

# STUDIES OF GLUTENIN. X. EFFECT OF FATTY ACIDS AND THEIR SODIUM SALTS ON SOLUBILITY IN WATER<sup>1</sup>

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## ABSTRACT

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Glutenin (freeze-dried) dissolves completely in water in the presence of relatively high concentrations of sodium palmitate or sodium stearate in comparison with the amount of glutenin. Sodium salts of shorter-chain fatty acids are less effective in this solubilizing action. Fatty acids do not show this effect except hexanoic which can solubilize some but not all the glutenin. Salts (*e.g.*, sodium

chloride) inhibit that ability of the sodium salts of long-chain fatty acids to dissolve glutenin. These results suggest that the insolubility of glutenin is largely due to hydrophobic interactions (which may involve flour lipids). It is postulated that these interactions play a key role in the functional properties of glutenin in breadmaking.

The insolubility of glutenin in highly dissociating solvents relative to gliadin has been attributed to its high-molecular weight and the presence of interpolypeptide S-S bonds (1,2), or to the formation of highly stable aggregates by cooperative noncovalent interactions (3,4). The fact that glutenin can be readily solubilized after reduction of disulfide bonds by reducing agents such as  $\beta$ -mercaptoethanol is generally cited as evidence for the presence of interpolypeptide S-S bonds. All of the work on the subunit structure of glutenin from our and other laboratories (5-7) included a reduction step. Kasarda *et al.* (3) argue that the S-S bonds are of the intrapolypeptide type giving subunit conformations which aggregate strongly through noncovalent interactions to form insoluble micelles. The nature of the noncovalent interactions has not been determined. So far, no one has succeeded in disrupting the glutenin "micelle" into subunits without reduction of S-S bonds, although there is some evidence that this may occur during dough mixing (8 and papers cited therein).

This paper presents results which show that the solubility of glutenin in water is markedly affected by sodium salts of long-chain fatty acids, suggesting that hydrophobic interactions may contribute significantly to the "insoluble" structure of the glutenin.

## MATERIALS AND METHODS

### Glutenin

The glutenin used was isolated from flour of the Canadian hard red spring wheat cv. Manitou by the pH precipitation method of Orth and Bushuk (9) and freeze-dried.

### Fatty Acids and Their Sodium Salts (Soaps)

Hexanoic, octanoic, decaanoic, dodecanoic, hexadecanoic (palmitic), and octadecanoic (stearic) acids were obtained from Sigma Chemical Co. Sodium

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salts of all but the dodecanoic and stearic acids were prepared in the laboratory by saponification with sodium hydroxide. Sodium dodecanoate and stearate were obtained from Sigma Chemical Co.

#### Solubilization Procedure

Various amounts of the solid fatty acid (or its sodium salt) were added to 5 or 10 mg of freeze-dried glutenin in 15-ml glass centrifuge tubes. For each 5 mg of glutenin, 1 ml of distilled-deionized water or buffer solution was added. Solubilization was achieved by rotating the tubes (attached to a circular disc) overnight. The mixtures were centrifuged at 4°C for 20 min at 17,000×*g* and the clear supernatant (which contained dissolved glutenin) was decanted. The insoluble residues were washed with 2 ml water (or buffer) per 5 mg glutenin by stirring for 2 hr, centrifuging as before, and decanting the supernatant. The amount of protein remaining in the precipitate was determined by the micro-Kjeldahl method. Each test was duplicated and reported as the average of the two values obtained.

#### Solutions Used

In addition to distilled water, the following solvents were used: 0.1*N* sodium chloride solution, 0.1*M* buffer solutions, Sorensen's glycine I (10) of pH 3.0, and Sorensen's glycine II (10) of pH 9.5, 10.0, 11.0, and 12.0, and 0.125*M* tris buffer of pH 8.9.

## RESULTS

#### Solubilization with Fatty Acids

*Distilled Water.* Figure 1 shows the solubility of glutenin in distilled water in the presence of fatty acids at two concentrations, 5 and 10 mg of fatty acid per 5 mg of glutenin.

*Effect of Ions.* The effect of ions was examined by determining glutenin solubility in tris buffer of pH 8.9 and Sorensen's glycine II buffer of pH 10.0 in the presence of 5 mg hexanoic acid only. The percentages of glutenin dissolved were 2 and 22% for the tris and glycine buffers, respectively. The equivalent figure for distilled water was 49%.

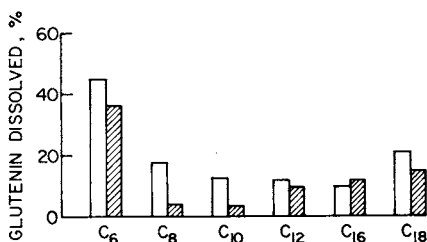


Fig. 1. Solubility of glutenin in water containing relatively high concentrations of fatty acids (□ 5 mg and ■ 10 mg fatty acid per 5 mg glutenin in 1 ml water).

### Solubilization with Sodium Salts of Fatty Acids

**Distilled Water.** Results showing the solubility of glutenin in aqueous solutions containing increasing quantities of the sodium salts of various fatty acids are shown in Fig. 2. In this experiment, 10 mg of glutenin were used with each 2 ml of water.

**Effect of Fatty Acid Chain length.** The relation between the length of the fatty acid carbon chain and its ability to dissolve glutenin is shown in Fig. 3 for two different concentrations of each soap.

**Effect of Dilution.** Experiments were carried out with the salts of dodecanoic and stearic acid only. The results are shown in Fig. 4. In this experiment, we used 5 mg of glutenin and 4 mg of soap and increasing volumes of distilled water from 1 to 8 ml.

**Effect of Ions.** Results of experiments made to examine the ability of sodium

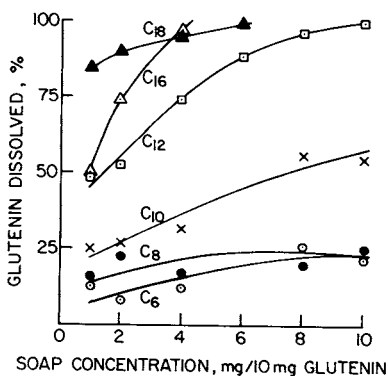


Fig. 2. Solubility of glutenin in water containing increasing quantities of various soaps (10 mg glutenin per 2 ml water).

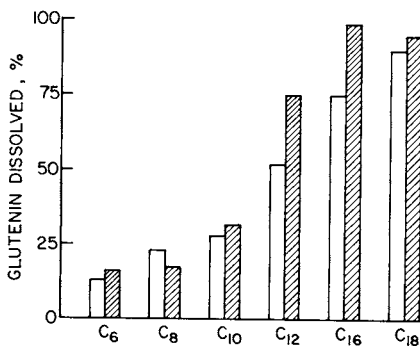


Fig. 3. Relation between glutenin solubility and fatty acid chain length at two soap concentrations in water ( $\square$  2 mg and  $\blacksquare$  4 mg soap per 10 mg glutenin in 2 ml of water).

salts of various fatty acids to dissolve glutenin in the presence of various ions (0.1*N* sodium chloride and glycine and tris buffers) are given in Table I. In these experiments, the amounts of glutenin and soap were 5 mg and 4 mg, respectively, in 1 ml of solvent.

*Effect of pH.* The solubility of glutenin in basic buffers with and without sodium stearate is presented in Fig. 5.

## DISCUSSION

### Effects of Fatty Acids

Only hexanoic acid showed any significant ability to increase the solubility of glutenin in water (Fig. 1). The solubility decreased slightly when the relative

**TABLE I**  
Percentage of Freeze-Dried Glutenin Dissolved in Water, Sodium Chloride Solution, and Various Buffer Solutions in the Presence of Various Soaps (5 mg Glutenin and 4 mg Soap per 1 ml Solvent)

Salt	Water	0.1 <i>N</i> NaCl	Glycine I	Tris	Glycine II	Glycine II
	%	%	pH 3.0 %	pH 8.9 %	pH 10.00 %	pH 11.00 %
Control, no soap	...	...	3	3	10	80
Na Decanoate (C <sub>10</sub> )	56	21	15	7	19	75
Na Dodecanoate (C <sub>12</sub> )	100	60	13	52	90	89
Na Palmitate (C <sub>16</sub> )	100	17	11	3	15	71
Na Stearate (C <sub>18</sub> )	100	2	19	1	25	68

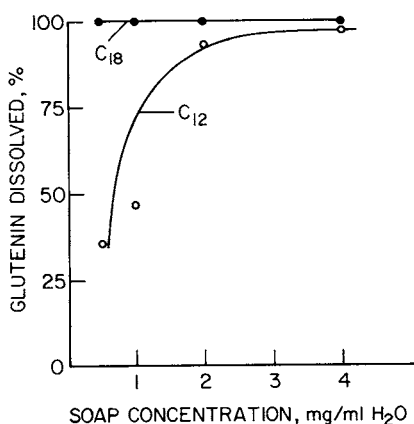


Fig. 4. Solubility of glutenin at varying dilutions of Na dodecanoate (C<sub>12</sub>) and sodium stearate (C<sub>18</sub>), but constant ratio soap:glutenin (4 mg soap per 5 mg glutenin in 1–8 ml water).

proportion of the fatty acid was doubled. Fatty acids with longer carbon chains all dissolved a relatively low percentage of the glutenin preparation used in this investigation; only the  $C_8$  and  $C_{10}$  acids gave a significantly lower solubility at the higher acid concentration of the fatty acid.

The addition of buffer ions decreased the dissolving power of hexanoic acid. This effect of small ions will be discussed in greater detail later.

It may be relevant to note that in hexanoic acid experiments, the insoluble glutenin pellet in the centrifuge tube had the appearance of a transparent gel. Obviously there was considerable modification of the structure of the glutenin in the presence of this fatty acid (pH 4-5).

The results discussed here are attributed to the ability of the fatty acids to disrupt hydrophobic interactions in the glutenin micelle. The limited solubility of the fatty acid in water may limit the magnitude of its solubilizing capacity.

#### Effects of Sodium Salts of Fatty Acids

Sodium salts of hexanoic and octanoic acids showed relatively low dissolving ability (Fig. 2); only about 20% of the glutenin was removed in the supernatant. The dissolving capacity increased rapidly with length of the carbon chain. With sodium stearate, essentially all of the glutenin dissolved at the lowest concentration of the soap used (1 mg/10 mg glutenin in 2 ml water). Indeed, very small additions of sodium stearate had a drastic solubilizing effect.

The marked increase in the glutenin dissolving capacity with length of the fatty acid carbon chain can be seen more readily from the presentation of results for two soap concentrations in Fig. 3. The  $C_{16}$  and  $C_{18}$  soaps are particularly effective in dispersing freeze-dried glutenin in water; the most rapid change in the solubility curve occurs in the  $C_{10}$ - $C_{12}$  region.

The relative effectiveness of soaps of different chain length can also be demonstrated by using different dilutions while the ratio of soap to glutenin is kept constant (Fig. 4). Sodium stearate ( $C_{18}$ ) maintained its dissolving capacity

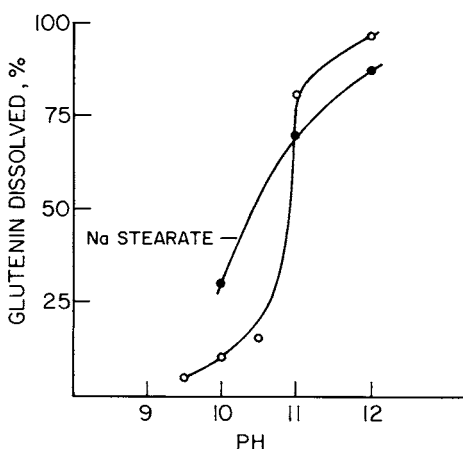


Fig. 5. Solubility of glutenin in solvents of different pH with and without (not labeled) 4 mg Na stearate per 5 mg of glutenin in 1 ml of solvent.

over the entire range of dilutions examined, whereas sodium dodecanoate rapidly lost its ability to solubilize glutenin when dilution reached a certain critical level (2 mg/1 ml water in our experiments).

In general, the addition of small ions to the mixture of glutenin and soap decreased the solubility of glutenin (Table I). This effect was particularly large when sodium chloride and tris buffer were added as the ions. Also, the effect seemed to increase with increasing chain length of the fatty acid. The high solubility in glycine II buffer at pH 11.0 is attributed to the well-known effect of highly alkaline pH on the solubility of glutenin. Glutenin is soluble at high pH (11.0 and 12.0) in the presence or absence of sodium stearate (Fig. 5).

The ability of soaps to dissolve glutenin can be attributed, in general, to the interaction between their hydrophobic chains and the hydrophobic regions of glutenin. However, unlike the case of fatty acids, the length of the hydrophobic chain of the soap must be more than ten C atoms in order to disrupt the hydrophobic interactions of glutenin sufficiently to cause dissolution. This effect is probably not due to solubility of the soap, since the salts of longer-chain fatty acids are less soluble in water. Our results may be compared with previous findings (11) which indicate that soaps with longer carbon chains are more effective than shorter-chain soaps in solubilizing various organic compounds in water.

It should be noted too, that when soaps are used for the solubilization of glutenin, the pH of the solution is slightly basic. Depending on the soap used and its concentration (in this study), the pH varied between 7 and 9. With hexanoic and octanoic acids the pH of the solutions was between 4 and 5. Table I shows that solvents (buffer solutions) of this pH do not dissolve glutenin. Furthermore, it is interesting to note that solvents of very high pH which can dissolve glutenin are less efficient in this action when soaps are added to the mixtures (see Fig. 5 and Table I). Accordingly, the phenomenon of glutenin solubilizations by soaps cannot be explained by the increase in pH. On the other hand, the decrease in the solubilizing power of bile salts and soaps (for various organic substances) on the addition of electrolyte has already been reported (11).

From the point of view of mechanism of the solubilizing action of the soaps, especially sodium palmitate and stearate, all that can be concluded from the preliminary results presented in this article is that hydrophobic interactions are probably involved. It is quite possible that flour lipids, which are extremely difficult to remove from glutenin and are usually present in glutenin preparations as contaminants, may also be involved in the solubilizing actions discussed here. Also, it remains to be established unequivocally that disulfide bonds were not cleaved under the conditions used in this study. Work is now in progress to examine those possibilities and also to determine the reversibility of the solubilization and the particle weight of the dissolved glutenin. The use of sodium palmitate and stearate solutions as solvents for flour proteins is under investigation. Results of this study have extremely important implications in the structure and function of glutenin and suggest new approaches to studies of breadmaking quality and its ramifications involving the gluten complex.

#### Literature Cited

1. NIELSEN, H. C., BABCOCK, B. E., and SENTI, F. R. Molecular weight studies on glutenin before and after disulfide-bond splitting. *Arch. Biochem. Biophys.* 96: 252 (1962).

2. EWART, J. A. D. A modified hypothesis for the structure and rheology of glutelins. *J. Sci. Food Agr.* 23: 687 (1972).
3. KASARDA, D. D., BERNARDIN, J. E., and NIMMO, C. L. Wheat proteins, p. 158. In: *Advances in cereal science and technology*, ed. by Y. Pomeranz. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1976).
4. DALEK-ZAWISTOWSKA, U., BARTOSZEWICZ, K., and KACZKOWSKI, J. The equilibrium between the high- and low-molecular fractions of wheat gluten. *Bull. Acad. Pol. Sci.* 23: 75 (1975).
5. BIETZ, J. A., HUEBNER, F. R., and WALL, J. S. Glutenin: The strength protein of wheat flour. *Baker's Dig.* 47: 26 (1973).
6. BUSHUK, W. Glutenin: Functions, properties and genetics. *Baker's Dig.* 48: 14 (1974).
7. HAMAUZU, Z., KAMAZUKA, Y., KANAZAWA, H., and YONEZAWA, D. Molecular weight determination of component polypeptides of glutenin after fractionation by gel filtration. *Agr. Biol. Chem.* 39: 1527 (1975).
8. TANAKA, K., and BUSHUK, W. Changes in flour proteins during dough-mixing. II. Gel filtration and electrophoresis results. *Cereal Chem.* 50: 590 (1973).
9. ORTH, R. A., and BUSHUK, W. Studies of glutenin. I. Comparison of preparative methods. *Cereal Chem.* 50: 106 (1973).
10. SOBER, H. A., ed. *Handbook of Biochemistry*, p. J-195. The Chemical Rubber Company: Cleveland (1968).
11. ELWORTHY, P. H., FLORENCE, A. T., and MacFARLANE, C. B. Solubilization by surface-active agents, pp. 62 and 85. Chapman and Hall: London (1968).

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