# Biochemical and Breadmaking Properties of Wheat Protein Components. II. Reconstitution Baking Studies of Protein Fractions from Various Isolation Procedures[1]

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**ABSTRACT**

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Two hard red spring wheat varieties, Prodax and Len, of poor and good breadmaking quality, respectively, were fractionated into various solubility fractions by three different procedures. Reconstitution baking studies were carried out to interchange fractions between varieties and between fractionation procedures to evaluate the breadmaking properties of the various fractions. Exchanging the water-soluble and starch fractions, separately, between the two varieties showed different effects on loaf volume depending on the fractionation procedure for isolating these fractions. Exchanging the gliadin and glutenin fractions, however, showed that the fractions that contained larger amounts of glutenin proteins gave the highest positive responses to loaf volume and were independent of the fractionation procedure. However, the magnitude of loaf volume response of the glutenin fraction was dependent on the fractionation procedure. Comparison of fractionation procedures showed that 70% (v/v) aqueous ethanol used to extract the gliadin fraction impaired the visco-elasticity of reconstituted doughs from both varieties. Starch fractions, however, did not seem to be affected by the fractionation procedures. Addition of various levels of total lipids to reconstituted fractions (obtained from defatted flour) showed differences in loaf volume response.

Recent reviews by MacRitchie (1980,1984), Pomeranz (1980), and Bushuk (1974, 1984) emphasize the extensive amount of work being conducted on the properties of the gluten proteins by solubility fractionation techniques. However, only a few researchers (Hoseney et al 1969b, Orth et al 1972, MacRitchie 1978, Booth and Melvin 1979, Preston and Tipples 1980) reported work concerning the relationship of specific gluten fractions to observed baking quality differences. A close look at their research work indicated differences in their interpretation of the functional properties of gluten proteins.

Hoseney et al (1969b) adopted the work of Shogren et al (1969) and dissolved gluten in 0.005*N* lactic acid. Hoseney et al (1969b) collected three fractions, the "insoluble fraction" (centrifugate at 1,000 × *g*), gliadin-rich (100-5S), and glutenin (100-5C) by ultracentrifugation at 100,000 × *g*. These three gluten protein fractions were reconstituted singly with their water solubles and starch (at original protein levels) and baked into bread. They concluded that the insoluble fraction had no specific role in breadmaking, whereas the gliadin-rich proteins (100-5S) controlled loaf volume and glutenin proteins (100-5C) governed the mixing requirements of the wheat flour.

MacRitchie (1978) fractionated gluten proteins by dissolving them in 0.1*M* acetic acid followed by centrifugation. The supernatant, which constituted 60% of the gluten, was referred to as the gliadin fraction, whereas the residue (pellet), which constituted 40% of the gluten, was called the glutenin fraction. These two gluten protein fractions were reconstituted singly with their water solubles and starch fractions and baked into bread. MacRitchie concluded that dough strength appeared to be a function of the molecular weight distribution of the gluten proteins. The more insoluble high molecular weight proteins were found to increase farinograph dough development time and extensigraph height and area, and to reduce farinograph dough breakdown. Reconstitution studies by MacRitchie (1978) and Legouar et al (1979) suggest that differences in the baking response of bread wheats that vary in quality may be closely related to the properties of the more insoluble high molecular weight glutenin proteins. The low molecular weight gliadin proteins decrease dough strength and mixing stability (MacRitchie 1972).

Marais and D'Appolonia (1981) carried out reconstitution studies using starch, gluten, tailings, and water solubles from wheat flours. They found that at optimum bromate levels, loaf volume correlated positively with total protein content but negatively with the percentage of glutenin and residue proteins.

Orth and Bushuk (1972) and Orth et al (1972) studied the solubility distribution of 26 wheat varieties that varied in breadmaking quality using a modified Osborne (1908) procedure. Remix loaf volumes were shown to be statistically highly correlated with the proportion of insoluble glutenin proteins. The level of this protein fraction was also shown to be highly correlated with dough strength as measured on the farinograph.

Preston and Tipples (1980) fractionated gluten proteins into acid-soluble and acid-insoluble fractions using 0.05*M* acetic acid. These two protein fractions were added singly to a base flour in increasing levels. They found that dough strengthening effects were mainly due to the acid-soluble fraction. They also noted that acid-soluble gluten proteins increased loaf volumes and acid-insoluble gluten proteins reduced loaf volumes.

The first paper (Chakraborty and Khan 1988) of this series dealt with the quantitative isolation and characterization by gel electrophoresis of various protein fractions. In this second paper we used the isolated fractions in reconstitution baking studies to explain further the role of the gluten proteins on dough rheology and breadmaking quality.

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## MATERIALS AND METHODS

### Flours

Two hard red spring wheat varieties, Prodax and Len, of poor and good breadmaking qualities (loaf volume), respectively, were grown at Dickinson, ND, and were milled into straight-grade flours on a Miag pilot mill (Miag, Braunschweig, Switzerland).

### Analytical Procedures

Moisture, protein, lipid, and ash were determined by AACC approved methods (1983). Protein content was obtained by multiplying the nitrogen content by the conversion factor 5.7 (Tkachuk 1969).

### Defatting of Flour Samples

Flour samples were defatted according to the procedure described by MacRitchie and Gras (1973).

### Protein Fractionation Procedures

Flours were fractionated into protein and starch (residue) fractions by the procedures described by Chen and Bushuk (1970), MacRitchie (1978), and Hoseney et al (1969a,b) as outlined by Chakraborty and Khan (1988). All fractions were freeze-dried (except for starch fractions from MacRitchie's procedure, which were air-dried), ground with a mortar and pestle in an ice bath, sifted through a seive (U.S. no. 70) and stored at −40° C.

### Reconstitution and Baking Procedures

Each fraction was carefully weighed on an equal protein and dry weight basis and pooled together in a baking tin, which was then sealed around the cover to prevent moisture uptake or loss. Fractions were then blended in a blender (type AR-400, Erweka, W. Germany) for 30 min at speed setting 1 and a 75° angle of inclination. This insured a thorough mixing of the fractions. All the reconstituted blends were tempered in a dry fermentation cabinet at 29° C overnight.

Moisture content of each fraction was predetermined before blending. Water absorption was calculated based on the moisture content of the blends. Mixing time was predetermined from several reconstituted mixing and baking trials. A constant mixing time of 35 sec, which gave acceptable dough consistency, was given to all reconstituted blends. Mixing time above or below 35 sec gave unsatisfactory dough properties. A four-pin laboratory mixer (National Manufacturing Co., Lincoln, NE) with a 25-g, two-pin bowl was used. A lean baking formula and procedure was used on 25 g of blend as described by D'Appolonia et al (1970).

### Treatment of Gluten with 70% Aqueous Ethanol

The 100-5S (gliadin-rich) fraction of Hoseney et al (1969b) and the acid-soluble (gliadin) fraction of MacRitchie's (1978) procedure were treated with 70% aqueous ethanol as follows: a 10-g sample was stirred in 40 ml of 70% ethanol for 3 hr, then dialyzed against distilled water at 4° C for 72 hr with six changes of water, and finally lyophilized.

### Determination of Starch Damage

The procedure for determining starch damage was that of Williams and Fegol (1969).

### Statistical Analysis

Data from this study were analyzed using the statistical analysis system as described by the SAS Institute (1982).

## RESULTS AND DISCUSSION

The quality parameters of the two hard red spring wheat varieties Prodax (poor breadmaking quality) and Len (good breadmaking quality) are shown in Table I. All parameters are reported as means of duplicates along with their standard deviation. These two varieties were well suited for this study, as indicated by their protein contents (close to each other), their soundness (high falling numbers), their wet gluten contents (fairly high), and their wide difference in loaf volume (30 $cm^3$).

Our first paper in this series described compositional differences (quantitation and gel electrophoresis) of the wheat protein fractions from the various isolation procedures used in this study (Chakraborty and Khan 1988). However, the most direct evidence of compositional differences between the protein fractions that affect functional properties should be seen in reconstitution baking experiments. Reconstitution baking studies were, therefore, carried out to test two major variables that could have contributed to the conflicting results found in the literature: first, differences due to different isolation procedures, and second, due to different varieties.

Composite blends were prepared by interchanging fractions between varieties and between fractionation procedures, reconstituted, and baked into 25-g pup loaves as described in Materials and Methods. Several test bakings of reconstituted fractions from the two varieties were performed, and their coefficients of variation (SD/mean) of the test bake loaf volume were 0.013–0.018. To minimize biased effects on loaf volume, the following precautions were taken: 1) the lyophilized protein fractions were weighed on an equal protein basis to avoid the influence of protein content differences on loaf volume; 2) a lean baking formula was used to avoid the preferential effect of dough conditioners (improvers) on specific protein fractions; and 3) no oxidizing agents such as bromate were used, also to avoid preferential effects on specific protein fractions.

### Effect of Variety on Breadmaking Quality

Baking data of reconstituted fractions isolated from the procedures of MacRitchie (1978) and Hoseney et al (1969a,b) are shown in Table II. These fractions were isolated and characterized as described in our earlier work (Chakraborty and Khan 1988).

### Water-Soluble Fraction

When the water-soluble fraction from Prodax (poor breadmaking quality) prepared by MacRitchie's procedure was substituted for that of Len (good breadmaking quality), there was a significant increase in loaf volume (+6 $cm^3$). Substituting the water-soluble fraction from Len into Prodax, however, did not improve the loaf volume of Prodax. In the Hoseney et al procedure opposite results were obtained, that is, the water solubles of Prodax substituted into Len did not improve the loaf volume of Len, but the loaf volume of Prodax was improved significantly (+9 $cm^3$) when the water-soluble fraction of Len was substituted into Prodax. The quantitative fractionation results of Chakraborty and Khan (1988) showed that the Prodax water-soluble fraction of the Hoseney et al procedure contained approximately 1.6% more of the total proteins than that of MacRitchie's procedure, whereas the opposite was found for Len, whose water-soluble fraction showed approximately 1.0% more proteins in MacRitchie's procedure than in that of Hoseney et al. The sodium dodecyl sulfate-polyacrylamide gel electrophoregrams of the water-soluble fractions (Chakraborty and Khan 1988) of the two procedures showed the varieties that contained more of the water-soluble fraction also contained more of subunits 1 and 2. These compositional differences in the water-soluble fractions, in

**TABLE I**
**Chemical Composition and Quality Parameters of Flours of Two Hard Red Spring Wheat Varieties[a]**

| | Wheat Varieties | |
|---|---|---|
| Component | Prodax | Len |
| Moisture, % | 16.3 ± 0.07 | 16.1 ± 0.07 |
| Protein,[b] 14% mb | 12.5 ± 0.07 | 13.2 ± 0 |
| Ash, 14% mb | 0.45 ± 0 | 0.52 ± 0 |
| Falling number, sec | 653 ± 2.8 | 512 ± 9.1 |
| Wet gluten content, % | 31.1 ± 0.22 | 35.3 ± 0.14 |
| Lipid, % | 0.84 ± 0.01 | 0.86 ± 0.01 |
| Loaf volume, $cm^3$ | 132 ± 2.90 | 162 ± 2.40 |

[a] Values reported are means of duplicates ± standard deviation.
[b] Kjeldahl protein (N × 5.7).

addition to other factors, may have contributed to the functional property differences observed in the interchange studies.

### Gliadin and Glutenin Fractions

When the gliadin (glutenin-rich) fractions of Prodax and Len from MacRitchie's procedure were exchanged, the Len gliadin had a significant positive effect (+11 $cm^3$) on the loaf volume of Prodax, whereas the Prodax gliadin had a significant negative effect (−9 $cm^3$) on the loaf volume of Len. When the glutenin fractions of MacRitchie's procedure were exchanged, both Prodax (+4 $cm^3$) and Len (+6 $cm^3$) showed positive effects on loaf volume, with Len having the more positive effect. Comparing the gliadin and glutenin fractions, the "gliadin" fraction of MacRitchie's procedure had the greater effect on loaf volume than the glutenin fraction. However, it should be clearly and unambiguously noted here that the so-called gliadin fraction of MacRitchie, in the present study, contained approximately 90–92% (w/w) of the gluten proteins, that is, this "gliadin" fraction contained almost all of the glutenin proteins as well.

When the gliadin fractions of Prodax and Len from the Hoseney et al procedure were exchanged, the trend in loaf volume response was the same as for MacRitchie's procedure, that is, Len gliadin had a significant positive effect (+13 $cm^3$) on the loaf volume of Prodax, whereas Prodax gliadin had a significant negative effect (−5 $cm^3$) on the loaf volume of Len. When the glutenin fractions were exchanged, the Len glutenin had a significant positive effect (+16 $cm^3$) on the loaf volume of Prodax, whereas Prodax glutenin had a significant negative effect (−5 $cm^3$) on the loaf volume of Len. In this study the glutenin fraction of the Hoseney et al procedure had a greater effect on loaf volume than the gliadin fraction. This is contrary to the results of Hoseney et al (1969b), who reported that the gliadin fraction had the greater effect on loaf volume. These differences in results are most likely due to compositional differences in the fractions. Table III shows the percent contribution to loaf volume of the gluten fractions of the two procedures. It can clearly be seen that those fractions that contained more glutenin proteins were the ones that contributed the greater positive response to loaf volume, that is, the so-called gliadin fraction of MacRitchie, which contained the majority of glutenin, and the glutenin (lactic acid insoluble or 100-5C) fraction of Hoseney et al. Therefore, these results seem to indicate that the glutenin proteins are responsible for loaf volume differences in bread wheats. This is in accord with the conclusion of MacRitchie (1978, 1985), but not that of Hoseney et al (1969b). Previously, Chakraborty (1986) interpreted these results to mean that the "gliadin" fraction (acetic acid-soluble of MacRitchie) was responsible for loaf volume differences, an interpretation based on nomenclature of fractions rather than on compositional differences.

### Starch Fractions

When the starch fractions from MacRitchie's procedure were exchanged between Prodax and Len, Prodax starch had a significant positive effect (+15 $cm^3$) on the loaf volume of Len, whereas Len starch had a significant negative effect (−5 $cm^3$) on the loaf volume of Prodax. These results were just opposite in the Hoseney et al procedure, that is, Len starch had a significant positive effect (+15 $cm^3$) on Prodax loaf volume, whereas Prodax starch had no significant effect (1 $cm^3$) on Len loaf volume. The quantitative protein recovery results (Chakraborty and Khan 1988) showed much less protein remaining in the starch fraction of MacRitchie than in that of Hoseney et al. For example, in MacRitchie's procedure, the starch fractions of Prodax and Len constituted 3.2 and 3.4%, respectively, of the total proteins, whereas in the Hoseney et al procedure, Prodax and Len constituted 10.9 and 6.6%, respectively, of the total proteins. These

**TABLE II**
**Evaluation of Breadmaking Quality of Two Hard Red Spring Wheat Varieties Using Reconstitution Baking Studies**

| Flours | Gliadin | Glutenin | Insoluble Fraction | Starch | Water-Soluble Fraction | Mixing Time (sec) | Baking Abs. (%) | Loaf Volume ($cm^3$) | Volume Change ($cm^3$) | *t* Value[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Unfractionated flour | | | | | | | | | | |
| Prodax | | | | | | 90 | 58 | 132 | | |
| Len | | | | | | 105 | 61 | 162 | | |
| Fractionated flour | | | | | | | | | | |
| **Solubility Fractions of the MacRitchie (1978) Procedure** | | | | | | | | | | |
| | Prodax | Prodax | ··· | Prodax | Prodax | 35 | 58 | 139[b] | | |
| | Len | Len | ··· | Len | Len | 35 | 61 | 149[b] | | |
| | Prodax | Prodax | ··· | Prodax | Len | 35 | 58 | 141 | + 2 | ± 1.488 |
| | Len | Len | ··· | Len | Prodax | 35 | 61 | 155 | + 6*[c] | ± 6.788 |
| | Len | Prodax | ··· | Prodax | Prodax | 35 | 58 | 150 | ±11* | ±16.315 |
| | Prodax | Len | ··· | Len | Len | 35 | 61 | 140 | − 9* | ±10.182 |
| | Prodax | Len | ··· | Prodax | Prodax | 35 | 58 | 145 | + 6* | ± 8.931 |
| | Len | Prodax | ··· | Len | Len | 35 | 61 | 153 | + 4* | ± 4.525 |
| | Prodax | Prodax | ··· | Len | Prodax | 35 | 61 | 134 | − 5* | ± 7.443 |
| | Len | Len | ··· | Prodax | Len | 35 | 58 | 164 | +15* | ±16.930 |
| **Solubility Fractions of the Hoseney et al (1969a,b) Procedure** | | | | | | | | | | |
| | Prodax | Prodax | Prodax | Prodax | Prodax | 35 | 58 | 128[b] | | |
| | Len | Len | Len | Len | Len | 35 | 61 | 143[b] | | |
| | Prodax | Prodax | Prodax | Prodax | Len | 35 | 58 | 137 | + 9* | ±11.067 |
| | Len | Len | Len | Len | Prodax | 35 | 61 | 144 | + 1 | ± 1.087 |
| | Len | Prodax | Prodax | Prodax | Prodax | 35 | 58 | 141 | +13* | ±15.986 |
| | Prodax | Len | Len | Len | Len | 35 | 61 | 138 | − 5* | ± 5.439 |
| | Prodax | Len | Prodax | Prodax | Prodax | 35 | 58 | 144 | +16* | ±19.67 |
| | Len | Prodax | Len | Len | Len | 35 | 61 | 138 | − 5* | ± 5.439 |
| | Prodax | Prodax | Len | Prodax | Prodax | 35 | 59 | 129 | + 1 | ± 1.229 |
| | Len | Len | Prodax | Len | Len | 35 | 61 | 142 | − 1 | ± 1.087 |
| | Prodax | Prodax | Prodax | Len | Prodax | 35 | 56 | 143 | +15* | ±18.446 |
| | Len | Len | Len | Prodax | Len | 35 | 62 | 142 | +-1 | ± 1.087 |

[a] Student-Fisher *t* statistical test.
[b] Controls.
[c] Significantly different at $\alpha = 0.05$; tabulated value, *t*: 3.182.

results clearly show the effect that differences in isolation procedures can have on the functional properties of the isolated fractions. It is also interesting to note that the same wheat variety showed a significant positive effect on loaf volume, with both its water-soluble and starch fractions, for example, Prodax in MacRitchie's procedure and Len in the Hoseney et al et al procedure.

### Effect of Alcohol on the Functionality of Gliadin

The protein fractions from the Chen and Bushuk (1970) procedure were weighed, blended, and reconstituted with the appropriate quantity of water. However, upon mixing, it failed to form a viscoelastic dough. It was suspected that functionality of protein fractions might have changed during the 70% ethanol extraction step. To establish whether or not 70% ethanol denatured the gliadin proteins, gliadins of the varieties Prodax and Len from the MacRitchie and Hoseney et al procedures were treated with 70% ethanol for 3 hr with constant stirring. After alcohol treatment, the suspensions were dialyzed at 4° C against distilled water for 72 hr with several changes of water to remove alcohol completely, and then lyophilized.

Alcohol-treated gliadin fractions from the MacRitchie (1978) and Hoseney et al (1969b) procedures were blended with the remaining fractions from their respective procedures, reconstituted, and baked into pup loaves. Prodax and Len loaves from the MacRitchie and the Hoseney et al procedures failed to produce satisfactory volume. Out of four bakes, the volume of three loaves could not be measured because they were less than 110 $cm^3$, and the fourth loaf was 112 $cm^3$. These loaf volumes were much below their respective controls. Therefore, aqueous ethanol at 70% concentration acted as a strong denaturing agent, strong enough to alter the functionality of the gliadin proteins. A similar conclusion was reached by Booth and Melvin (1979).

### Colorimetry of Starch Fractions

It is well established that a high damaged starch content in flour can have many adverse effects on breadmaking quality (Farrand 1964). Colorimetric and scanning electron microscopy procedures were, therefore, used to determine damaged starch content in flour residue (pellet) samples recovered after fractionation. Fractionation of flour with different types of solvents, vigorous stirring during extraction, freeze-drying, and grinding might produce a higher starch damage content of the residue (pellet) over the original flour, thereby influencing baking results.

**TABLE III**
**Contribution of Gluten Protein Fractions to Differences in Loaf Volume of Two Flours Found by Interchange of Fractions**

| Fraction | Percent of Total Gluten Protein | Contribution to Loaf Volume Difference (%) |
|---|---|---|
| MacRitchie "gliadin"[a] | 90–92 | 65 |
| MacRitchie glutenin | 3–4 | 35 |
| Hoseney et al gliadin | 58–63 | 45 |
| Hoseney et al glutenin | 12–13 | 55 |

[a] This fraction contained almost all glutenin proteins plus gliadins.

The colorimetric procedure (Table IV) showed that starch damage content of Prodax, Len and Coteau (included for comparison purposes) flours fractionated by Chen and Bushuk's and the Hoseney et al procedures were similar to the unfractionated flour. However, flours fractionated by MacRitchie's procedure gave higher starch damage values than the other two procedures. A possible explanation could be that flours fractionated by MacRitchie's procedure were air-dried at room temperature with frequent grinding and sifting. Also, toward the end of the drying period, coarser starch particles became hard and were ground for a longer time with a mortar and pestle which, perhaps, increased the mechanical damage to the starch granule. However, scanning electron micrographs (results not shown) of starches from Prodax and Len. fractionated by the three procedures (Chen and Bushuk 1970, MacRitchie 1978 and Hoseney et al 1969b) showed no apparent gross mechanical damage of the visible starch granules over unfractionated flours. It is, therefore, assumed that starch damage did not appreciably influence the baking results.

### Effect of Whole (Total) Flour Lipid on Loaf Volume of Reconstituted Fractions

In the literature there are reports on the effect of whole (total) lipid and lipid fractions on loaf volume using defatted flour (Daftary et al 1968, MacRitchie and Gras 1973). In a review, MacRitchie (1984) reported that most lipid-extracting solvents adversely affect the functional properties of flour. MacRitchie (1978) reported that removal of flour lipid by chloroform has very little effect on the dough development requirements of a flour. In our study, the effect of addition of whole flour lipid on loaf volume of fractionated and reconstituted flour was investigated.

Three types of reconstituted blends were prepared separately from defatted flour of the two varieties Prodax and Len. Blend one consisted of starch, gluten, and water solubles (gluten was not fractionated); blend two consisted of unfractionated defatted flours; blend three consisted of starch, water solubles, gliadin and glutenin (gluten was fractionated into gliadin and glutenin). Whole flour lipids were added in varying amounts from 0 to 350 mg to the three blends of each variety. Blends were reconstituted with the required amounts of distilled water, mixed with salt, sugar, and yeast (D'Appolonia et al 1970) and baked into pup loaves. Loaf volumes were plotted against the weight of added whole lipid and are shown in Figure 1 (top, Prodax; bottom, Len).

At zero lipid level, loaf volumes in all blends were higher than in loaves that contained normal lipid levels (shown by arrows in Fig. 1). There seems to be no uniform increase or decrease in loaf volume in all the blends. The inconsistency in loaf volume could be attributed to the following factors: lipid distribution may not be homogeneous during mixing; and there may be an alteration in lipid-protein and starch-protein interactions due to solvents. Loaf volume decreased beyond 300 mg of added lipid for the variety Len (Fig. 1, bottom), whereas loaf volume remained the same or increased slightly beyond 300 mg of added lipid in Prodax (Fig. 1, top). Defatted flour (curve B) did not produce the inverted bell-shaped curve reported by MacRitchie and Gras (1973). It is apparent from Figure 1 that lipid could have influenced reconstituted loaf volume to some extent, because in the procedure

**TABLE IV**
**Determination of Damaged Starch[a] in Starch-Containing Fractions From Various Protein Solubility Fractionation Procedures**

| Variety | Unfractionated Flour | Fractionation Procedures: Chen and Bushuk (1970) Dialyzed[b] | Chen and Bushuk (1970) Undialyzed | MacRitchie (1978) | Hoseney et al (1969a,b) |
|---|---|---|---|---|---|
| Prodax | 15.4 ± 0.28[c] | 16.2 ± 0.27 | 15.1 ± 0.07 | 23.3 ± 0.42 | 16.5 ± 0 |
| Len | 16.1 ± 0.28 | 18.2 ± 0.35 | 19.3 ± 0.56 | 25.1 ± 0.07 | 17.1 ± 0.14 |
| Coteau | 16.9 ± 0.07 | 18.6 ± 0.28 | 15.4 ± 0.56 | 24.4 ± 0.28 | 17. 8± 0.8 |

[a] Williams and Fegol (1969). Values are reported in Farrand equivalent units.
[b] Starch fraction slurried in distilled water and dialyzed at 4° C for 72 hr.
[c] Values in parentheses are standard deviations.

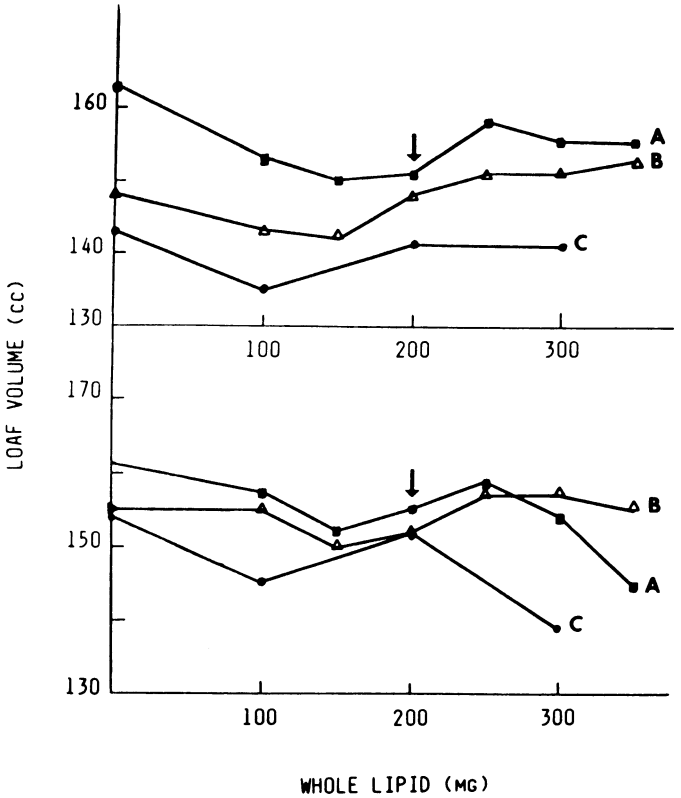


**Fig. 1.** The effect on loaf volume of addition of whole (total) flour lipids to fractionated flour of the hard red spring wheat varieties Prodax (top) and Len (bottom): reconstituted water-soluble, gluten, and starch fractions (**A**); unfractionated defatted flour (**B**); and reconstituted water soluble, gliadin, glutenin and starch fractions (**C**). Arrow indicates natural lipid content.

of Hoseney et al (1969a,b) defatted flour was not used for isolation of the protein fractions.

## CONCLUSION

The results of this study showed that compositional differences in protein fractions such as albumins and globulins (water solubles), gliadins, glutenins, and residue can result in functional property differences such as loaf volume. These compositional differences resulted from variations in the procedures used to extract these protein fractions. However, from comparison of the protein fractions from two different fractionation procedures, it was concluded that loaf volume seemed to be associated with the glutenin fraction. Furthermore, our results seem to indicate that a certain portion of the glutenin fraction, when in combination with gliadin, is responsible for producing optimum loaf volume. Further research is needed to isolate, characterize, and evaluate the functional (breadmaking) properties of this specific glutenin fraction.

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