DETERMINATION OF β-GLUCAN IN OATS AND BARLEY[1]

P. J. WOOD, D. PATON, and I. R. SIDDIQUI, Food Research Institute, Agriculture Canada, Ottawa, Canada

ABSTRACT

Cereal Chem. 54(3): 524-533

A specific colorimetric procedure was developed for the determination of glucose in gum from oats (*Avena sativa* L.) and barley (*Hordeum vulgare* L.). The difference between this and the starch content, as measured enzymatically, gave the β-glucan content of the gum. Extraction of the flour with alkali obviated the necessity for any pretreatment to inactivate β-glucanases. Results for a number of oat cultivars are reported. A considerably shortened, though somewhat less rigorous, version of the assay is also described.

Two major components, namely pentosan and β-glucan, have been identified in the nonstarchy, gummy polysaccharides of cereals (1,2). Of the common cereals, oats and barley contain the largest proportion of β-glucan, which has been broadly established as a linear molecule composed of β-1,3- and β-1,4-linked D-glucopyranosyl units. Because of its high viscosity in aqueous solution, barley β-glucan has caused filtration problems in the brewing industry, and has consequently been the subject of considerable study in the last 20–25 years (3).

Although it was known that oats also contained significant quantities of β-glucan, which reportedly differed little, if at all, from barley β-glucan (4–6), until recently there was little practical interest in pursuing studies of this polysaccharide in oats. The development of high-protein cultivars of oats and an increasing interest in novel food protein sources has changed this situation, and our studies of oat gum stem from attempts to separate starch and protein from a recently developed high-protein strain named Hinoat.

During the course of this study, Fleming *et al.* (7) published a procedure for determination of β-glucan in barley flours. The present report is based on the same general principle, namely extraction of gum and specific determination of total glucan and starch, but extraction procedures and methods for determination of total glucan are considerably modified.

MATERIALS AND METHODS

Oat flour, sieved through a 30-mesh screen, was prepared from dehulled seed by milling in a coffee mill. Whole barley was processed in a Retsch mill successively through 5-mm and 0.75-mm screens. Flour was used immediately or stored frozen before use. When required, enzyme inactivation was carried out by twice refluxing in 70% ethanol for 2 hr.

Analytical grade chemicals were mostly used, but L-cysteine hydrochloride was Fisher Scientific Co. Reagent Grade, and arabinose and xylose were Calbiochem A grade. Laminaran and lichenan were obtained from the Sigma Chemical Co. The following enzymes were used: Amyloglucosidase Type II (*Rhizopus*) (1,4-α-D-glucan glucohydrolase, 3.2.1.3), α-amylase Type IA (hog pancreatic) (1,4-α-D-glucan glucohydrolase, 3.2.1.1), glucose oxidase Type II

[1]Contribution No. 285, Food Research Institute, Agriculture Canada, Ottawa, Ont.

(*Aspergillus*) (1.1.3.4), and peroxidase Type I (Horseradish) (1.11.1.7) from the Sigma Chemical Co.; Amyloglucosidase, "Agidex" powder (*Aspergillus*) from British Drug Houses; glucose oxidase, Cat. 15424 (fungal) and peroxidase, Cat. 15302 EPAB (horseradish) from Boehringer-Mannheim; "Glucostat Special" reagent from ICN Pharmaceuticals; and a purified amyloglucosidase which was a gift from J. J. Marshall, Miami, Florida.

Acid Hydrolysis and Paper Chromatography

Samples (~5 mg) were hydrolyzed in M-H_2SO_4 at 100°C for 3 hr and neutralized by passage through small columns of a barium carbonate-celite mixture. Paper chromatography was performed on Whatman No. 1 paper by the descending method, using ethyl acetate-pyridine-water (8:2:1), and sugars were detected by aniline hydrogen phthalate.

Extraction of Gum

Water (500 ml) was added to flour (50 g), and the suspension was immediately adjusted to pH 10 with sodium carbonate (20% w/v; in some earlier experiments 10.6% w/v) and stirred vigorously for 0.5 hr at 45° C. The mixture was centrifuged (15 min at 15,000 × g; 4° C), the residue retailed for further extraction, and the cooled supernatant adjusted to pH 4.5 with 2M-HCl and centrifuged (20 min at 21,000 × g; 4° C). This residue was discarded and the cold supernatant made 50% with respect to 2-propanol (IPA), which was added slowly with vigorous stirring. The precipitate was allowed to settle overnight, collected by centrifugation, resuspended in IPA, and the rubbery pellet disintegrated in a Virtis homogenizer. The sample was washed with IPA on a suction filter and air-dried with gentle warming to prevent moisture condensation and the development of a horny texture to the gum.

The residue from the initial extraction was subjected to further extraction under the same conditions as the first. Routinely, three extractions were carried out, giving three extracts referred to, in order, as gums (or extracts) 1, 2, and 3.

In some experiments, only two extractions were made. These were combined prior to adjusting to pH 4.5, and the gum was isolated as before by IPA precipitation.

Glucan Determination by the Cysteine-Sulfuric Acid Reaction

A modification [A. R. Law and P. J. Somers, unpublished work; reported by Kennedy and Butt (8)] of the Dische *et al.* (9) cysteine-sulfuric acid reaction was used, but absorbance was measured at 428 nm. Ice-cold sulfuric acid (86% v/v; 2 ml) containing L-cysteine hydrochloride (700 μg/ml) was added from an automatic dispensing pipet to the sample (400 μl; containing 0–25 μg glucose) with immediate thorough mixing. After 3 min at 100° C, the solution was held at about 20° C for 40 min before absorbancies were read.

Starch Determination

Routinely, BDH amyloglucosidase, "Agidex" from *Aspergillus* (7) was used to hydrolyze starch to glucose, essentially as described by Banks *et al.* (10). Also used were Sigma amyloglucosidase (*Rhizopus*), purified amyloglucosidase (from J. J. Marshall), and Sigma hog pancreatic α-amylase. Use of the latter, as recommended by Banks *et al.*, did not appear to be necessary with the BDH

"Agidex."

In general, the glucose oxidase procedure described by Lloyd and Whelan (11) was used to measure released glucose, and the Boehringer-Mannheim enzymes were found to be more suitable than those from Sigma. In some cases, the "Glucostat Special" reagent was used.

In most of these assays, the final volume prior to removal of aliquots for glucose assay was 50 ml rather than 250 ml, as described by Banks *et al.* (10) for starch samples. The higher concentration of salt in these aliquots resulted in a cloudiness when the Lloyd and Whelan (11) glucose oxidase reagent was added, but this dispersed when acid was added to complete the reaction and did not interfere with the assay.

Shortened Analytical Procedure

Samples of flour (~1 g) were suspended in sodium carbonate-bicarbonate buffer (pH 10 at 25°C; 10 ml) and stirred vigorously at 45° ± 3°C for 0.5 hr. Samples were centrifuged (5 min at 24,000 × *g*) and the supernatant was retained (cold) while two further similar extractions were carried out. The three extracts were combined, diluted to 50 ml with water, an aliquot adjusted to pH 4.5, and centrifuged (24,000 × *g*; 10 min). The supernatant and washings were combined, heated at 100°C for 10 min to inactivate enzymes, cooled, and dialyzed against tap water (16 hr) and distilled water (2 hr). Following appropriate dilution samples were assayed by the cysteine-sulfuric acid reaction.

α-Amylase Treatment of Gum

Gum extract (151 mg) was refluxed 2 hr with 70% ethanol and the solvent was dried to give an enzyme-inactivated product (146 mg). The enzyme-inactivated gum (100 mg) was dissolved in 50 ml phosphate buffer, pH 6.8, and 10μl hog pancreatic α-amylase was added. After 2 hr stirring at room temperature, the sample was dialyzed against tap water for 44 hr. IPA (4 vol) was added to the dialysate and the precipitate was dried following solvent exchange to give a starch-free product (94 mg).

RESULTS

Acid hydrolysis and paper chromatography of gum extracts from a number of cultivars of oats grown under different environments showed glucose as the major monosaccharide component, with lesser amounts of arabinose and xylose and some (tentatively identified) uronic acid. Visual estimations from the chromatograms indicated less than 10% pentosan. The mean value of the difference (7%) between glucan as determined by the nonspecific phenol-sulfuric acid reaction (12) and the glucose specific cysteine sulfuric acid reaction confirmed this estimate for the cultivars Hinoat and Rodney. In one instance, galactose was detected in trace amounts from an oat grown under abnormal (zero nitrogen fertilizer application) conditions.

The modification of the cysteine-sulfuric acid reaction described here thus allowed specific determination of glucose in oat gum. The results (Fig. 1) show that arabinose and xylose, even when present at the same concentration as glucose, essentially do not interfere in the assay when absorbancies are read at 428 nm. The chromophore given by the pentoses at 390 nm unfortunately faded

by 58% in 2 hr, thus making a dichromatic assay procedure for simultaneous estimation of glucose and pentose inconvenient. The glucose chromophore, following an initial slight fade, was stable for at least 2 hr. The standard error of the glucan estimation was less than ±2% in triplicate assays of four separately prepared solutions of three different gum extractions (76.4 ± 1.8, 89.3 ± 1.6, 89.4 ± 0.8% glucan). Most of the error in the procedure arises from preparation of the sample solution rather than from assay replicates, so long as great care is taken during pipetting of aliquots. Thus, poor reproducibility of replicates was found

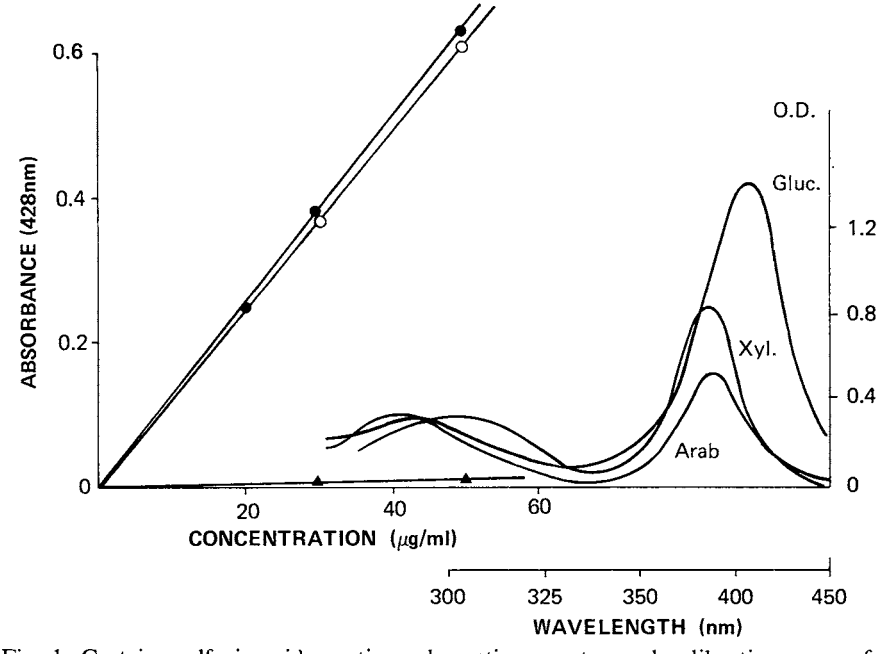

Fig. 1. Cysteine-sulfuric acid reaction: absorption spectra and calibration curves for glucose o, arabinose or xylose ▲, and glucose + arabinose + xylose •. In the latter, each sugar is present at the concentration shown by the abscissa.

TABLE I
Starch Analysis of β-Glucans Using Sigma and BDH Amyloglucosidase

Sample	Enzyme Used and wt mg		% "Starch" Analyzed
Laminaran	Sigma	1	31
Laminaran	Sigma	5	65
Laminaran	BDH	1	0.8
Laminaran	BDH	5	1.2
Lichenan	Sigma	1	13
Lichenan	Sigma	5	46
Lichenan	BDH	1	2.2
Lichenan	BDH	5	2.7

when a plunger-operated semiautomatic pipet was used rather than the Lang Levy constriction micropipets.

The BDH amyloglucosidase from *Aspergillus* was shown (Table I) to be β-glucanase free (more precisely, unable to release significant quantities of free glucose from β-glucans under the assay conditions described), unlike the enzyme from *Rhizopus* (Sigma). It can be seen that using the latter enzyme, the mixed linkage glucan, lichenan, might appear to contain almost 50% of starch but, in fact, contained less than 3% α-linked glucan (possibly isolichenan). Activity against contiguous β-1,3-linkages (as in laminaran) was also demonstrated for the *Rhizopus* enzyme. It is unlikely that the laminaran was contaminated with α-linked glucan, consequently the BDH enzyme must have some β-1,3-glucanase activity.

Misleading results arising from impure enzymes might also occur with the glucose oxidase procedure. The Lloyd and Whelan method (11) with the Boehringer-Mannheim enzymes specified, or the "Glucostat Special" reagent, gave identical results with oat gums and was essentially free from interferences by glucosidase ("maltase," "cellobiase") and glucanase activities. However, when the Sigma glucose oxidase and peroxidase were used in the Lloyd and Whelan method (11), applied directly to a glucose-free solution of laminaran, a color

TABLE II
Yields of β-Glucan in Pooled Material from Twice-Extracted Flour

Variety and Description	Gum Yield[b] %	Glucan Content[a] %	Starch Content[a] %	β-Glucan Yield[b] %
Hinoat; Regina 1972	3.50	78	1	2.69
Hinoat; Glenlea 1973	3.46	80	0	2.77
Hinoat; zero nitrogen	0.63	57	2	0.35
Rodney; Morden 1972	2.24	80	1	1.77
Rodney; Morden 1973	2.43	77	1	1.85
Rodney; Glenlea 1973	2.64	77	2	1.98
Stormont	2.54	84	2	2.08
Harmon	1.52	75	2	1.11
Gary	1.34	77	1	1.02

[a]Percentage, dry weight basis, in gum extract.
[b]Percentage, dry weight basis, in flour.

TABLE III
Analysis of Four Replicate Samples of Hinoat Oat Flour

Extract	Mean Gum Yield[b] % ± S.E.	Mean Starch Content[a] % ± S.E.	Mean Glucan Content[a] % ± S.E.	Mean β-Glucan Yield[b] % ± S.E.
1	2.25 ± 0.05	1.10 ± 0.05	75.5 ± 2.7	1.68 ± 0.08
2	1.25 ± 0.06	1.75 ± 0.15	92.7 ± 1.6	1.14 ± 0.06
3	0.67 ± 0.02	3.64 ± 0.46	79.0 ± 2.2	0.50 ± 0.02
Total	4.19 ± 0.10	1.68 ± 0.09	81.0 ± 1.7	3.32 ± 0.08

[a]Percentage, dry weight basis, in gum extract.
[b]Percentage, dry weight basis, in flour.

yield equivalent to 8% glucose content was found. Thus, the Sigma glucose oxidase and/or peroxidase must contain β-glucanase activity. The results (Tables II and III) show that starch contamination of oat gum extracts was small, reaching a maximum of about 4% in the third extract. Treatment of two gum samples, starch content 1.6% and 2.7%, with hog pancreatic α-amylase reduced this starch content to 0.5% and 0.6%, respectively, confirming that these figures give a true estimate of starch (at least above ~ 0.5%) rather than reflect some small β-glucanase impurity in the BDH amyloglucosidase used.

Gums from pooled extracts 1 and 2 from a number of different cultivars grown in different locations were analyzed (Table II). There appear to be some varietal differences, but there are insufficient samples to allow assignment of any statistical significance. Since environment may considerably affect yield and analytical results (see, for example, Hinoat grown under zero nitrogen fertilizer application), locational variance might well obscure varietal variance, although in a number of the samples, location and year of growth have apparently not greatly affected results.

The relative yields of successive alkaline extractions of oat flour (Fig. 2) show that two or three extracts do not represent the total gum content, but it is impractical to attempt further extractions on a routine basis. In contrast (Fig. 2),

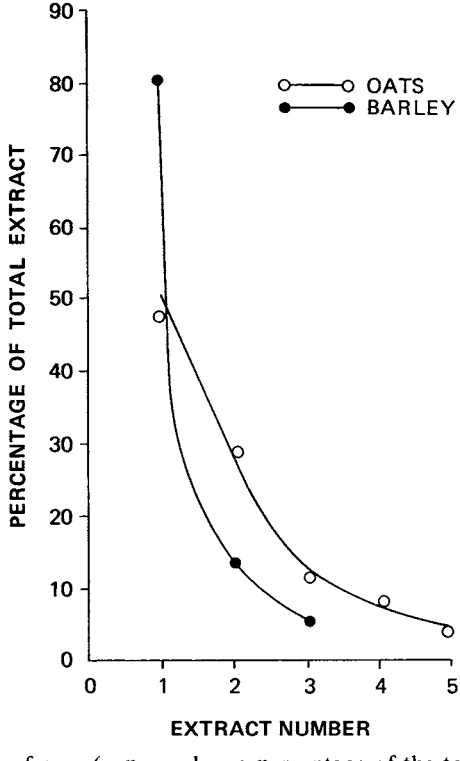

Fig. 2. Distribution of gum (expressed as a percentage of the total extracted) between successive extractions of oats o, and barley •.

the progress of extraction of gum from 6-row and 2-row barley flour (Conquest and Betzes) was much more rapid.

The overall reproducibility of extraction and analysis was examined using four replicates of a sample of Hinoat flour, which appeared to be a high gum-yielding cultivar. The three extracts, separately analyzed (Table III), showed reasonable overall reproducibility. The greatest error occurs in the extraction procedure rather than in the analytical procedures themselves, leading to coefficients of variation in the β-glucan content of the individual extracts of 10%. However, the coefficient of variation for the total yield of gum and total yield of β-glucan is only 5%; thus, variations in individual extracts (as might be expected) tend to cancel out.

Samples of Hinoat and Rodney flour were treated with refluxing 70% ethanol to inactivate enzymes prior to extraction of the gum. The results (Table IV) showed that enzyme inactivation resulted in a reduced total yield of β-glucan for both cultivars. Starch content increased somewhat, particularly for Rodney, with the result that total crude gum yield was essentially unchanged.

In the shortened version of the procedure, triplicate analysis of five different extractions gave a glucan content and standard error of $3.80 \pm 0.05\%$ of flour, dry weight basis. This compared with $3.40 \pm 0.09\%$ for the more rigorous isolation procedure ($P = 0.01$).

DISCUSSION

Studies by Morris (13), Acker *et al.* (4), and Peat *et al.* (5) established the presence in oats of a water-soluble glucan composed of β-1,3- and β-1,4-glucopyranosyl units. The presence of xylose and arabinose in hydrolysates of unpurified aqueous extracts was also noted, and in comparative studies of wheat, rye, corn, barley, and oats, Preece and Mackenzie (1) showed that aqueous

TABLE IV
β-Glucan Content of Enzyme Inactivated Hinoat (H) and Rodney (R) Flour

Sample	Extract	Gum Yield[b] %	Starch Content[a] %	Glucan Content[a] %	β-Glucan Yield[b] %
	1	1.95	2	71	1.34
H[c]	2	1.21	3	78	0.91
(Enzyme inactivated)	3	0.76	4	80	0.57
	Total	3.92	3	75	2.82
	1	1.93	8	73	1.26
R	2	1.17	5	78	0.86
(Enzyme inactivated)	3	0.47	13	75	0.29
	Total	3.57	8	75	2.40
	1	2.38	1	81	1.89
R	2	0.81	3	87	0.70
(Untreated flour)	3	0.43	4	81	0.33
	Total	3.62	2	82	2.92

[a]Percentage, dry weight basis, in gum extract.
[b]Percentage, dry weight basis, in flour.
[c]See Table III for analysis of untreated Hinoat flour.

extracts of oat flour contained mainly glucan with 13% contaminating pentosan ("xylan" and "araban"). In the present study, similar results were obtained with the alkaline extracts from oats. In only one instance was galactose detected. It seemed possible, therefore, that glucose might be measured by a simple and direct colorimetric procedure, thus eliminating the need for hydrolysis, neutralization, and assay, and this proved to be so with the modification of cysteine-sulfuric acid reaction described.

It was essential, for these studies, to use an amyloglucosidase preparation that was unable to release free glucose from β-glucan, and, following the advice of M. Fleming (Department of Brewing and Biological Sciences, Heriot-Warr University, Edinburgh, Scotland), the BDH preparation was found to meet this requirement, as did a purified amyloglucosidase provided by J. J. Marshall. Although care in the use of such enzymes is fairly obvious in the present context, it is perhaps less so when the prime interest is in starch measurement. It is worth noting that certain flour fractions or fabricated foodstuffs might contain significant amounts of β-glucan, leading to erroneously high starch values if the amyloglucosidase is not carefully selected.

Yields of β-glucan extractable by alkali (Tables II and III) are higher than amounts previously reported for oats or barley (1,7,14,15) although Acker *et al.* (4) reported amounts of about 4% in oats, and Luchsinger *et al.* (16) reported about 5% in barley. It is unlikely that all of this glucan can reside in that fraction of the endosperm normally isolated as the cell wall, although at least 50% of cell wall in barley is composed of mixed linkage β-glucan (17).

It seems that oats, like barley (14,15), are subjected to varietal differences, though without more data on the statistical significance of environmental effects (an extreme of which is the effect of zero nitrogen fertilizer), apparent varietal differences must be treated with caution. Even with the extensively studied barley, there is little information available on environmental as opposed to varietal differences. Bourne and Pierce (3) reported considerable variation from area to area, but varietal ranking was unchanged. Bendelow (18), using viscosity as a measure of glucan content, also found considerable locational variance, and in this instance cultivar ranking also changed. Our findings on the effect of zero nitrogen fertilizer application suggest that further study of environmental influence might be worthwhile.

The high protein cultivar Hinoat was used to study reproducibility of the analytical procedure. Three extractions were chosen, although extraction is not complete at this stage (Fig. 2). As with the method of Fleming *et al.* (7) for barley, this is probably a factor in some of the variability of results noted in Table III, but it is not practical to continue with an exhaustive extraction. It is worth noting (Fig. 2) that a complete extraction of barley was more easily achieved, suggesting the potential utility of this procedure for analysis of barley as well as oats.

It should be emphasized that methods described here for β-glucan analysis were essentially devised for monitoring extracts prepared by potentially commercial procedures. However, for routine analysis of flour, such as might be required in a breeding program, isolation of the gums is time-consuming. In the shortened version of the procedure, therefore, isolation of the gums was omitted and β-glucan was determined directly on a combined dialyzed extract. The starch determination could be included, but since all analyses of normal flour indicated starch contents of less than 2% in the gum, the error in equating total glucan with

β-glucan was small. The dialysis step was necessary to remove interference from low-molecular-weight carbohydrates normally present in the flour, since this method, unlike that of Fleming *et al.* (7), did not include a prior treatment with 80% ethanol. Despite this, the shortened version of the assay gave a significantly higher value. It is likely that dialysis retains some low-molecular-weight polysaccharide, possibly fructosan, which is not precipitated by 50% isopropanol.

It is presumed that the indigenous β-glucanase enzymes are essentially inactive at the extraction pH (19). Nevertheless, alkaline extracts slowly lose viscosity on standing. It is uncertain at present whether this reflects enzymatic or chemical action, although conditions may be suitable for alkaline degradation (20) or hydroxy radical induced cleavages (21). We have shown with crude enzyme extracts that the extraction treatment reduces oat "gumase" activity (measured viscometrically at pH 5.3) by about 90%. However, the situation with soluble extracts and flour suspensions may be different and, thus, the effect of enzyme inactivation on two varieties of flour was checked. Since the yield of β-glucan obtained decreased somewhat, it was concluded that when alkaline extraction is used enzyme inactivation is of no advantage.

It would seem, therefore, that alkaline extraction methods originally developed for commercial pilot plant studies offer improvement over traditional milder methods, since enzyme inactivation of flour is no longer necessary and, in the case of barley, extraction approaches completion more rapidly. In modern practice, when milder techniques suffice, it is normal to avoid such harsh methods of extraction, with the concomitant risk of degradation. In this instance, however, the harsher extraction conditions, on the basis of viscosity data, yield minimally degraded material. Kinematic viscosities (details to be published elsewhere) in excess of 1000 cSt have been found for 0.5% (w/v) solutions of oat gum in dimethyl sulfoxide, which is considerably greater than values previously reported for either oat or barley gum.

The modified extraction procedure and rapid colorimetric glucan assay offer the advantage of simplification over the method of Fleming *et al.* (7). Methods based on viscosity correlations (18), although simple and rapid, require a constant relation between viscosity and concentration for the cultivars in question. Oat extracts obtained in this study show considerable differences between the viscosities given by different cultivars at the same concentration.

Studies of viscosity and other physicochemical properties of oat β-glucan and factors relating to its extraction and degradation are in progress.

Acknowledgments

The authors thank J. Weisz and H. Hamilton for technical assistance, and J. J. Marshall, Miami, Florida, for amyloglucosidase.

Literature Cited

1. PREECE, I. A., and MACKENZIE, K. G. Non-starchy polysaccharides of cereal grains. II. Distribution of water-soluble gum-like materials in cereals. J. Inst. Brew. 58: 457 (1952).
2. PREECE, I. A., and MACKENZIE, K. G. Non-starchy polysaccharides of cereal grains. III. Higher molecular gums of common cereals. J. Inst. Brew. 59: 385 (1952).
3. BOURNE, D. T., and PIERCE, J. S. β-Glucan and β-glucanase-Review. Tech. Quart. Master Brew. Ass. Amer. 9: 151 (1972).

4. ACKER, L., DIEMAIR, W., and SAMHAMMER, E. The lichenin of oats. I. Properties, preparation and composition of the muciparous polysaccharides. Z. Lebensm. Unters. Forsch. 100: 180 (1955).
5. PEAT, W., WHELAN, W. J., and ROBERTS, J. G. The structure of lichenin. J. Chem. Soc. (London) 1957: 3916.
6. PARRISH, F. W., PERLIN, A. S., and REESE, E. T. Selective enzymolysis of poly-β-D-glucans, and the structure of the polymers. Can. J. Chem. 38: 2094 (1960).
7. FLEMING, M., MANNERS, D. J., JACKSON, R. M., and COOKE, S. C. The estimation of β-glucan in barley. J. Inst. Brew. 80: 399 (1974).
8. KENNEDY, J. F., and BUTT, W. R. Periodate oxidation studies of human pituitary follicle-stimulating hormone. Biochem. J. 115: 225 (1969).
9. DISCHE, Z., SHETTLES, L. B., and OSNOS, M. New specific color reactions of hexoses and spectrophotometric micromethods for their determination. Arch. Biochem. 22: 169 (1949).
10. BANKS, W., GREENWOOD, C. T., and MUIR, D. D. The characterisation of starch and its components. Part 2. The semi-micro estimation of the starch content of cereal grains and related materials. Staerke 22: 105 (1970).
11. LLOYD, J. B., and WHELAN, W. J. An improved method for enzymic determination of glucose in the presence of maltose. Anal. Biochem. 30: 467 (1969).
12. DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1: 350 (1955).
13. MORRIS, D. L. Lichenin and araban in oats (*Avena sativa*). J. Biol. Chem. 142: 881 (1942).
14. BOURNE, D. T., and PIERCE, J. S. β-glucan and β-glucanase in brewing. J. Inst. Brew. 76: 328 (1970).
15. SPARROW, D. H. B., and MEREDITH, W. O. S. Malt cylolytic activity of barleys of diverse origins and its relation to maltability. J. Inst. Brew. 75: 237 (1969).
16. LUCHSINGER, W. W., ENGLISH, H., COCHRANE, D. G., and KNEEN, E. Autolytic action of gumases in malting and brewing. Amer. Soc. Brew. Chem., 1958, p. 40.
17. SMITH, M. M., and STONE, B. A. Chemical composition of the cell walls of *Lolium Multiflorum* endosperm. Phytochemistry 12: 1361 (1973).
18. BENDELOW, V. M. Determination of non-starch polysaccharides in barley breeding programmes. J. Inst. Brew. 81: 127 (1975).
19. BASS, E. J., MEREDITH, W. O. S., and ANDERSON, J. A. Enzymes that degrade barley gums. II. Preparation from barley and green malt. Cereal Chem. 30: 313 (1953).
20. WHISTLER, R. L., and BeMILLER, J. N. Alkaline degradation of polysaccharides. Advan. Carbohyd. Chem. 13: 289 (1958).
21. SCOTT, J. E., and TIGWELL, M. J. Periodate induced viscosity decreases in aqueous solutions of acetal and ether-linked polymers. Carbohyd. Res. 28: 53 (1973).

[Received August 13, 1976. Accepted October 12, 1976]