stirring did not affect viscosity significantly.

Extracts from Hinoat (1973a) and Rodney were compared at 0.2% w/v in DMSO; significant differences in kinematic viscosity for each extract were noted (Table V). The highest protein content was in extract 1, which may account in part for the lower viscosity of this extract, since the β -glucan content was lower. Difference in β -glucan content of extracts 2 and 3 was small. The summed analysis of protein, starch, ash, and β -glucan was close to 100%. The remaining

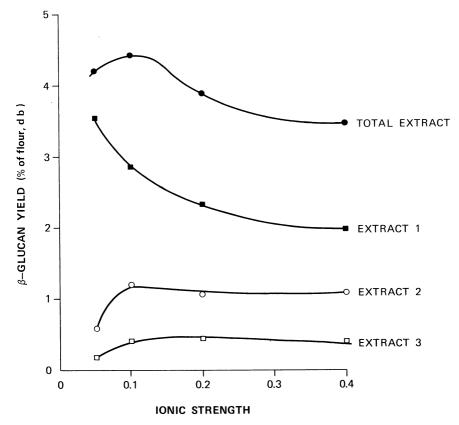


Fig. 2. Effect of ionic strength on yield of β -glucan extracted at pH 10.0 from oats (Hinoat, 1975). Average of duplicate extractions at each ionic strength.

material was mostly pentosan, since acid hydrolysis and paper chromatography of each extract showed arabinose and xylose in addition to glucose.

Although the influence of protein, pentosan, and the like remains to be determined, differences in kinematic viscosities seem likely to depend on the physicochemical characteristics of the β -glucan itself; the differences in limiting viscosity numbers suggest that molecular weight is one factor involved. Little information appears in the literature on limiting viscosity numbers for similar polysaccharides. Extrapolation of the results of Djurtoft and Rasmussen (13) for a supposedly high-viscosity barley β -glucan would show a value less than $5 \, \text{dl/g}$. Amylose is normally less than $10 \, \text{dl/g}$ (14).

To determine limiting viscosity numbers, viscosities of dilutions down to 0.025% (w/v) were required, since the plot of intrinsic viscosity against concentration was nonlinear above 0.1% (w/v). High viscosities were observed in dilute solutions in DMSO, water, and 7M urea in which the viscosity of Hinoat (1973a) extract 2, was 49 cSt (0.2% w/v) and the limiting viscosity number was 17.5 dl/g.

TABLE III

Effect of Defatting and Enzyme Deactivation on Yield of β -Glucan Extracted From Oats (Hinoat, 1975)

Average of Duplicate Extractions

Treatment	Extract	Glucan Yield (% db of Flour)	Starch Content (% db of Glucan)	β-Glucan Yield (% db of Flour)
Defatted	1	2.44	4.23	2,32
	2	1.20	5.09	1.13
	3	0.39	11.40	0.34
Total		4.02	5.18	3.78
Enzyme deactivated	1	2.20	2.97	2.10
	2	0.98	5.04	0.93
	3	0.53	10.49	0.47
Total		3.71	4.99	3.50

TABLE IV
Published Viscosities of Aqueous Solutions of Oat and Barley β -Glucan

Sample	Reference	Concentration (%)	Temperature (°C)	Viscosity (cSt) ^a	
Oats	Preece & MacKenzie (6)	0.5	25	2.1	
Oats	Preece & Hobkirk (7)	0.5	25	3.4	
Barley	Bourne & Pierce (8)	1	•••	200	
Barley	Preece & Hobkirk (7)	0.5	25	5.5	
Barley	Meredith et al (9)	0.5	30	3.0	
Barley	Meredith et al (10)	1	30	752	
Barley	Preece & Hobkirk (11)	0.5	25	16.1	

^aLiterature values quoted in terms of specific viscosity or in centipoise. For comparisons we have assumed $\eta H_2O = 1$ cSt and that solution density is approximately equal to solvent density (ie, 1.0 for H_2O).

The differences shown in Table V between Hinoat and Rodney should not be attributed at this stage to a genetic cause, since environment and seed batch (Table VI) can affect yield and viscosity of gums. The extracts from Rodney flour were among the lower viscosity samples found and Hinoat among the higher; the values in Table V are thus indicative of a range to be expected.

Highest yields were obtained at pH 9 (I, 0.1 and 0.2) or pH 10 (I, 0.1). At pH 9, however, yields of the high-viscosity extracts 2 and 3 were low. Furthermore, carbonate—bicarbonate buffer causes considerable foaming during neutralization, particularly at higher I, which delays manipulations and increases the risk of degradation by β -glucanases even after addition of octyl alcohol. Consequently, for routine extractions in which samples were to be accumulated for further physicochemical studies, 20% sodium carbonate was used for pH adjustment as previously described (2).

In a series of extractions from untreated flour and enzyme-deactivated flour (Table VI), the latter provided extracts of higher viscosity, differing significantly

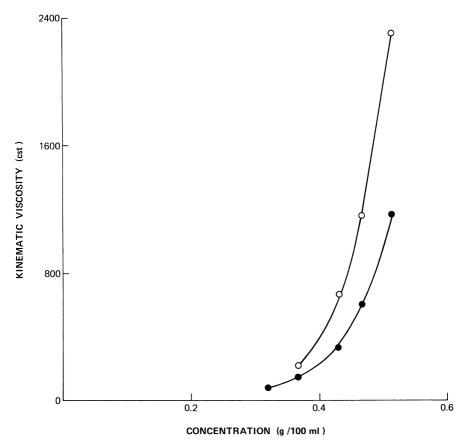


Fig. 3. Effect of concentration on viscosity of extract 2 from enzyme-deactivated Hinoat (1973b) flour. O——O, In water; •——•, in DMSO.

TABLE V Analysis of Hinoat 1973a (H) and Rodney (R) Gum Extracts (Average of Duplicate Extractions and Analysis)

		Gum	Starch	Protein	Ash	Ash Total	β-Glucan Yield ^a (%)	Viscosity	
Sample	Extract	Extract Yield Conte	Content ^b (%)	Content ^b /c Co	Content ^b Analyzed ^b (%)	Kinematic ^d (cSt)		Limiting ^c $[\eta]_{\hookrightarrow}$ (dl/g)	
	1	2.22	1	20	0.7	93	1.55	11.4	10.3
H	2	1.33	2	7.1	0.8	99	1.16	43.1	16.9
	3	0.69	4	15	2.6	102	0.53	41.9	17.8
Total		4.24	2	15	1.0	96	3.24		
	1	2.14	1	10	0.9	93	1.70	5.7	5.8
R	2	0.86	2	5.6	0.7	93	0.71	21.9	13.9
	3	0.47	4	14	1.4	97	0.35	32.1	16.2
Total		3.47	2	9.4	0.9	94	2.76		

^aPercentage, dry weight basis, in flour. ^bPercentage, dry weight basis, in gum extract.

 $^{^{\}circ}$ Nitrogen \times 6.25.

d0.2% w/v in DMSO.

[°]In DMSO.

from extracts from undeactivated flour (extract 2, P < 0.01; extract 3, P < 0.01). Examination of gum yields showed that only extract 1 was changed significantly by enzyme deactivation (P < 0.05). With or without enzyme deactivation, extract 1 was always of lowest viscosity.

Mention has been made regarding β -glucanase activity in extracts. Therefore, presence of such enzyme activity in flour from ungerminated oats must be shown and some retention of activity after treatment with alkali demonstrated. Crude enzyme extracts from each sample of flour contained endo β -1,3-glucanase, endo β -1,4-glucanase, and endo β -1,3—1,4-glucanase activities. Hinoat flours showed

TABLE VI Yields and Viscosities of Gum Extracted From Oats (Hinoat, 1973b)

	No. of		Gum Yield ^b		Kinematic Viscosity (cSt) ^c	
Sample	Extractions	Extract	Mean	SD	Mean	SD
		1	1.83	0.03	9.09	0.6
1	6	2	1.21	0.03	37.8	1.6
		3	0.79	0.03	41.5	3.2
		Total	3.82	0.05		
		1	1.66	0.13	23.1	1.7
2	6	2	1.18	0.13	49.2	6.3
		3	0.82	0.09	50.8	4.0
		Total	3.66	0.20	•••	

[&]quot;1 = Untreated flour, 2 = enzyme-deactivated flour.

TABLE VII

Analysis and Viscosity of Conquest (C) and Betzes (B) Barley Gums

Cultivars	Extract	Gum Yield ^a (%)	Starch Content ^b (%)	β-Glucan Yield ^a (%)	Kinematic Viscosity (0.2% w/v) (cSt) ^c
С	1	2.05	2	1.49	4.8
	2	0.35	4	0.17	6.9
	3	0.15	•••	•••	•••
Total		2.55	•••	1.66	
В	1	2.23	3	1.44	9.3
	2	0.38	3	0.27	9.0
	3	0.16	•••	•••	•••
Total		2.77		1.71	

[&]quot;Percentage, dry weight basis, of flour.

^b% (db) of flour.

^{°0.2% (}w/v) in DMSO.

^bPercentage, dry weight basis, of gum extract.

In DMSO.

high endo β -1,3-glucanase activities (45 and 49 min⁻¹ per 100 g of flour) compared with Rodney (13 min⁻¹ per 100 g of flour). Hinoat (1973a) showed the lowest endo β -1,3; β -1,4-glucanase activity (3.3 min⁻¹ compared with 3.5 and 9.5 min⁻¹ per 100 g of flour for Rodney and Hinoat [1975], respectively). Further work would be required to determine if these figures can be related to yields and quality of extracted β -glucan. Such relationships have been difficult to prove in the case of barley β -glucan (12). Treatment of crude enzyme preparation from Hinoat (1973a) for 0.5 hr at 45° C and pH 10 resulted in retention of about 12% of the mixed linkage β -glucanase activity.

Samples of oat gum, which were not resuspended immediately in 100% IPA after precipitation in 50% IPA but allowed to remain at room temperature in 50% IPA, showed viscosity losses. A partition of water may have been between solution and "precipitate" phase and possibly enzymatic activity was sufficient to affect viscosity of the product. Certainly aqueous solutions of oat gum can show viscosity losses with time. Therefore, the alkaline extraction procedure must be extracting active enzymes. To test this, a sample of untreated flour was extracted at pH 10 as normal, and the enzyme activity in the fraction from which gum is usually obtained was estimated. Appreciable endo β -1,4-, endo β -1,3-, and gumase activities were found.

Barley β -glucan has been the subject of considerable research over the last 20-25 years because of its importance to the brewing industry. Therefore, examining two barley cultivars, namely Betzes, a 2-row cultivar, and Conquest, a six-row cultivar was of interest (Table VII). Because of the more rapid extraction of gum from barley, insufficient material was available in extract 3 to warrant analysis or measurement of viscosity. Acid hydrolysis of the gum extracts and paper chromatography of the hydrolysates showed major amounts of glucose. with lesser amounts of arabinose and xylose and traces of uronic acid (tentative identification). Betzes also showed traces of galactose, and Conquest showed traces of galactose and mannose. The β -glucan content, similar for the two cultivars, was somewhat less than that found for most oat samples, but the biologic significance of this cannot be assessed since the method of flour preparation was different. Viscosities of extract 2 (in DMSO) were lower than those for oat extracts. In water, the viscosity (3.2 cSt) of a 0.5% (w/v) solution of Betzes, extract 2 (previously refluxed in 70% ethanol to inactivate possible traces of enzyme) was of the same order as some previously published values (7.9) and was low compared with a simultaneously prepared similar solution of Hinoat (1973a), extract 2, which showed a viscosity of 867 cSt.

In conclusion, alkaline extraction of dehulled oat flour yields gum extracts that give solutions of greater viscosity than do similar extracts from barley and (at 0.5% w/v) most commercially used gums (15). Using consecutive extractions, maximum viscosity is obtained with the second and third extracts, which comprise about 40% of the total. From the viewpoint of retaining protein functionality and starch granule integrity, enzyme deactivation in refluxing ethanol is obviously undesirable and commercially impractical. Preliminary attempts to inactivate or preextract enzymes using N-bromosuccinimide or a short extraction in the cold at 5°C did not promise improvement. The commercial steam heating treatment used to inactivate enzymes in rolled oats possibly may achieve a satisfactory balance between enzyme deactivation and starch and protein damage.

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