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THE RELATIONSHIP BETWEEN THE VISCOSITY OF AN ACID FLOUR EXTRACT OF BARLEY AND ITS β-GLUCAN CONTENT

by

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The relationship between the viscosity of an acid flour extract and the acid-soluble, acid-insoluble and total β-glucan content of barley flour has been investigated. The extract viscosity was found to be closely related (R^2 = 0.99, S.E.E. = 0.19) to the content of soluble β-glucan. It is concluded that the acid-soluble β-glucans from 14 of the 18 different genotypes studied have a similar structural nature. Genotypes containing the lys-3a allele were found to have very low extract viscosities and β-glucan contents. Quick and accurate methods are described for measuring viscosities of acid extracts made from flour derived from grains of a single plant and flour derived from a single grain. These methods can be used to screen plants for their soluble β-glucan content in a plant breeding program.

1. INTRODUCTION

Mixed-linked β-glucans, referred to as β-glucans in the present communication, are found primarily in the seeds of cereals, especially those of barley and oats (3), but are also present in the vegative parts of given plants (2). These glucans are polymers of β-D-glucopyranose (41) in which about 30% of the glucosidic linkages are in the 1,3 position, the remainder being 1, 4 (11, 20). They form part of the endosperm and in barley they account for 75% of the endosperm cell walls (18, 23). When situated in the cell walls the β-glucans are linked to proteins forming molecules of very high molecular weight (4×10^7 daltons, 23). However, 50% of the β-glucans can be removed by water without destroying the wall structure (23). The water soluble β-glucans and pentosans have often been referred to as »gums« (4, 25, 32, 34, 42, 44). The β-glucans have been extracted in many ways (3, 7, 29), and this fact has made the term soluble β-glucan very vague and unprecise. Not only the amount but also the molecular structure appears to depend on the extraction conditions, e.g.

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temperature, pH, solvents (3, 7, 23, 29). In the present study the soluble β-glucans are defined as those which are extracted at room temperature (approx. 22 °C) from flour by a HCl-KCl buffer (pH 1.5). Extracts prepared in this way were used for the viscosity determinations. In extracts of barley flour the β-glucans appear to be free and no longer bound to proteins (23). These molecules have molecular weights, ranging from $2 \times 10^5 - 2 \times 10^7$ daltons (9, 13) and give rise to very viscous solutions.

In normal germinating seeds the β-glucans are hydrolyzed by enzymes including at least 3 β-glucan-endo-hydrolases (2, 50). In current brewery practice the β-glucans escape complete degradation by enzymes during the malting and wort extraction procedures. The residual β-glucans with molecular weights of $7 \times 10^4 - 6 \times 10^5$ daltons (22, 25) are found both in wort and beer where their large size contributes to viscosity and filtration problems (5, 15, 17, 24, 42, 43, 44, 45). These problems are exacerbated in the case of strong beers (25) and become much worse when barley is used as an adjunct (6, 8, 16, 31, 32) because of the higher molecular weight of β-glucans from unmalted barley. The β-glucan content of the wort does not necessarily correlate well with the amount in the unmalted barley (51, 52), due to the varying amounts and activities of the enzymes present in the grains (9, 33).

Since β-glucans cause trouble in the breweries as well as in feeding of monogastic animals (10, 26, 27), it will be profitable to breed for a barley with as little soluble β-glucan as possible. The present communication presents a method by which the viscosity of an acid flour extract prepared from the grains of a single plant, a single grain or even half a grain can be rapidly and accurately determined. By analysing 18 barley genotypes with widely different viscosities, it is shown that the viscosity of the acid flour extract is highly correlated to the soluble β-glucan content, but not to the insoluble β-glucan content.

2. MATERIALS AND METHODS

2.1. Plant material

The investigations were carried out on 18 genotypes of barley, which include five commercial varieties and 13 experimental lines (Table I). These genotypes were selected after the harvest of 1976 because they had either very low or very high viscosities of their acid flour extracts. Nine of each type were chosen. This same plant material was used in the investigations concerning the effect of temperature on the extraction procedure.

The seeds used for parallel β-glucan and viscosity determinations were harvested from plants grown at the Crop Research Division (D.S.I.R.) Research Farm, Lincoln, New Zealand in the period September 1977 – February 1978.

The seeds from the oat variety Astor harvested from plants grown in 1976 were obtained from Qvade A/S, Maribo, Denmark.

2.2. β-glucanase

The 1,3:1,4-β-glucan 4-endo-hydrolase (E.C. 3.2.1.73), hereafter referred to as the β-glucanase, used for the β-glucan determinations was a kind gift from B. A. STONE, Biochemistry Department, La Trobe University, Bundoora, Victoria, 3083, Australia and from B. S. ENEVOLDSEN and K. ERDAL, Department of Brewing Chemistry, Carlsberg Research Laboratory. They purified it from bacterial amylase Novo 1000 S (Novo Industri A/S, Copenhagen, Denmark) and it had the following specifications: no α-amylase activity, a β-glucanase activity of 235 $U \cdot ml^{-1}$ at pH 7.5 and an endo-barley-β-glucanase activity of 572 $U \cdot ml^{-1}$ at pH 6.5.

2.3. Preparation of the acid flour extract and pellet

The procedure used was based on the method of GREENBERG and WHITMORE (29) modified to the following method: a suitable number of grains was ground in a cyclone sample mill (Udy Analyser Company, Boulder, Colorado, U.S.A.) using a 0.2 mm mesh screen. From the resulting flour, 0.2 g, was accurately weighed into a plastic tube (70 × 11 mm) and suspended in 2 ml of a HCl-KCl buffer, pH 1.5 (29). The extract was prepared by shaking the tube, held in a rubber bung for 1 hour at room temperature in a React-R-shaker (Tecator, Höganäs, Sweden),

Table I.

Origin of 18 barley genotypes selected after the harvest of 1976 for high and low viscosity of their acid flour extracts.

Genotypes	Origin[a]	Place of[b] growth	Viscosity of acid flour extract (cP)
Commercial varieties			
Triumph	**DDR,** Veb, Saat-und Pflanzgut, Berlin	All	3.8
Proctor	**U.K.,** Plant Breeding Institute, Cambridge, England	Hyld	4.5
Zephyr	**The Netherlands,** Veredelingsbedrijf N.V. Westpolder (Gr)	All	24
Lami	**Denmark,** Landbrugets Kornforædling, Sejet, Horsens	Hyld	68
Minerva	**The Netherlands,** Fonds Bevordering Veredeling Landbouwgewassen, Wageningen	Høj	210
Experimental lines			
1392[c]	Gerkra[2] × Risø 1508	Hyld	1.5
1382[c]	Gerkra[2] × Risø 1508	Hyld	1.8
1396[c]	Cilla[2] × Risø 1508	Hyld	1.8
M 205	Marker genes LK and log 1 (chromosome 2)	Høj	2.9
Risø 1508[c]	High lysine mutant in Bomi	All	3.0
Nirasaki (nijo, 9)	Beka × Kanta nijo, 12	Hyld	3.8
Ca 1289	Kristina × (Heine 4808 × Dana)	All	4.4
V1 75–271	Background unknown	Høj	100
149	H. tetrast. v. viol	Høj	140
1450[d]	Sv 71/667 × Sv 71/11399	Hyld	160
1351[d]	(Bomi × Hiproly) × Minerva	Hyld	330
1356[d]	(Bomi × Hiproly) × Minerva	Hyld	560
1352[d]	(Bomi × Hiproly) × Minerva	Hyld	980

a) Place of origin is given for the commercial varieties. For the experimental lines, the cross from which the lines were derived or the name of the mutant is given.
b) Research farms: Allingemaglegård (All) and Hyldagergård (Hyld) of Carlsberg Plant Breeding and Højbakkegård (Høj) of the Royal Veterinary and Agricultural University, Copenhagen.
c) Genotypes containing the allele of the high lysine gene from Risø 1508 called lys-3a (30).
d) Genotypes containing the allele of the high lysine from Hiproly called lys (30, 38).

followed by centrifugation at 1000 g for 20 min at room temperature in a Sorval GLC-2B centrifuge. The resulting supernatant is referred to as the acid flour extract. The pellet was resuspended in the HCl-KCl buffer, shaken for 10 min and centrifuged for 10 min under the conditions given above. This procedure was repeated until the viscosity of the resulting supernatant solution was the same as the viscosity of the buffer, indicating that all soluble material contributing to the viscosity had been extracted. The residual material is referred to as the acid flour pellet.

2.3.1. Modification for single grains

The method described was modified for application to single grains as follows: Flour was prepared by grinding a single grain in a ball mill. About 54 mg of flour was weighed into a plastic tube having a volume of 1.2 ml, and the exact weight (w mg) noted. The flour was suspended in (w × 15) µl of the HCl-KCl buffer.

2.3.2. Modification for extraction at different temperatures

To study the effect of different extraction temperatures on the viscosity of the acid flour extract, the extract was shaken in a conical flask at the desired temperature and transferred to a plastic tube before centrifugation.

2.3.3. Modification for treatment with β-glucanase

The grains were boiled in 80% ethanol for 30 min to inactivate any β-glucanase present, and dried on filter paper for a week at room temperature before grinding. Maleate buffer, 0.25 M, pH 6.5 used in the β-glucan determination (see section 2.5) was substituted for the HCl-KCl buffer. The extract is referred to as the maleate flour extract.

2.4. Viscosity determinations

A 400 µl sample of the acid flour extract was transferred to the cup of a Contraves Low Shear 100 viscometer (Contraves AG, Zürich, Switzerland). The viscosity was measured at 20 °C. All results are recorded in centipoises (cP) and based on duplicate determinations.

2.4.1. Modification for treatment with β-glucanase

The viscometer was connected to a recorder to allow continuous measurement of the viscosity. A 400 µl sample of the maleate flour extract was transferred to the cup of the viscometer, and after 12 min, 10 µl of the β-glucanase (see sections 2.2 and 2.5) was added. The incubation was stopped when no further change in the viscosity could be detected.

2.5. β-glucan determinations

The total β-glucan content of the flour before extraction was measured as described by ANDERSON et al. (3). This involves enzymic hydrolysis of the β-glucans followed by extraction and hydrolysis of the liberated oligosaccharides and estimation of the amount of glucose released by a glucose oxidase-peroxidase reagent (Boehringer Mannheim Gmbh, Mannheim, West Germany).

All results are based on triplicate measurements. That is, the sample was divided into three aliquots following enzyme treatment and before drying in the vacuum oven (see ANDERSON et al., 3). Control measurements were made using the maleate buffer blank. Glucose was used as a standard.

2.5.1. Modification for soluble β-glucans

Soluble β-glucans, those present in the acid flour extract, were measured by a modification of the method for total β-glucan. A 1 ml sample of the acid flour extract and 1 ml of 0.025 M-NaOH were transferred to a stoppered centrifuge tube and titrated with NaOH to pH 7. This ensured that no hydrolysis of the β-glucans would occur and that the pH would be at an optimum for the β-glucanase activity. The sample was then freeze-dried. To inactivate any enzymes 4 ml of 80% ethanol was added and the sample boiled for 30 min. The ethanol concentration was maintained at approx. 80% by replacing the evaporated liquid with 96% ethanol. Thereafter the sample was washed 3 times in 80% ethanol to remove the low molecular weight oligosaccharides and free sugars. This involved adding 4 ml of 80% ethanol, shaking at room temperature for 30 min and centrifuging at 1000 g for 10 min at room temperature. Finally the freeze-dried neutralized acid flour extract was dried in an oven at 50 °C. The subsequent steps of the procedure were essentially those of ANDERSON et al. (3).

2.5.2. Modification for insoluble β-glucans

Insoluble β-glucans, those present in the acid flour pellet, were measured by the following modification of the method of ANDERSON et al. (3). The acid flour pellet was suspended in 80% ethanol and transferred to a Soxhlet extraction thimble (Whatman cellulose, 22 × 80 mm). Quantitative transfer of the pellet to the thimble was ensured by washing 3 times in 80% ethanol and adding the washings to the suspension in the thimble. The remainder of the procedure was essentially that of ANDERSON et al. (3).

2.6. Determination of the main constituents of the acid flour extract

2.6.1. Total carbohydrates

The method of DUBOIS et al. (14) was modified as follows. A 4 ml aliquot of the acid flour extract was neutralised with NaOH and diluted to 12.5 ml with distilled water. Then 12.5 ml of 2 M-H_2SO_4 was added and the sample held for 20 min at 100 °C. The sample was next diluted to 50 ml with distilled water. A 0.1 ml aliquot of the sample was mixed with 0.7 ml of distilled water, 0.04 ml of 80% phenol and 2 ml of concentrated H_2SO_4 and allowed to stand for 15 min at room temperature. The absorption was then measured at 490 nm. Glucose was used as the standard.

2.6.2. Protein

Protein content of the extracts was measured by the Kjeldahl-method using the Tecator System (Tecator, Höganäs, Sweden) with a selenium catalyst (Kjeltabs, Bie and Berntsen, Copenhagen, Denmark).

2.6.3. Ash and buffer salts

These were determined by weighing the freeze-dried acid flour extract before and after incineration in an oven at 900 °C for four hours and cooling one hour in a desiccator.

2.6.4. Fats

Freeze-dried acid flour extract (10 ml) was dried and weighed before and after extraction with petroleum ether (b.p. 40–60 °C) in a Rafatec extraction unit (Tecator, Höganäs, Sweden). The fat content was then determined from the difference between the two measurements.

2.7. Standard errors

The exponential nature of the viscosity (see Figure 2) indicated that the standard error was dependent on the level of the viscosity. This was tested by comparing the variances (ω^2) at two different levels (~17 cP and ~40 cP). As expected the variances showed no difference (F-test), if ln(extract viscosity, cP) was used. The standard error, S_v, was calculated from the formula:

$$S_v = \frac{1}{n}\sqrt{e^{[2\ln(\text{extract viscosity}) + \omega^2(e^{\omega^2}-1)]}}$$

where n is the number of determinations. In the case of the β-glucan determinations a similar study was made, and it was found that the standard error of the β-glucan contents was also level-dependent so that an analogous formula could be used.

3. RESULTS

3.1. Effect of extraction temperature on the viscosity of the acid flour extract

In arriving at the method used to measure the viscosity of the acid flour extracts, the effect of the extraction temperature on the viscosity was examined. Extracts from all 18 genotypes were made at seven different temperatures ranging from 15 to 70 °C. Figure 1 illustrates the results from six genotypes selected to represent the range of viscosities as reported in Table I. The viscosity of the extracts was found to increase as the extraction temperature was raised from 15 to 50 °C for 14 of the 18 genotypes. Further increases in temperature gave lower extract viscosities, and at 70 °C the actual viscosities of each of these genotypes were similar. However,

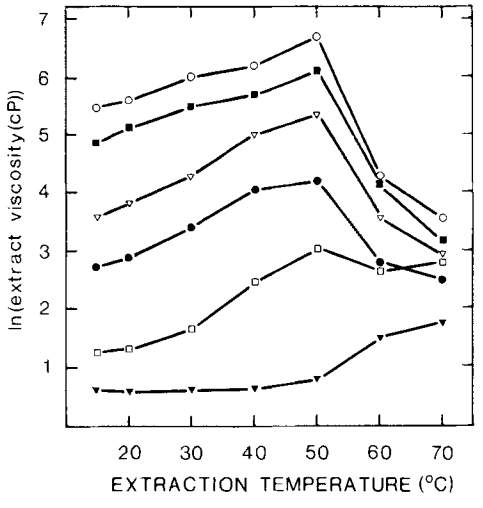

Figure 1. The dependence of the viscosity of the acid flour extract on the extraction temperature is illustrated for six barley genotypes. O—O = 1351, ■—■ = Minerva, ▽—▽ = Lami, ●—● = Zephyr, □—□ = Triumph, ▼—▼ = 1392.

the extract viscosities of the four genotypes containing the lys-3a allele (Risø 1508, 1382, 1392 and 1396) were little affected by temperatures below 50 °C. Thereafter they increased slightly, but were still less than those of the other 14 genotypes. A most important result is that at each of the temperatures up to and including 50 °C, the 18 genotypes could be arranged in the same order with respect to their extract viscosities.

3.2. Viscosity of the acid flour extract and the content of total, soluble and insoluble β-glucans

In Table II the 18 barley genotypes are arranged with respect to their measured extract

Table II.

Viscosity of acid flour extracts and contents of total, soluble and insoluble β-glucans from 18 barley genotypes grown in New Zealand in 1977/78.

Genotype	Viscosity (cP)[a]	β-glucan (% of dry flour)[b]			
		Total[c]	Soluble[d]	Insoluble[e] calculated	Insoluble[f] measured
1392[g]	1.81	2.00	0.14	1.86 ± 0.09	1.60 ± 0.06
1396[g]	2.12 ± 0.08	2.62	0.20	2.42	
Risø 1508[g]	2.22 ± 0.09	2.60	0.23	2.37	
1382[g]	2.57 ± 0.10	2.65	0.36	2.29	
Nirasaki	2.75 ± 0.11	3.56	0.42	3.14 ± 0.16	3.21 ± 0.12
Triumph	7.84	4.44	0.67	3.77 ± 0.20	3.67 ± 0.14
Proctor	12.2	4.73	1.02	3.71	
1356[h]	39.6 ± 1.6	4.43	1.27	3.16	
Ca 1289	40.3 ± 1.6	5.16	1.34	3.82	
1450[h]	53.0	5.80	1.36	4.44	
Zephyr	58.3	5.55	1.45	4.11	
M 205	66.2 ± 2.6	4.63	1.42	3.21 ± 0.23	3.19 ± 0.12
149[i]	68.4 ± 2.7	6.19	1.47	4.72	
1352[h]	96.7	4.72	1.53	3.19 ± 0.24	3.56 ± 0.13
1351[h]	156	5.10	1.74	3.36	
V1 75–271	246 ± 10	5.28	1.84	3.44	
Lami	256 ± 10	5.22	1.85	3.37 ± 0.27	3.67 ± 0.14
Minerva	632	6.36	2.34	4.02	

[a] Standard error can be calculated from the formula described in section 2.7. For the viscosity only the standard errors necessary for comparison are given.

[b] Standard error can be calculated from the formula described in section 2.7. For the β-glucan content only the standard errors of insoluble β-glucans are given for comparison.

[c] $\frac{\text{mg β-glucan in flour} \times 100}{\text{mg dry flour}}$

[d] $\frac{\text{mg β-glucan in acid flour extract} \times 100}{\text{mg dry flour}}$

[e] Total β-glucan ÷ soluble β-glucan.

[f] $\frac{\text{mg β-glucan in acid flour pellet} \times 100}{\text{mg dry flour}}$

[g] Genotypes containing the lys-3a allele.

[h] Genotypes containing the lys allele.

[i] Naked genotype.

viscosities from genotype 1392, which had the lowest viscosity (1.81 cP) to Minerva, which had the highest viscosity (632 cP). Although the standard error of a viscosity measurement is less than 4%, it is not possible to distinguish 1396 from Risø 1508, 1382 from Nirasaki, 1356 from Ca 1289, M 205 from 149 or Vl 75–271 from Lami. In the original screening of material grown in Denmark in 1976, nine of the genotypes had very low and nine very high extract viscosities (Table I). The same extreme difference between the two groups is not as evident in the material grown in New Zealand in 1977/78 (Table II). Nevertheless, a division of these genotypes into two groups of equal size by drawing a line between Ca 1289 and 1450 (Table II) results in the same distribution as found for the 1976 analyses with two exceptions, M 205 and 1356. Thus, the ranking with respect to extract viscosities was in general reproducible between the two harvests except for the genotypes having the lys allele, which were lower in the New Zealand material.

The total and soluble β-glucan contents were determined for all 18 genotypes grown in New Zealand. The total β-glucan content increases more than three-fold when comparing 1392 at the top of the table to Minerva at the bottom, while the soluble β-glucan content of Minerva is sixteen-fold greater than that of 1392 (column 3 vs 4 of Table II). The β-glucan contents are expressed in percent of original flour sample on a dry weight basis. It is expected that the total β-glucan equals the soluble β-glucan plus the insoluble β-glucan. Thus for all 18 genotypes the insoluble β-glucan content was calculated by subtraction with the results shown in column 5 of Table II. Only a two-fold variation can be seen. To verify the correctness of this assumption, the content of insoluble β-glucans was also determined directly for six genotypes representing the total range of measured viscosities (column 6 of Table II). In five of the six cases a reasonable agreement between the values in the last two columns of Table II can be seen. The exception is 1392, which has both a low extract viscosity and measured amount of insoluble β-glucans, suggesting that measurement of small amounts of β-glucans in an acid flour pellet is more prone to error.

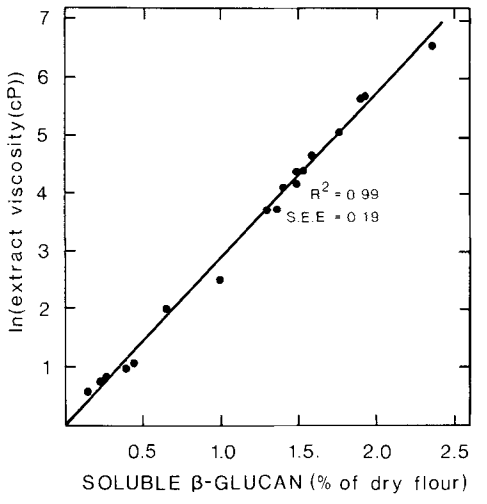

Figure 2. The logarithmic relationship between the viscosity and the β-glucan content of the acid flour extracts from 18 barley genotypes.
R^2 = correlation coefficient. S.E.E. = standard error of estimate.

3.2.1. Viscosity of the acid flour extract related to its content of β-glucan

The range of values obtained for the content of soluble β-glucans, those present in the acid flour extract, of the 18 genotypes is very narrow when compared to the range of the viscosity measurements (column 4 vs 2 of Table II). An excellent correlation (R^2 = 0.99, S.E.E. = 0.19), however, exists between these two types of measurements, as illustrated in Figure 2. The logarithmic relationship (55) can be expressed by the following formula:

$$\text{Soluble β-glucan (\% of dry flour)} = \frac{\ln(\text{extract viscosity}) - \ln(1.049)}{2.85} \sim \frac{\ln(\text{extract viscosity})}{3}$$

To ensure that this relationship was only a concentration effect, the viscosity of several acid flour extracts were measured at all speeds covering the range of the low shear viscometer. The extracts showed Newtonian behaviour (49), i.e., the viscosity is independent of the actual velocity of flow of the liquid.

The logarithmic nature of the relationship between viscosity and concentration of soluble β-glucans implies that the sensitivity of the viscosity measurements increases with the concentration of the soluble β-glucans. This means that for medium and high concentrations of β-glucans, very small differences in amount of the latter can be distinguished by the viscosity method. Thus, a 14% increase in soluble β-glucan content gives a 60% increase in viscosity (1351 and 1352 of Table II). The absolute standard error also increases with increase in viscosity. Since the standard error is constant in terms of %, however, the accuracy of the measurements is independent of the amount of β-glucan present in the extract (see section 3.3).

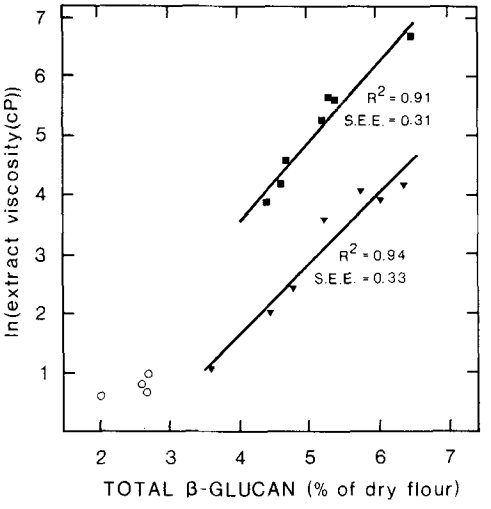

Figure 3. The 18 barley genotypes can be divided into three groups, when the logarithm of the viscosity is related to the total β-glucan content.

○ = high lysine crosses with Risø 1508 having the lys-3a allele. ■ = genotypes with relatively high percentage of soluble β-glucans. ▼ = genotypes with relatively low percentage of soluble β-glucans.

3.2.2. Viscosity of the acid flour extract compared to total and insoluble β-glucan contents

An inspection of data in column 2 and 3 of Table II reveals that a good correlation does not exist between the total β-glucan content and the viscosity of the acid flour extract ($R^2 = 0.78$). However, if the material was divided into smaller groups, better correlations were obtained. This division was based on a visual examination combined with calculations of different R^2 values. The four genotypes with the lys-3a allele, which are very low in both β-glucan content and extract viscosity, seems to represent a group by themselves (Figure 3). The most reasonable division of the remaining 14 genotypes was into two groups with seven members in each. One group consists of genotypes having relatively high extract viscosities compared to the amount of total β-glucan (1356, M 205, 1352, 1351, VI 75–271, Lami and Minerva; $R^2 = 0.91$, S.E.E. = 0.31; Figure 3). The other group consists of those having relatively low extract viscosities compared to the amount of total β-glucan (Nirasaki, Triumph, Proctor, Ca 1289, Zephyr, 1450 and 149; $R^2 = 0.94$, S.E.E. = 0.33; Figure 3). It is notable that Minerva and all genotypes with Minerva background (1351, 1352, 1356 and Lami) are found in the first group.

No correlation was found between the viscosity of the acid flour extract and the amount of insoluble β-glucan in the flour (column 2 vs 5 of Table II) even if the genotypes are divided into two groups on the basis of their content of insoluble β-glucan. The first group consists of the four genotypes with the lys-3a allele having 1.9 to 2.4% insoluble β-glucan. The 14 other genotypes have 3.1 to 4.7% insoluble β-glucan.

3.3. Viscosity of the acid flour extract related to the amount of flour

Given the excellent correlation between the ln(extract viscosity) and the soluble β-glucan content of the flour it is expected that varying the amount of flour extracted per unit of buffer should yield similar correlation of the latter with the ln(extract viscosity). Results of such an experiment are shown in Figure 4 for the three genotypes Minerva, Zephyr and Triumph whose

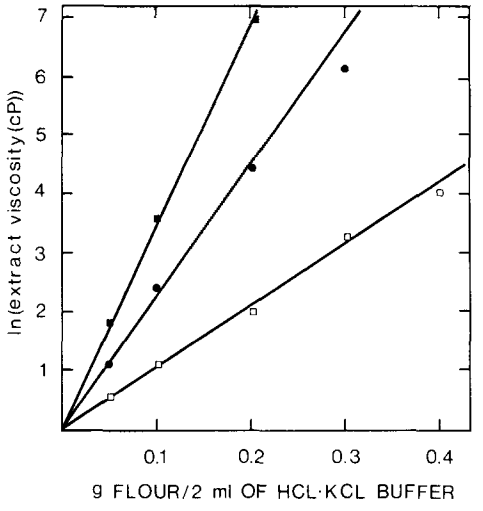

Figure 4. The logarithmic relationship between the amount of flour and the viscosity of the acid flour extracts from three genotypes.
■ = Minerva, ● = Zephyr, □ Triumph.

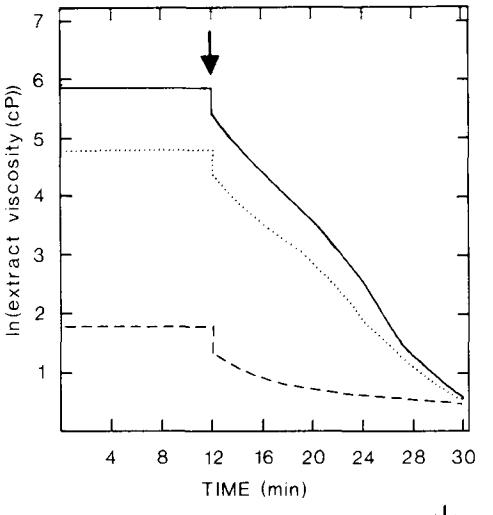

Figure 5. Addition of 10 µl of pure β-glucanase (↓) to 400 µl of the maleate extract results in a tremendous decrease in viscosity within 18 min. —— = Minerva, ····· = Lami, – – – = Triumph.

acid flour extracts represent the range of studied viscosities (Table II). The ln(extract viscosity) is linearly related to the amount of the flour extracted, but only up to a certain weight. This limiting weight of the flour is considerably lower (approx. 0.2 g) for the high viscosity genotype Minerva than it is for the low viscosity genotype Triumph (approx. 0.4 g). These limitations are presumably due to the ability of the buffer to extract the flour, the restrictions imposed by the viscometer and/or that the interactions between molecular chains of the β-glucan become so great that the viscosity becomes non-Newtonian.

3.4. Viscosity of the acid flour extract attributable to the β-glucans

The correlation observed between the extract viscosity and the soluble β-glucan intimates, as has often been assumed (7, 8, 9, 15, 29, 36), that the extract viscosity is due to the β-glucan. If this is true then treatment of the flour extract with the β-glucanase (see section 2.6) should result in a marked decrease in viscosity. Three varieties; Triumph, having a rather low extract viscosity, and Lami and Minerva, with the two highest extract viscosities, were chosen for investigation. A different extraction procedure employing a maleate buffer (pH 6.5) was used. The viscosity of this extract remained stable for at least two hours. In all cases addition of the enzyme to the extract resulted in an immediate drop in viscosity (Figure 5). Approx. the same very low viscosity of 1.7 to 1.9 cP was reached by all three samples within 18 min. The viscosity of the blank was 1.0 cP, indicating that 99.5, 98.5 and 70.0% of original viscosity of the maleate flour extract was due to β-glucan in Minerva, Lami and Triumph, respectively.

3.5. The non-β-glucan constitutents of the acid flour extract

In Table III the results of the analysis to determine the amount of non-β-glucan comprising carbohydrates, protein, fats and ash are presented together with the β-glucan contents. The same three representative genotypes Minerva, Lami and Triumph were investigated. Examination of the results reveals that the total amount of none of the other measured constituents within the acid flour extract can be directly correlated with the viscosity measurements. The most interesting observations concern the carbohydrates. Whereas Lami and Triumph have the same amount of total extractable carbohydrates (4.6

Table III.

Main constituents of the acid flour extracts from three of the barley varieties[a] analyzed in Table II.

Variety	β-glucan mg · ml^{-1}	Total carbohydrate mg · ml^{-1}	Protein mg · ml^{-1}	Fat mg · ml^{-1}	Ash + buffer salt mg · ml^{-1}
Triumph	0.63 ± 0.03	4.55 ± 0.14	2.51 ± 0.05	0.36 ± 0.02	2.40 ± 0.08
Lami	1.75 ± 0.07	4.60 ± 0.14	2.78 ± 0.05	0.44 ± 0.02	2.43 ± 0.08
Minerva	2.21 ± 0.09	5.41 ± 0.16	3.47 ± 0.07	0.34 ± 0.02	2.43 ± 0.08

[a] Methods used in these analyses are described in section 2.

mg · ml^{-1}), Lami has approx. three times as much β-glucan as Triumph. In Minerva the increased amount of extractable β-glucan is paralleled by an increase in the total extractable carbohydrates to 5.4 mg · ml^{-1} so that the β-glucan as in Lami accounts for approx. 40% of the carbohydrates.

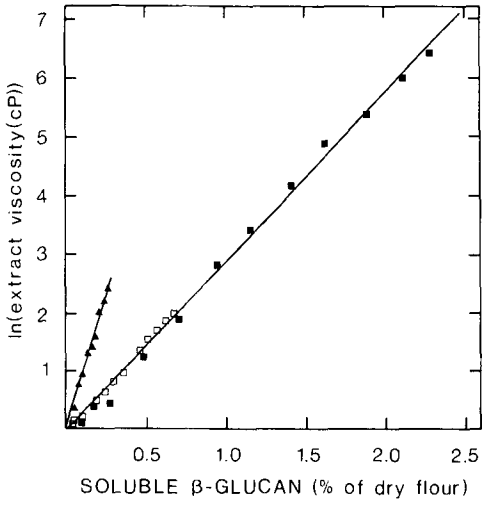

Figure 6. An apparently similar biochemical nature of the soluble β-glucans in the 18 barley genotypes is suggested by comparing the logarithmic relationship between the viscosity and the soluble β-glucan content of the 18 genotypes (solid line, from Figure 2) and the logarithmic relationship between the viscosity and the soluble β-glucan content obtained from dilution curves of the two acid flour extracts from Minerva (■) and Triumph (□).

Included as a control is the logarithmic relationship between the soluble β-glucans obtained from a dilution series of an acid flour extract prepared from the oat variety Astor (▲——▲).

3.6. Dilution studies

Dilution series were prepared from the acid flour extracts of Minerva, Lami, Zephyr, Triumph and the oat variety Astor, and the extract viscosities were measured. The ln of the resulting viscosities were plotted against the calculated β-glucan contents to yield dilution curves. Figure 6 shows the results for Minerva, Triumph and Astor. No significant difference between the dilution curves from the four barley genotypes and the genotype-curve obtained by relating the viscosities and the β-glucan contents of the 18 barley genotypes (Figure 2) can be seen. Intrinsic viscosities (21) were also calculated for the investigated genotypes with the following results: Minerva = 21.3 dl · g^{-1}, Lami = 24.0 dl · g^{-1}, Zephyr = 18.0 dl · g^{-1}, Triumph = 24.7 dl · g^{-1} and Astor 80 dl · g^{-1}. The correlation coefficient (R^2) of the curves from which the intrinsic viscosities are extrapolated were in all cases between 0.96 and 0.98. When calculating the intrinsic viscosity from the results obtained for all 18 genotypes, however, it was necessary to delete the four lys-3a allele containing genotypes in order to obtain an R^2 = 0.96 for an intrinsic viscosity of 19.5 dl · g^{-1}. If the four genotypes containing the lys-3a allele were included a value of 29.4 dl · g^{-1} was obtained but the correlation coefficient was poor (R^2 = 0.80).

3.7. Single grain determinations

Single grains > 2.5 mm in diameter were used for the viscosity determinations, because deleting the small grains gave less interkernel error. In Table IV the results are presented from the analysis of 15 or 20 grains of four genotypes selected to represent the range of viscosities

Table IV.

The viscosities from single grain determinations of four barley genotypes are compared to those obtained using 0.2 g of flour.

Genotypes	Single grains			0.2 g flour[b]
	Number	Range (cP)	Mean[a] (cP)	(cP)
1392	15	1.0 – 1.7	1.2	1.81
Nirasaki	20	1.7 – 5.5	3.5	2.75
1352	15	40 – 68	55	96.7
Minerva	20	96 – 230	130	632

a) The mean was calculated as $e^{\left(\frac{\ln x_1 + \ln x_2 + \ldots + \ln x_n}{n}\right)}$
where n is the number of grains and $x_1 \ldots\ldots x_n$ are the values of the viscosity measurements.
b) Data from Table II.

studied at the 0.2 g level. The observed mean values of the single grain determinations have the same relative order as when 0.2 g of flour was used. The observed lower viscosities in three cases are those expected because of the decreased flour to buffer ratio (see section 3.3). In the fourth case, Nirasaki, the result is slightly higher than expected.

4. DISCUSSION

4.1. Relationship between the extract viscosity and the β-glucans in the flour

The primary objective of the present investigation was to develop a quick method to estimate accurately the β-glucan content in flour from individual barley plants and individual barley grains. Since direct measurements of β-glucans are laborious (3, 19), GREENBERG and WHITMORE (29) had approached this question by assaying the viscosity of an extract prepared from 2 g of flour at 40 °C, pH 1.5. Surprisingly, given the often reported observation that viscosity of barley flour extracts are highly dependent on the environmental conditions in which the plants were grown (e.g. 46), GREENBERG and WHITMORE (29) and GREENBERG (28) were able to correlate their viscosity measurements with previously published data of BOURNE and PIERCE (9) on the content of β-glucan extracted at 65 °C from the same varieties grown another year. BENDELOW (7) also used viscometric analysis to estimate β-glucan contents. His flour extract was prepared using α-amylase, papain and TCA at 50 °C. However, when ANDERSON et al. (3) subsequently estimated the soluble and total β-glucans by a direct enzymatic procedure in the same samples, no correlations were observed; and PALMER (39) states in the light of an investigation of extracts from Julia and Proctor that the viscosity of a barley extract may not be an accurate measure of the concentration of the gum material it may contain.

The conflicting results obtained by these and other workers (52, review) are not unexpected given our present limited knowledge of β-glucan structure and cellular distribution combined with the wide diversity of methods used to assay β-glucans (1, 3, 7, 9, 19, 29). In the present study an acid extract (pH 1.5) of the flour was used since the viscosity of such extracts remain stable with time (29). In parallel with measurements of the viscosity of the extracts, the amount of β-glucan in the flour, in the extract and in several cases also those remaining in the flour after extraction were directly determined using modifications of the enzymic β-glucan assay of ANDERSON et al. (3). The total β-glucan content in the flour was found to represent 2.00 to 6.36% of the dry weight which is comparable to other reported values: 1.9–7.4% (7) and 3.77–6.44% (3) using various techniques. The viscosity measurements, made for 18 barley genotypes grown on a single occasion at the same place,

ranged from 1.81 to 632 cP. Thus, it was possible to demonstrate that the ln(extract viscosity) divided by three was approx. equal to the % of β-glucan in the extract referred to as the soluble β-glucan. Furthermore, since an apparently random two-fold variation in the amount of non-extractable β-glucan in the flour, i.e., insoluble β-glucan, was found for the 18 genotypes, the correlation between the total β-glucan in the flour and the viscosity of the respective acid flour extract was less strong.

4.2. Properties of the acid flour extract

4.2.1. Non-β-glucans

ANDERSON et al. (3) have pointed out that other constituents of barley extracts might contribute to the viscosity. To determine what proportion of the total viscosity was due to β-glucan, maleate flour extracts were treated with the specific 1,3:1,4 β-glucan endo-β-glucanase. The viscosities of the maleate flour extracts were 10 to 17% less than those of the respective acid extracts prepared from the same flour. The lower viscosities may be due to an alteration of the β-glucan by the ethanol as has been reported by POLYAKOV et al. (40). Within 18 min of the addition of the β-glucanase, the viscosity was reduced to less than two cP which represents 30% of the starting viscosity in the extract of a low-viscosity genotype and as little as 0.5% of the starting viscosity in the extract of a high-viscosity genotype. This result indicates that maleate extractable flour constituents other than β-glucan contribute relatively little to the viscosity. Since the exact mechanism of the acid and maleate extractions is not understood, it can not be stated definitely from this experiment that in the acid flour extract substances other than β-glucan contribute little to the viscosity. Such a conclusion seems reasonable, however, given the specificity of the enzyme and the fact that the initial viscosities of the acid and maleate extracts were of the same order of magnitude. Additional support for this conclusion comes from the quantification of the primary non-β-glucan constituents of the acid flour extract. The amounts of these soluble components did not correlate with the viscosity measurements. Furthermore, the results are in agreement with SMITH et al. (47) who analysed an acid flour extract (35) prepared essentially as described by GREENBERG and WHITMORE (29). Most interestingly, the total amount of carbohydrates in Triumph and Lami extracts was the same, but 14% was β-glucan in the former and 38% in the latter. As discussed in the next section the soluble β-glucans of Lami and Triumph are of similar if not identical molecular structure. Since the difference in amount of β-glucan in the two genotypes corresponded to the measured viscosity differences, one can conclude that at least 24% of the acid soluble non-β-glucan carbohydrate in Triumph does not contribute at all to the viscosity of the extract.

4.2.2. Structural nature of the β-glucans

The question can be asked whether or not the excellent correlation between the viscosity and the β-glucan content of the acid flour extracts indicates that the soluble β-glucans in all 18 investigated genotypes have the same structure with regard to their molecular weight distribution as well as their 1,3 and 1,4 linkage distribution. If this is true the intrinsic viscosity of all investigated genotypes should be similar to one another and to the intrinsic viscosity calculated from the data relating the β-glucan contents to the extract viscosities of the 18 genotypes. This was indeed the case for Minerva, Lami, Triumph and Zephyr providing the four lys-3a allele containing genotypes were not included when calculating the intrinsic viscosity from all the investigated genotypes.

The intrinsic viscosity calculated from a dilution series prepared from an acid flour extract of the oat variety Astor was significantly greater than all the barley intrinsic viscosities. The present work supports the conclusion of WOOD et al. (54) that the soluble β-glucans of oats are structurally different from the barley β-glucans, perhaps in having a higher molecular weight. It should be noted that WOOD et al. made their comparison using alkaline extracts whereas the present analysis used an acid extract. Unfortunately no information is presently available to demonstrate how modifications, other than in molecular weight range (54) affect the intrinsic viscosity of β-glucans. Furthermore, whether similar intrinsic viscosity measurements reflect the in vivo β-glucan structure or is an artifact brought about by the extraction

procedure cannot be decided at present. However, the result is in agreement with SPARROW and MEREDITH (48), who found that gum material extracted from different varieties was similar in structure. Thus, one can only tentatively conclude that whereas no structural differences in the β-glucans of the acid flour extracts from the fourteen investigated genotypes have been detected by the present methodology, there is a high probability that the β-glucans in the four lys-3a allele containing genotypes have a different structure.

4.2.3. Extraction temperatures

As the extraction temperature was raised from 15 to 50 °C there was an increase in extract viscosity for 14 of the investigated genotypes ranging from 3.5 to 20 cP for a low-viscosity type (Triumph) and from 123 to 450 cP in a high-viscosity type (Minerva). This presumably reflects, as others (20, 40, 54) have concluded, the increasing solubility of the β-glucans as the temperature rises.

POLYAKOV et al. (40) have shown that the β-glucan concentration of an aqueous barley extract is linearly related to the extraction temperature over the range from 5 to 45 °C. In the present study an approx. linear relationship was found between the ln(extract viscosity) and the extraction temperature as it increased from 15 to 40 °C. Taken together, these results suggest that in any one of the 14 given genotypes the β-glucans extracted at 15, 20 and 30 °C are of the same structural nature as those extracted at 40 °C. If this is true then one can estimate, using the formula derived in section *3.2.1.* that for a low-viscosity genotype such as Triumph an increase in temperature from 15 to 40 °C results in a 83% increase in soluble β-glucan. For a high-viscosity genotype such as Minerva the soluble β-glucan is only increased by 23%.

The decrease in viscosities of the 14 acid flour extracts when the temperatures were increased above 50 °C may be due to non-enzymatic degradation of the β-glucans to which the low pH of the extract contributes. Heat lability of pure β-glucan has been shown by MORGAN (37) who found that heating β-glucan from room temperature to 85 °C gave solutions of greatly reduced viscosities compared to solutions made from untreated β-glucan. On the other hand, FORREST and WAINRIGHT (23) showed that heating a solution of β-glucans isolated from barley endosperm cell walls to 100 °C did not disassociate the molecules.

The effect of the extraction temperature on the viscosity of the extract was markedly different for the four genotypes containing the lys-3a allele. Practically no increase in viscosity of the extracts was detected as the temperature increased from 15 to 50 °C, but thereafter an increase did occur. The question can be asked, why these four genotypes differ from the other 14. A number of possibilities come to mind. Is it due to a different chemical nature of the β-glucans in these four genotypes as suggested above? Is it due to the fact that the observed increase and decrease in viscosities as the extraction temperature was increased is not really a result of increased solubility and degradation of the β-glucans in the 14 genotypes? Is it related to the observation that these four genotypes also differed from the others by having a smaller amount of insoluble β-glucan at room temperature? That is, are these four genotypes missing β-glucans bound in such a way that they can be released by increasing temperature? Certainly further investigations are required to determine whether one or more of these possibilities are responsible for the observed differences.

4.3. Sensitivity of the technique

One of the important observations made in this study was that varying the extraction temperature from 15 to 50 °C did not affect the ranking of the genotypes with respect to their extract viscosities. The soluble β-glucan content, however, increased as the temperature did as discussed above. This means that in this aspect the present method is less sensitive than that of GREENBERG and WHITMORE (29) who worked at 40 °C. To illustrate, increasing the temperature from 20 to 40 °C increased the measured soluble β-glucan of Triumph from 0.46 to 0.75% and those of Minerva from 1.71 to 1.97%. This corresponds to increases in viscosity of 4.0 to 9.5 cP for Triumph and 169 to 370 cP for Minerva. The lower sensitivity arising from the use of a lower temperature is more than counterbalanced by the five-fold increase in the ratio of flour to

buffer employed in the present method. The resulting [ln(extract viscosity)/3]-fold (see section *3.2.1*) increase in the viscosity measurements can be illustrated by expanding the above example. The lower amount of flour used by GREENBERG and WHITMORE (29) at 40 °C would give viscosities of 1.6 cP for Triumph and 3.3 cP for Minerva. These values are certainly more difficult to discriminate between than the 4.0 and 169 cP of the present analysis at 20 °C.

The logarithmic nature of the relationship between viscosity and concentration of β-glucans shown in this study is in agreement with the results of several authors, e.g. BOURNE et al. (8), who related concentrations of pure β-glucan solutions to their viscosity . The high correlation of the viscosity of the extracts to the soluble β-glucans reported herein, makes the viscosity measurement suitable for a screening program in a plant breeding effort to reduce the content of soluble β-glucan. By choosing in a given variety the optimal ratio of flour to buffer and the temperature of the extraction, a small increase in β-glucan content can be made to give rise to a large increase in viscosity. In this way it will be possible to select for and discriminate among the low viscosity genotypes. In practice an initial selection against the high viscosity genotypes can be made without actually measuring the viscosity by simply turning the tube (see 2.3) containing the extract upside down to detect the extremely viscous samples.

When the viscosity measurements are made starting with 0.2 g flour, 150 determinations are possible in a day. The capacity of the method is reduced to 75 determinations per day when the analysis is carried out on single grains. Nevertheless, this capacity has proved adequate when examining F_2 grains in a study of the inheritance of high versus low viscosity (S. AASTRUP: Inheritance of mixed-linked β-glucan content in barley grains (in preparation)). The method can also be extended to the non-destructive selection of F_2 grains by using only half a grain for the viscosity measurement, and then permitting the embryo half to germinate.

These investigations explain the relationships between the viscosity of the acid flour extract and the total and soluble β-glucan contents of the flour. Further studies are needed to determine these relationships in malting and feeding experiments and to relate them to the present observations. It is generally considered that β-glucans giving rise to high viscosity cause difficulties in the brewing process and in feeding of monogastic animals (5, 10, 15, 22, 26, 27, 32, 52). A low content of soluble β-glucans in the grains can therefore be expected to remove one source of the problem. To overcome the other source, namely solubilization of water insoluble glucans during malting, for example, requires a different approach.

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REFERENCES

1. ALLISON, M. J., I. A. COWE & R. MCHALE: The use of infra red reflectance for the rapid estimation of the soluble β-glucan content of barley. J. Inst. Brew. 84, 153–155 (1978)
2. ANDERSON, R. L., M. A. ANDERSON & B. A. STONE: Some structural enzymatical aspects of non-starchy polysaccharides in cereal grains. Inst. Brew. Aust. N.Z. Sec. Proc. 14th conv. 153–160 (1976)

3. ANDERSON, M. A., J. A. COOK & B. A. STONE: Enzymatic determination of 1,3:1,4 β-glucans in barley grains and other cereals. J. Inst. Brew. 84, 233–239 (1978)
4. ASPINAL, G. O. & C. T. GREENWOOD: Aspects of the chemistry of cereal polysaccharides. J. Inst. Brew. 68, 167–177 (1962)
5. BARRETT, J., J. F. CLAPPERTON, D. M. DIVERS & H. RENNIE: Factors affecting wort separation. J. Inst. Brew. 79, 407–413 (1973)
6. BATHGATE, G. N. & C. E. DALGLIESH: The diversity of barley and malt beta-glucans. ASBC Proceedings 33, 32–36 (1975)
7. BENDELOW, V. M.: Determination of non-starch polysaccharides in barley breeding programmes. J. Inst. Brew. 81, 127–130 (1975)
8. BOURNE, D. T., M. JONES & J. S. PIERCE: β-glucan and β-glucanase in malting and brewing. MBAA Technical Quarterly 13, 3–7 (1976)
9. BOURNE, D. T. & J. S. PIERCE: β-glucan and β-glucanase in brewing. J. Inst. Brew. 76, 328–335 (1970)
10. BURNETT, G. S.: Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. British Poultry Science 7, 55–75 (1966)
11. CLARKE, A. E. & B. A. STONE: Chemistry and biochemistry of 1-3-glucans. Rev. Pure Appl. Chem. 13, 134–156 (1963)
12. DALGLIESH, C. E. & J. R. HUDSON: Research Foundation Report. J. Inst. Brew. 84, 61–62 (1978)
13. DJURTOFT, R. & K. L. RASMUSSEN: Studies on barley gum preparations including determination of the molecular weight. EBC Proc. 17, 17–25 (1955)
14. DUBOIS, M., K. GILLES, J. K. HAMILTON, P. A. REBERS & F. SMITH: Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356 (1956)
15. ERDAL, K. & P. GJERTSEN: Beta-glucans in malting and brewing II. The fate of beta-glucans during mashing. EBC Proc. 11th congr. 295–302 (1967)
16. ERDAL, K. & P. GJERTSEN: Beta-glucans in malting and brewing III. The action of endo-beta-glucanases. EBC Proc. 13th congr. 49–57 (1971)
17. EWERTSON, G.: Protein content and grain quality relations in barley. Agri Hortique Genetica, bandXXXV, 58–61 (1977)
18. FINCHER, G. B.: Morphology and chemical composition of barley endosperm cell walls. J. Inst. Brew. 81, 116–122 (1975)
19. FLEMMING, M., D. J. MANNERS, R. M. JACKSON & S. C. COOKE: The estimation of β-glucans in barley. J. Inst. Brew. 80, 399–404 (1974)
20. FLEMMING, M. & K. KAWAKAMI: Studies of the fine structure of β-D-glucans of barley extracted at different temperatures. Carbohydrate Research 57, 15–23 (1977)
21. FLORY, P. J.: Principles of polymer chemistry. VII-4a, pp. 308–311, Cornell University Press, Ithaca, New York (1953)
22. FORREST, I. S. & T. WAINWRIGHT: Differentiation between desirable and troublesome β-glucans. Eur. Brew. Conv. Congr. pp. 401–412 (1977)
23. FORREST, I. S. & T. WAINWRIGHT: The mode of binding of β-glucans and pentosans in barley endosperm cell walls. J. Inst. Brew. 83, 279–286 (1977)
24. GJERTSEN, P.: Filtration of beer and factors influencing its filterability. Brew. Dig. XLV, 68–80 (1970)
25. GJERTSEN, P.: Beta-glucans in malting and brewing. I. Influence of beta-glucans on the filtration of strong beers. ASBC. Proc. 113–120 (1966)
26. GOHL, B., S. ALDEN, K. ELWINGER & S. THOMKE: Influence of β-glucanase on the feed value of barley for poultry and moisture content of excreta. Br. Poult. Sci. 19, 41–47 (1978)
27. GOHL, B. & I. GOHL: The effect of viscous substances on the transit time of barley digesta in rats. J. Sci. Food. Agric. 28, 991–1006 (1977)
28. GREENBERG, D. C.: β-glucan and extract viscosity. J. Inst. Brew. 80, 435 (1974)
29. GREENBERG, D. C. & E. T. WHITMORE: A rapid method for estimating the viscosity of barley extracts. J. Inst. Brew. 80, 31–33 (1974)
30. JENSEN, J. & H. DOLL: Gene symbols for barley high-lysine mutants. Barley Genetics Newsletter 9, 33–37 (1979)
31. KOSZYK, P. F. & M. J. LEWIS: Unmalted grains as malters' adjuvant and brewers' adjunct. J. Am. Soc. Brew. Chem. 37, 77–81 (1977)
32. MEILGAARD, M. C.: Wort composition: With special reference to the use of adjuncts. MBAA Technical Quarterly 13, 78–90 (1976)
33. MEREDITH, W. O. S.: The significance of malt analysis in industry and plant breeding. Inst. Brew. Aust. N.Z. Sec. Proc. 13th Conv. 29–40 (1974)
34. MORRALL, P. & D. E. BRIGGS: Changes in cell wall polysaccharides of germinating barley grains. Phytochemistry. 17, 1495–1502 (1978)
35. MORGAN, A. G.: The relationship between barley extract viscosity curves and malting ability. J. Inst. Brew. 83, 231–234 (1977)

36. MORGAN, A. H. & P. G. GOTHARD: A rapid, simple viscometric technique for indirect estimation of soluble β-glucan content of raw barley. J. Inst. Brew. 83, 37–38 (1977)
37. MORGAN, K.: Heat lability of barley β-glucan. J. Inst. Brew. 77, 509 (1971)
38. MUNCK, L.: High lysine barley – a summary of the present research development in Sweden. Barley Genetics Newsletter 2, 54–59 (1972)
39. PALMER, G. H.: Influence of endosperm structure on extract development. ASBC Proc. 33, 174–180 (1975)
40. POLYAKOV, V. A., V. N. UGRYUMOVA & I. YA. VESELOV: Determination of β-glucan content in barley and malt. Fermentn. Spirt. Prom-st. 7, 38–39 (1976)
41. PREECE, I. A. & K. G. MACKENZIE: Non-starchy polysaccharides of cereal grains I. Fractionation of the barley gums. J. Inst. Brew. 58, 353–362 (1952)
42. PREECE, I. A. & K. G. MACKENZIE: Non-starchy polysaccharides of cereal grains II. Distribution of water-soluble gum-like materials in cereals. J. Inst. Brew. 58, 457–464 (1952)
43.. SCOTT, R. W.: The viscosity of worts in relation to their contents of β-glucans. J. Inst. Brew. 78, 179–186 (1972)
44. SCHUSTER, K., L. NARZISS & J. KUMADA: Über die Gummistoffe der Gerste und ihr Verhalten während der Malz- und Bierbereitung. I. Problemstellung und Analysemetoden. Brauwissenschaft 20, 125–135 (1967)
45. SCHULSTER, K., L. NAZISS & J. KUMADA: Über die Gummistoffe der Gerste und ihr Verhalten während der Malz- und Bierbereitung. II. Gummistoffe in der Gerste und ihre Veränderungen beim Mälzen und Maishen. Brauwissenschaft 20, 185–206 (1967)
46. SMART, J. G.: The β-glucan content of New Zealand barley. Inst. Brew. Aust. N.Z. sec. Proc. 14th Conv. 161–168 (1976)
47. SMITH, D. B., A. G. MORGAN & A. A. GILL: The major biochemical constituents of an acid flour extract of barley flour as used in viscosity determinations by the falling ball method. J. Inst. Brew. (in press) (1979)
48. SPARROW, D. H. G. & W. O. S. MEREDITH: Malt cytolytic activity of barley of diverse origins and its relation to maltability. J. Inst. Brew. 75, 237–242 (1969)
49. TANFORD, C.: Physical chemistry of macromolecules. Chap. 6, 19f, pp. 328–329, John Wiley & sons, inc., New York, London (1961)
50. THOMPSON, R. G. & D. E. LABERGE: Barley endosperm cell walls: A review of cell wall polysaccharides and cell wall-degrading enzymes. MBAA Technical Quarterly 14, 238–243 (1977)
51. WAINWRIGHT, T.: Barley analysis and malt quality. MBAA Technical Quarterly 15, 184–189 (1978)
52. WAINWRIGHT, T. & G. K. BUCKEE: Barley and malt analysis – a review. J. Inst. Brew. 83, 325–347 (1977)
53. WOOD, P. J., D. PATON & I. R. SIDDIQUI: Determination of β-glucan in oats and barley. Cereal Chem. 54, 524–533 (1977)
54. WOOD, P. J., I. R. SIDDIQUI & D. PATON: Extraction of high-viscosity gums from oats. Cereal Chem. 55, 1038–1049 (1978)
55. YANG, J. T.: Adv. Protein Chem. 16, IIB, pp. 328–239, Academic Press, New York, London (1961)