DISTRIBUTION OF CARBOHYDRATES IN EARLY HARVESTED BARLEY GRAIN

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ABSTRACT

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Layers of early-harvested barley grain (65% dry matter at harvest, immediately dried afterwards) were successively removed by abrasive milling. Fractions collected after about 25 to 55% of the kernel weight had been abraded gave viscous water suspensions. A maximum in viscosity was found in the fraction where 42–52% of the kernel weight was removed. The fractions removed were extracted with 80% ethanol followed by extraction with water. The extracts and the residues after water extraction were analyzed for carbohydrates. The ethanol extracts contained mostly sucrose together with stachyose, raffinose, glucose, fructose, glucitol, and myoinositol. Trace amounts of xylose were found in the fractions making up the first 25% of the material abraded. Acid hydrolysates of the water extracts contained mostly glucose, together with galactose, mannose, rhamnose, arabinose, and xylose. Hydrolysis of the extraction residues yielded mainly glucose, except in the outer layers where galactose, arabinose, and xylose were identified. The distributions of crude protein, lignin, crude fat, and ash were also determined.

In feeding experiments with broilers, Thomke (1) showed that barley harvested at yellow ripeness had a lower feeding value than barley harvested at combine-harvesting ripeness. No differences in the proximate composition of the barley harvested at the two stages of ripeness could be demonstrated. Water extracts and water suspensions showed that early-harvested barley had a much higher viscosity than the late-harvested barley. It has been known for some time that viscous barley has a lower nutritive value than nonviscous barley when given to poultry (2). The viscosity is generally attributed to a soluble β-glucan (3,4), but its location in the kernel was not known. The aim of the present investigation was to demonstrate the location of the viscous factor in barley grain, and to investigate whether factors other than β-glucans may be involved.

MATERIAL AND METHODS

Material

The barley used was a two-row spring barley of the cultivar Ingrid harvested at yellow ripeness (65% dry matter). It was dried immeidately after harvest. In an earlier report (1) this barley was denoted as U-69.

Milling

The barley grain was abraded in a Scott-Strong Seedburo mill in 30-g portions. At predetermined time intervals, the abraded material was removed and collected for analysis. The percentage of each fraction is given in Table I. The abrasion is not concentric, as the tips of the grain are more quickly abraded than the body.

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Chemical Analyses

Crude protein, crude fat, ash, and starch were determined according to AOAC (5). Lignin and nonextractable carbohydrates were determined according to Sjöström *et al.* (6).

Viscosity Determinations

Viscosity was measured in an Eprecht Rheomat 15, using system "B" at pH 3.0 and 35°C. The viscosity was measured in a suspension of 20 g of the abraded material mixed with 70 ml 0.1*M* phosphate-citrate buffer. Care was taken to measure the viscosity at the same time interval (5 min) after mixing the meal with the buffer.

Extractions

The defatted, abraded fractions were extracted four times with 200, 150, 100, and 50 ml, respectively, of boiling 80% ethanol for 10 min with centrifugation between each extraction. The extracts from each fraction were pooled and evaporated to dryness by vacuum drying. The residues after extraction with ethanol were extracted four times with 40 ml water in an ultrasonic bath for 20 min with centrifugation between each extraction. Extraction with water gave the same results as extraction with 0.1M phosphate-citrate buffer, pH 7.0. The combined extracts from each fraction were freeze-dried and stored at +4°C until further treatment. The residues were washed and dried with ethanol and ether.

Chromatographic Procedures

Gas-liquid chromatography (glc) was conducted with a Varian Model 2700 instrument, fitted with a flame-ionization detector. Separations were performed

TABLE I
Chemical Composition of Early-Harvested Barley Fractions[a]

% of Kernel Abraded	Crude Protein[b] %	Crude Fat %	Ash %	% EtOH[c]-Extractable Carbohydrates	% Water-Extractable Carbohydrates	% Non-Extractable Carbohydrates	Lignin %	Starch %
0– 6.5	7.1	2.1	7.2	3.0	0.3	39.1	15.5	5.1
6.6– 9.9	15.2	4.9	8.7	3.1	0.6	36.6	6.4	6.8
10.0–23.5	19.8	6.8	5.1	3.2	1.0	41.0	5.1	18.4
23.6–28.6	20.0	6.0	4.4	2.8	1.5	42.3	5.0	33.9
28.7–34.0	19.6	5.1	3.6	2.8	1.7	43.0	4.6	42.8
34.1–38.5	19.5	3.7	2.9	2.3	1.9	46.1	4.1	49.8
38.6–42.4	19.5	2.9	2.5	2.2	1.9	49.2	2.4	52.2
42.5–51.1	18.6	1.9	1.7	1.0	1.8	57.3	1.5	59.0
51.2–58.1	16.5	1.4	1.2	1.1	1.5	61.4	0.8	64.1
58.2–64.9	14.6	1.3	1.0	1.8	1.3	64.5	0.7	65.6
65.0–70.1	13.4	1.4	0.8	1.5	2.6	66.0	0.5	68.3
70.2–74.6	12.5	1.0	0.7	1.4	2.8	66.9	0.5	68.7
74.7–100	9.3	0.6	0.3	1.1	1.4	71.9	0.4	73.2

[a]All figures are given on dry matter basis.
[b]N × 6.25.
[c]80% EtOH:20% water, v/v.

on glass columns (240 × 0.15-cm) containing 3% OV-1 on Varaport 30 (100–120 mesh) at 100°–275°C, 6°/min, N_2 35 ml/min, for the trimethylsilylated derivatives; 3% OV-17 on Chromosorb W (80–100 mesh) at 150°–325°C, 8°/min, N_2 50 ml/min, for quantification of trimethylsilylated stachyose according to Theander and Åman (7); and 3% OV-225 on Gas Chrom Q (100–120 mesh) at 190°C, N_2 25 ml/min, for acetylated derivatives. For quantitative evaluations of the results, obtained by glc, an Autolab Minigrator was used.

The dried ethanol extracts were trimethylsilylated according to Sweeley *et al.* (8). Myoinositol was used as an internal standard. As the original extracts contained myoinositol, a sample with a known amount of myoinositol added was analyzed and the analysis was compared with the analysis of the original extract. The amount of natural myoinositol was calculated by difference. Response factors and silylation yields were measured by silylating known amounts of the different saccharides together with myoinositol.

The water extracts evaporated to dryness were subjected to 1) weak acid hydrolysis, and 2) strong acid hydrolysis, as follows. 1) A 100-mg sample was treated with 10 ml 0.025*N* H_2SO_4 for 1 hr at 100°C. The solution was cooled, neutralized with $BaCO_3$, and filtered. The hydrolysate was evaporated to dryness and silylated and analyzed as above. 2) A 100-mg sample was treated with 10 ml 0.5*N* H_2SO_4 for 12 hr at 100°C. The solution was cooled, neutralized with $BaCO_3$, filtered, reduced with $NaBH_4$, and cationized with ion-exchanger Dowex 50 WX 8 (H+). After evaporation to dryness with methanol three times to

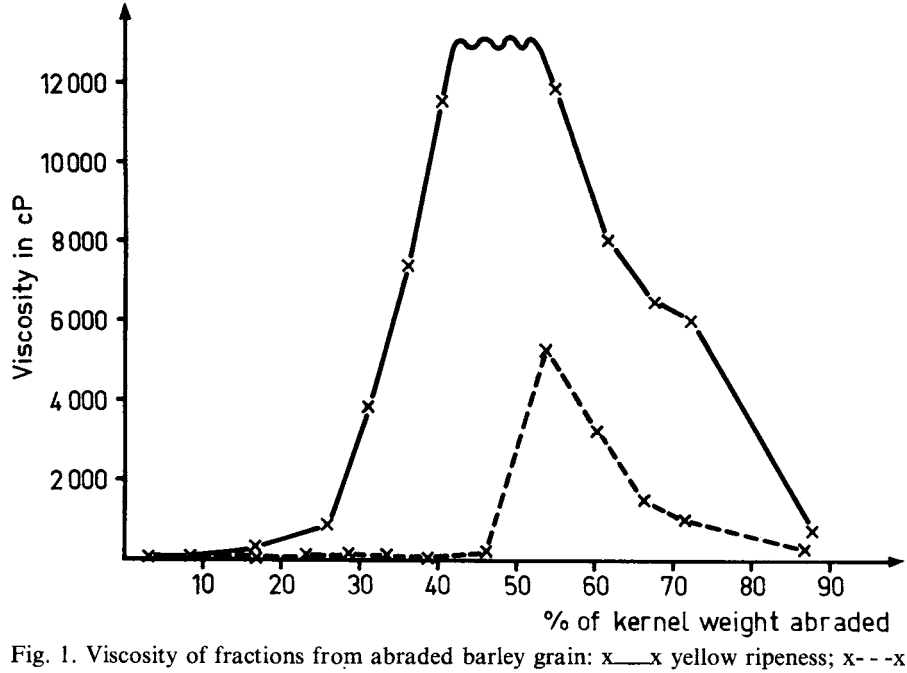

Fig. 1. Viscosity of fractions from abraded barley grain: x___x yellow ripeness; x- - -x combine-harvesting ripeness.

remove boric acid, the alditols were acetylated with acetic anhydride in pyridine and analyzed by glc (9). Myoinositol was used as an internal standard. A 100-mg sample of the solid residue of each fraction remaining after ethanol and water extractions was treated with 72% H_2SO_4 (6), which was successively diluted to 0.358*M*. This solution was refluxed for 6 hr. The hydrolysate was then analyzed for carbohydrates as alditolacetate derivatives as above. Corrections for detector responses and losses during preparation were made according to Bethge *et al.* (10). The remaining solids were dried, quantified, and referred to as lignin (Klason lignin). Paper chromatography (PC) was carried out on Whatman No. 1 paper using ethyl acetate:acetic acid:water (13:1:1, v/v/v) as eluent. Visualization of the spots was performed with *p*-anisidine hydrochloride and silver nitrate-sodium hydroxide.

RESULTS AND DISCUSSION

The concentration of crude protein in the kernel was low in bran and endosperm and higher in the intermediate layers (Table I). The ash was found mainly in the first 15% of the kernel weight abraded, while the concentration of crude fat reached its maximum in the fraction between 10 and 25%. As expected, the maximum concentration of lignin, 15.5%, was found in the outermost layer. The factor causing viscous water suspensions was concentrated in the layers between the bran and the center of the kernel (Fig. 1). For some of the fractions, the viscosity was too high and could not be measured under the conditions used. Viscosity of fractions from the same cultivar harvested at combine-harvesting ripeness later in the same year was measured for comparison (Fig. 1). The outer layers produced extracts with low viscosity.

Ethanol Extract

The total amount of ethanol-extractable carbohydrates decreases from about 3% in the outer layers to about 1% in the center (Table I). The relative distribution of carbohydrates in the ethanol extract is given in Fig. 2. In addition, small amounts of xylose were found in the outer part of the kernel and traces of myoinositol could be detected in all fractions.

Stachyose was found only in the inner part of the kernel. It appeared when ~40% of the kernel had been abraded, and increased toward the center of the kernel. About 10% of the ethanol-extractable carbohydrates from the endosperm is stachyose. Its presence in wheat kernels has been reported previously (11).

Raffinose is present in all fractions in increasing amounts toward the center of the kernel. At the most, 28% of the extracted carbohydrates is raffinose.

Sucrose is the main component in all extracts except those originating from bran. A maximum, where 81% of the carbohydrates is sucrose, was found when slightly more than 30% of the kernel weight had been removed. The relative amount then decreased to 46% in the central part.

Glucose was found in all extracts in relative amounts ranging from 7 to 17% of the carbohydrate content.

Fructose accounts for 23% of the carbohydrates in the bran, and the amount decreases toward the inner part of the kernel.

Glucitol is the only alditol found in the extracts, and its contribution to the

content of low-molecular carbohydrates in the outer layers (~40%) is notably high. To our knowledge, the presence of glucitol in barley has not been reported previously. It is otherwise of widespread occurrence in plants, particularly in fruits and berries. The richest sources are the *Sorbus* and *Crataegus* species.

The presence of free maltose in maturing barley is controversial. Presumably, the amounts are variable and depend on the extent of enzymatic degradation of starch during preparation (12). In the present investigation, maltose could not be detected in any fraction, indicating that complete enzyme deactivation had been

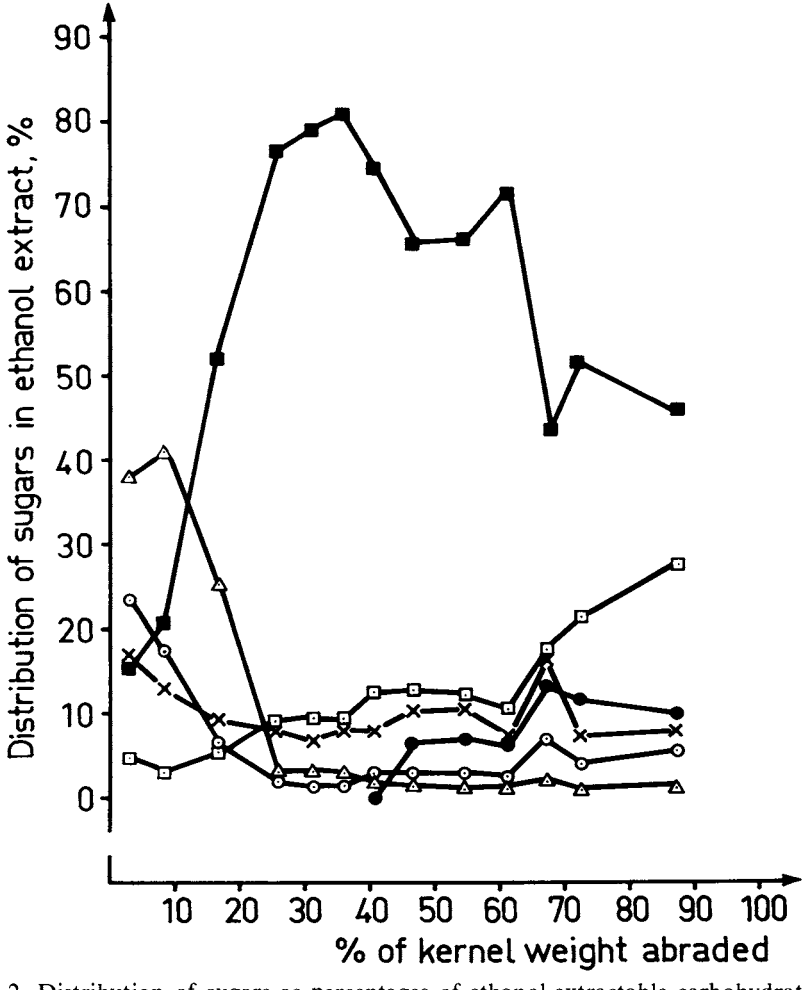

Fig. 2. Distribution of sugars as percentages of ethanol-extractable carbohydrates in fractions abraded from early-harvested barley grain. x—x Glucose; ■—■ sucrose; •—• stachyose; ⊡—⊡ raffinose; ◬—◬ glucitol; ⊙—⊙ fructose. Traces of xylose were found in the first 25% of material abraded and about 1% of inositol was found in all fractions.

achieved by the ethanol treatment. In preliminary experiments, free maltose was found in the inner part of the kernel, but the ethanol treatment immediately after milling inactivated starch-degrading enzymes and prevented maltose formation. Ethanol-soluble fructosans may well be present in the ethanol extracts. The glc-analysis method used does not, however, give any information about such components.

Water Extract

To verify that the low-molecular-weight carbohydrates had been successfully extracted with ethanol, the water extracts were analyzed by glc as above and also

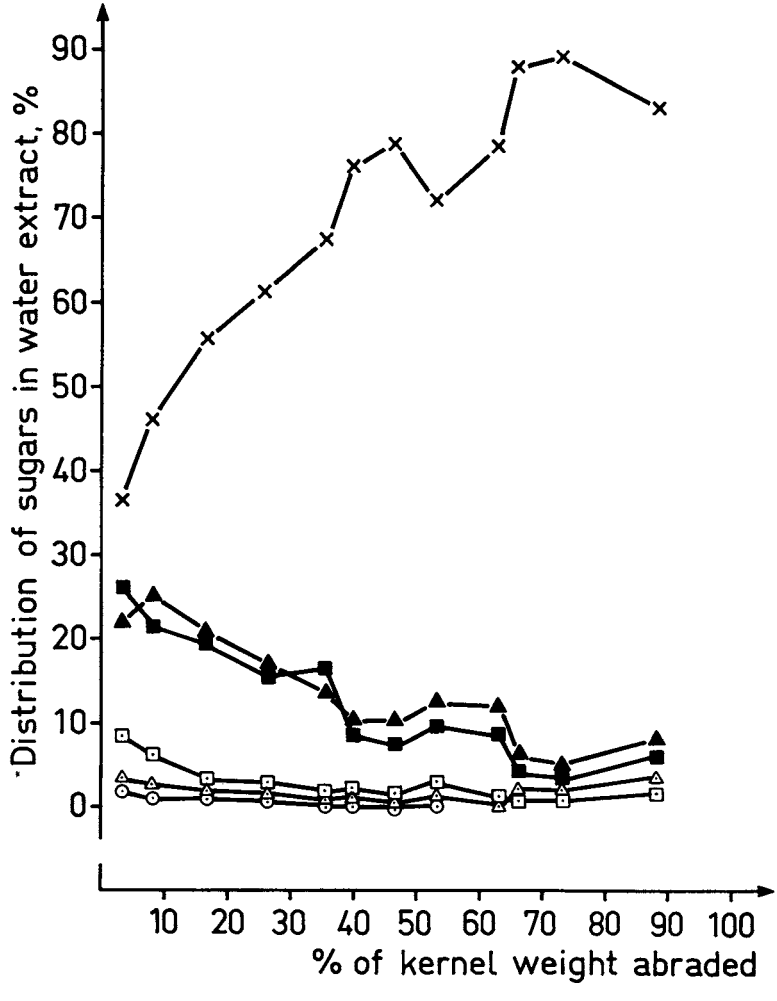

Fig. 3. Distribution of sugars after strong hydrolysis as percentages of water-extractable carbohydrates in fractions abraded from early-harvested barley grain. x—x Glucose; ■—■ arabinose; ▲—▲ xylose; ⊡—⊡ galactose; ◬—◬ mannose; ⊙—⊙ rhamnose.

analyzed by PC. It was confirmed that the extraction of low-molecular saccharides with ethanol was complete. Examination of the weak hydrolysates (0.025*N* H_2SO_4) of water extracts by glc and PC verified a successful ethanol extraction. Under the conditions used, acid-labile furanosidic linkages would be hydrolyzed, and the results show that the water extracts are essentially free from fructosans and other polysaccharides containing such linkages. Under the more

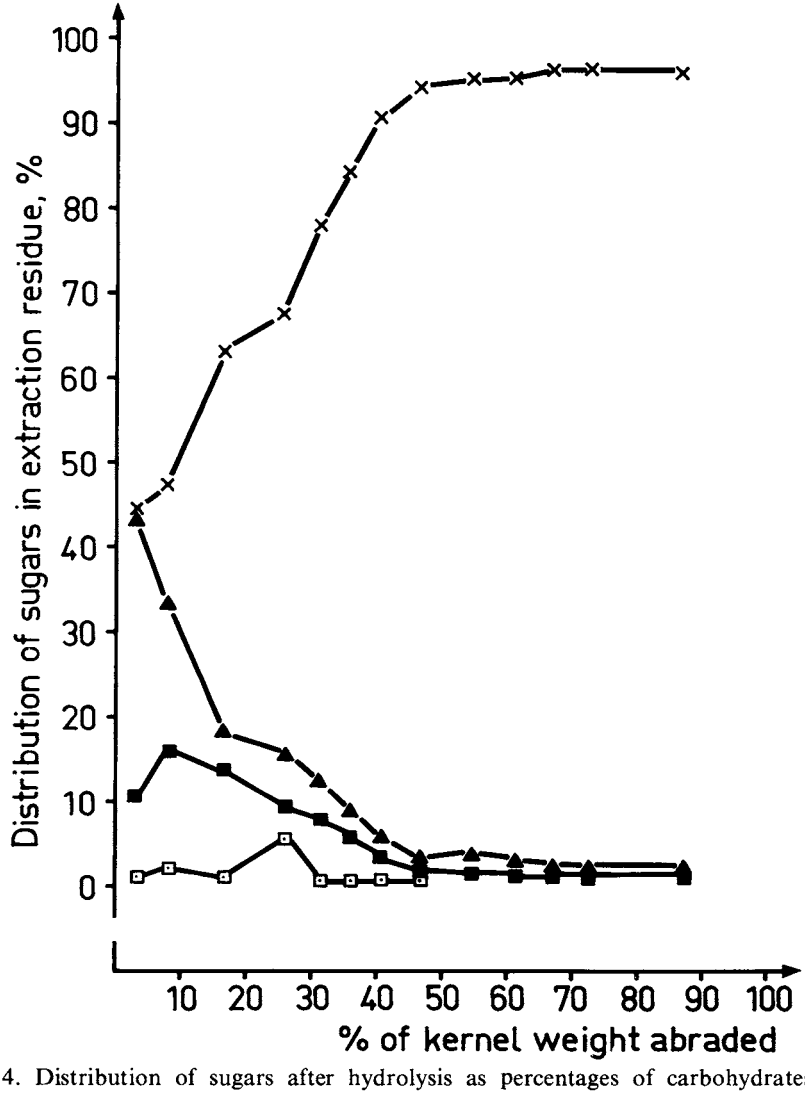

Fig. 4. Distribution of sugars after hydrolysis as percentages of carbohydrates in extraction residues of fractions abraded from early-harvested barley grain. x—x glucose; ■—■ arabinose; ▲—▲ xylose; ⊡—⊡ galactose. Mannose was found in all fractions in amounts of less than 1% after hydrolysis.

drastic hydrolytic conditions, monosaccharides were liberated. As can be seen in Fig. 3, glucose dominates in all fractions in increasing concentrations toward the center of the kernel. Part of the glucose present in the water extract probably originates from starch, but when comparing the starch content in the fractions (Table I) with the distribution of glucose in the water extracts, it can be seen that other glucose-containing polysaccharides may well be present in the water extracts. Appreciable amounts of arabinose and xylose, together with small amounts of mannose, galactose, and rhamnose, were also found.

Water extracts from barley grain contain a mixture of water-soluble polysaccharides and some of them have been prepared in pure form (3). Parrish *et al.* (13) demonstrated the presence of β-glucan composed of (1 → 3) and (1 →4)-linked glucose units. A feature of this polysaccharide is its high viscosity in solution. According to Neukom and Markwalder (14), a soluble arabinoxylan is responsible for the high viscosity of aqueous wheat-flour extracts. Aspinall and Ferrier (15) fractionated some of the barley arabinoxylans after acetylation and suggested that the furanosidic arabinose units are attached to a backbone of pyranosidic xylose units in a linear structure. This is in contrast to our findings, since no arabinose was released during the weak hydrolysis.

Extraction Residue

The results produced by the hydrolysis of the residues after water extraction are shown in Fig. 4. Glucose from starch and cellulose is, of course, the dominating carbohydrate component in the hydrolysates. In the outer region, hemicelluloses yield xylose and arabinose upon hydrolysis. After 50% of the kernel had been abraded, galactose could not be detected in the fractions. By comparing the glucose distribution (Fig. 4) and the starch content, it can be seen that cellulose is present exclusively in the outer regions, together with the hemicelluloses. It is also evident that the conventional method of starch determination by specific rotation measurements of hydrolysate is not sufficiently accurate.

Acknowledgments

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