Glutenin in Developing Wheat Grain[1]

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ABSTRACT

This article describes an examination of the possibility that glutenin might arise during the drying stages of grain ripening as an aggregation product of smaller molecules in the endosperm. Grain of hard red spring, soft white winter, and durum wheats was harvested at 4-day intervals during development. The frozen undried grain was extracted with a dissociating solvent and glutenin, gliadin, albumin, and small molecules were fractionated quantitatively by gel filtration. Glutenin was present at all stages of maturity. The proportion of glutenin was not affected by drying the grain. A low-molecular-weight glutenin appeared for the bread wheats, but not the durum in the late stages of maturity. Its presence appears to be related to baking quality.

Since the early days of cereal chemistry, gluten has been regarded as a mixture of two protein components, gliadin and glutenin. Gliadin has been well characterized, since its lower molecular-weight (MW) range makes it more amenable to common techniques of protein fractionation than glutenin. On the other hand, many aspects of the composition of glutenin are still obscure. Recent studies suggest that glutenin may be a polymer of gliadin, together possibly with other molecules (1,2,3). This concept leads to the further suggestion that glutenin may not exist as such originally in the grain, but that it may form when the grain dries out in the late stages of ripening, or that it is perhaps an artifact of classical extraction and preparation procedures.

Examination of the glutenin content in undried developing grain, using a disaggregating solvent, should test these possibilities. Previous studies of glutenin content in developing grain lack agreement (3–8). Furthermore, they involve conventional extraction of artificially dried grain. In the present study, immature grain was studied before and after drying. To avoid possible protein aggregation during extraction, a strongly dissociating solvent was used which gave complete protein extraction. The proportions of glutenin, gliadin, and albumin were determined on the basis of MW distribution (9).

MATERIALS AND METHODS

Three varieties of wheat were grown in the glasshouse: Manitou (hard red spring), Talbot (soft white winter), and Stewart 63 (amber durum). Heads were labeled at the half-bloom stage and harvested at 4-day intervals from 10 days after flowering to fully ripe. Immediately after harvesting, heads were placed into a cold room at -20°C. Grain was threshed frozen on a small-scale Vogel thresher.

Undried grain was ground in the frozen state and the equivalent of 1.5 g. dry weight was extracted in a Potter and Elvejhem homogenizer for 1 hr. with 26 ml. of the AUC solvent of Meredith and Wren (9) (aqueous solution containing 0.1M

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acetic acid, 3M urea, and 0.01M cetyl trimethylammonium bromide). The mixture was centrifuged for 20 min. at 20,000 X g and the residue re-extracted twice with AUC and finally with 0.1N sodium hydroxide (NaOH). The first AUC extract was used for gel filtration after centrifugation for 30 min. at 100,000 X g.

Gel filtration was performed on a 2.5 X 34-cm. bed of Sephadex G-150 with AUC as solvent. Sephadex G-150 was substituted for G-200 used by Meredith and Wren (9) because it had better flow properties and yet gave a satisfactory resolution. Sample volume was 3 ml. (about 20 mg. protein). By adapting the column for upward flow (10) and restricting the pressure head to about 10 cm., a flow rate of about 25 ml. per hr. could be maintained for a number of runs. Proteins of known MW were used to calibrate the column for MW distribution. The various parts of the elution curve were designated as suggested by Meredith and Wren (9): glutenin, material over 100,000 in MW; gliadin, 25,000 to 100,000; albumin, 10,000 to 25,000; and nonprotein material, less than 10,000. The quantitative distribution of material between these groups was determined by comparing areas under relevant portions of the continuously monitored elution curve and by measuring the absorbancy at 280 nm. of pooled fractions.

Sedimentation velocity experiments were performed at 59,940 r.p.m. in a Spinco model E analytical ultracentrifuge equipped with a phase plate schlieren analyzer and R.T.I.C. unit. A standard 12-mm. Kel-F center piece was used. Measurements were taken from 20 to 60 min. after reaching speed.

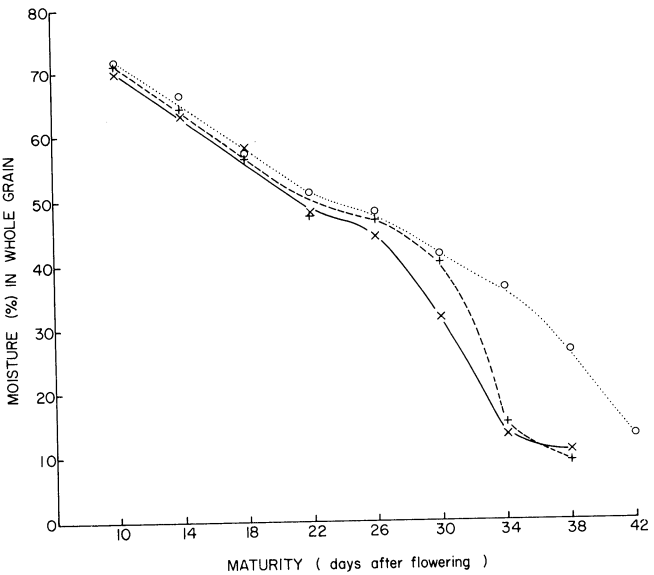

Fig. 1. Moisture content of developing grain for the varieties: solid lines, Manitou; broken lines, Talbot; dotted lines, Stewart 63.

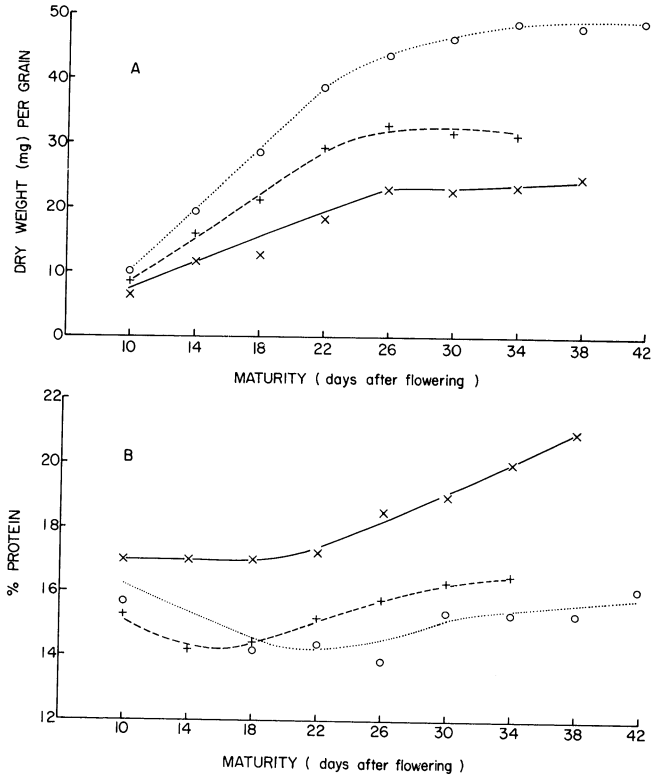

Fig. 2. A, dry weight per grain for developing grain; B, percentage of protein on a dry-weight basis for developing grain. Varieties designated as in Fig. 1.

RESULTS

Analytical Results

The moisture content of the maturing grain samples is shown in Fig. 1. Talbot wheat ripened early, at 34 days after flowering. Manitou was slightly later, and Stewart 63 ripened at about 42 days. Figure 2, A shows the increase in dry weight per grain during ripening. Protein content on a dry-weight basis (Fig. 2, B) was relatively constant during the early stages when dry weight increased rapidly. Thereafter, protein content increased steadily through to maturity.

The milling data for the freeze-dried grain (Fig. 3) suggest that even in the early stages of ripening, the endosperm represented a large proportion of the grain. However, the high ash content (Fig. 3, B) of the flour milled from immature samples indicated some contamination with non-endosperm material. It has not yet been definitely established if high ash content of flour from immature wheat is directly related to bran contamination, although this is the case for mature wheat. Only in the fully ripened grain is baking quality properly developed in the Manitou sample, as judged by the Zeleny sedimentation test (AACC Approved Method 56-60; Fig. 4). Although the sedimentation test is not necessarily an accurate guide

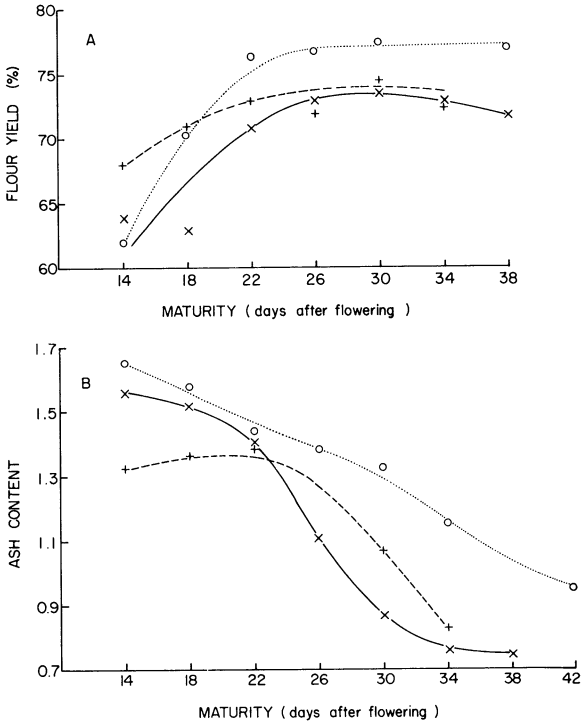

Fig. 3. A, yield of flour after milling of freeze-dried developing grain on a Brabender Quadrumat Junior mill; B, ash content of flour milled from freeze-dried developing grain. Varieties designated as in Fig. 1.

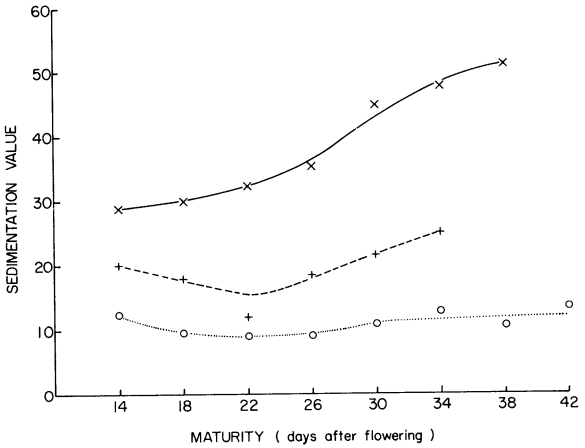

Fig. 4. Zeleny sedimentation values for flour samples described in Fig. 3. Varieties designated as in Fig. 1.

to baking quality when comparing very similar samples, it is a reasonable guide in ranking samples of widely different quality. Accordingly, its use is justified in this case.

Protein Extraction

Because of difficulty in determining protein content of AUC extracts, the efficiency of protein extraction was determined through estimating, by the biuret method, the amount of protein extractable with dilute NaOH following AUC extraction. This amounted to only about 2%, indicating almost complete extraction by AUC.

Gel Filtration

Gel filtration elution profiles for selected samples are shown in Fig. 5. There were no significant qualitative differences in the shape between varieties for immature grain. The profile for Talbot extract is shown as an example. However, at about 30 days after flowering, a shoulder appeared to the right of the main glutenin peak for Manitou and Talbot. The shoulder was more pronounced for ripe grain and was larger for Manitou than for Talbot; it was absent for Stewart 63, even at full maturity. The position of the shoulder corresponded to a MW of 200,000 to 500,000. Examination in the analytical ultracentrifuge indicated a MW of 370,000 by the approach to equilibrium method.

Figure 6 shows the proportions of glutenin, gliadin, and albumin in the AUC extracts of the undried developing grain. In most cases the results are means of at least two determinations. Nonprotein ultraviolet absorbing material (low MW) is expressed as a percentage of, and in addition to, the total protein content. The gradual decline in the proportion of this material reflected the progressive decrease in the metabolic activity of the grain. There was also a distinct, but less dramatic, decrease in the albumin fraction.

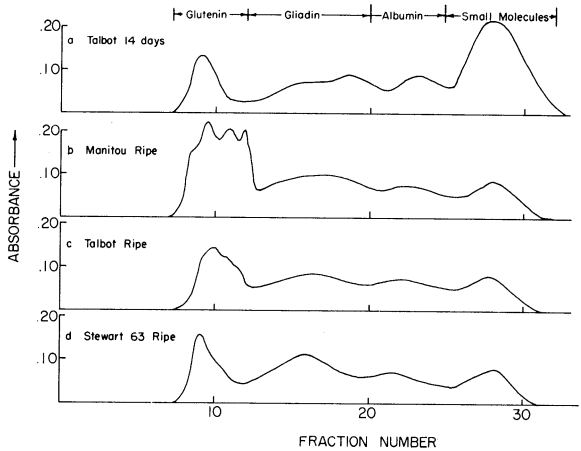

Fig. 5. Gel filtration of AUC extracts of ground grain on Sephadex G-150. Elution profiles (absorbance at 280 nm.) for: a) Talbot (undried) harvested 14 days after flowering, b) ripe Manitou, c) ripe Talbot, d) ripe Stewart 63.

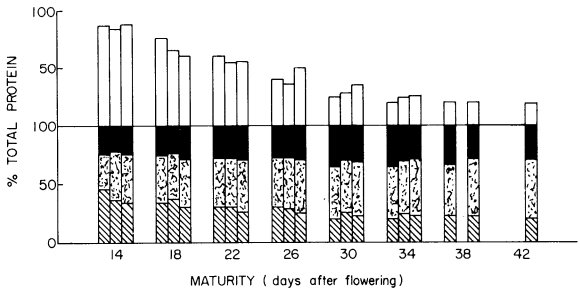

Fig. 6. Proportions of grain protein as glutenin (solid column), gliadin (stippled), albumin (cross hatched), and small UV-absorbing molecules (open) for undried developing grain. The columns at each stage of development represent Manitou (left), Talbot (center), and Stewart 63 (right).

Gliadins and glutenins, evident at all stages examined, both increased with the decrease in the proportion of albumin (see Fig. 6). Ratios of gliadin to glutenin (Fig. 7) suggest that in the early stages glutenin content increased slightly more rapidly than did gliadin for Talbot and Stewart. The trend in the ratios for mature samples is due partly to the late increase for Manitou and Talbot in the low MW glutenin.

Freeze-drying of immature grain of Talbot and Stewart 63 produced no significant changes in the proportions of glutenin, gliadin, and albumin, and only minor changes in the content of nonprotein material (generally a slight decrease).

Ultracentrifugation

When an AUC extract of grain was examined by sedimentation velocity, the schlieren pattern showed only one peak which seemed to correspond to the boundaries for albumin and gliadin proteins. The sedimentation coefficient increased steadily with grain maturity (Fig. 8). This increase can be fully explained by the shift in the weight-average MW of combined albumin and gliadin proteins as the proportion of albumin decreased with development.

DISCUSSION

Classically, the major protein groups of flour (glutenin, gliadin, and albumin)

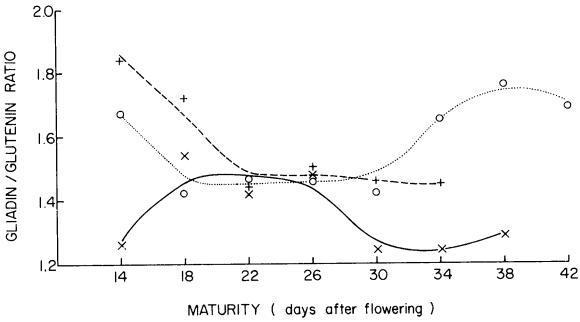

Fig. 7. Ratios between gliadin and glutenin for undried developing grain. Varieties designated as in Fig. 1.

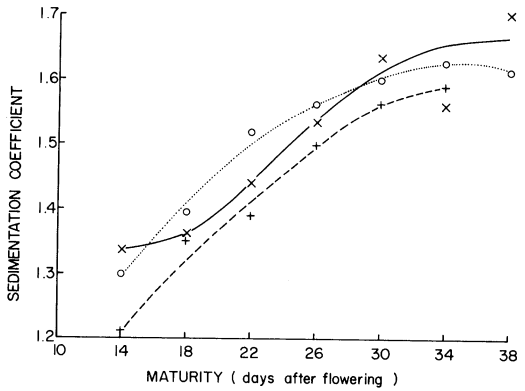

Fig. 8. Sedimentation coefficients ($s_{20,w}$) for AUC extracts of developing grain. Varieties designated as in Fig. 1.

have been defined according to their solubility properties. Characterization according to this criterion did not suit the aim of the present investigation. On the other hand, characterization according to MW distribution using a dissociating solvent (9) was particularly appropriate to the aim of preventing possible protein aggregation during extraction. To determine whether definition according to MW was a satisfactory criterion for extracts from whole immature grain, glutenin, gliadin, and albumin were prepared by the AUC procedure from grain harvested 14 days after flowering. Each preparation had the solubility and viscoelastic properties characteristic of it when prepared conventionally from mature grain.

The main purpose of the experiments reported was to examine the possibility that glutenin might result from an aggregation of smaller endosperm proteins during the drying stages of ripening. The results showed clearly that glutenin was present even in the early stages of development and that its content was not affected by drying at any stage of maturity. It does not seem likely that the low-MW glutenin that appeared on ripening for Manitou and Talbot is produced only by the drying process, since its appearance could not be duplicated by artificial drying (freeze-drying) of immature grain. It is still possible that different results might be obtained with a drying procedure more closely approximating conditions experienced by the growing plant.

The appearance of low-MW glutenin only in the late stages of maturity and the correspondence of its amount in the varieties to their baking quality suggest a relationship between this material and baking quality. However, more direct evidence would be required to establish such a relationship. This material is probably similar to the "high-molecular-weight gliadin" described by Beckwith et al. (11) and material, excluded from Sephadex G-100, which penetrated starch gel to give a "streak" pattern on electrophoresis (12).

Acknowledgment

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