Studies on Vitality of Commercial Gluten. II. Solubility Fractionation, Electrophoresis, and Fluorescence Results[1]

C. K. WADHAWAN[2] and W. BUSHUK

ABSTRACT

Osborne solubility fractionation of commercial vital glutens showed that samples containing more insoluble residue protein gave poorer baking performance (i.e., lower vitality). Devitalized glutens prepared by heating at elevated temperature contained the greatest amount of insoluble residue. The proportion of acetic acid soluble protein was directly correlated to vitality of gluten. Electrophoresis patterns were altered upon devitalization. Intrinsic fluorescence of dry gluten increased upon devitalization; vitality was highly significantly negatively correlated to the intrinsic fluorescence value. Fluorescence of solution of acetic acid soluble fractions of vital gluten was highly significantly correlated with vitality as measured by the baking test.

Cereal Chem. 66(6):461-466

The first paper (Wadhawan and Bushuk 1989) reported physical, chemical, and technological properties of commercial wheat glutens in relation to their "vitality" for supplementation of low-protein flours for breadmaking. This paper presents results of several physicochemical techniques for measuring protein denaturation, attempting to discern information on the molecular nature of gluten vitality and thereby a means of practical measurement of vitality in gluten manufacturing.

MATERIALS AND METHODS

Samples

Commercial glutens (as-is), commercial glutens artificially devitalized by dry heating, and laboratory-prepared glutens used in this study were described previously (Wadhawan and Bushuk 1989).

Modified Osborne Solubility Fractionation

Fractionation of gluten protein by solubility in various solvents was performed using the modified Osborne procedure of Chen and Bushuk (1970) with some minor modifications. Gluten (2 g) was extracted sequentially with 0.5*M* NaCl, 70% ethanol, and 0.05*M* acetic acid. The salt solution extracts, which contain albumins and globulins, were treated as a single fraction. Extracts were freeze-dried and their nitrogen content determined by the micro-Kjeldahl procedure (method 46-13, AACC 1983). Analysis of variance was calculated on the results, and least significant differences were computed at the 5% level.

Protein Solubility in Acetic Acid Solution

The method of Orth and O'Brien (1976) was modified as follows: 1 g (db) of gluten was extracted with 25 ml of 0.05*N* acetic acid for 1 hr and centrifuged at 4,500 × *g* for 20 min at room temperature. The supernatant was removed and the extraction was repeated twice. The supernatants were combined and freeze-dried. Moisture and protein contents of both fractions (soluble and insoluble) were determined.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) in aluminum lactate buffer at pH 3.1 of the 70% ethanol solution extracts (gliadins) was performed using the apparatus and method described by Sapirstein and Bushuk (1985). PAGE in the presence of sodium dodecyl sulfate (SDS-PAGE) was carried out at pH 8.3 according to the method of Ng and Bushuk (1987).

Fluorescence Measurements

Intrinsic fluorescence of dry gluten was measured using the front surface accessory of the Perkin-Elmer model LS-5 fluorescence spectrometer. Samples (10 g) were sifted through a U.S. standard sieve no. 100 (149 μm) for 10 min on a Ro-tap sieve shaker to eliminate the effect of coarse material present in most samples. The sample (0.3 g) was held in the sample holder with a quartz window.

Packing of the sample in the sample holder should be as consistent as possible. The geometry of the accessory allows light from the excitation monochromator to strike the sample at an angle of 60°. Fluorescence emitted from the sample is directed to the emission monochromator at an angle of 30° to the surface of the sample. A pre-scan mode on the spectrophotometer can be deployed to automatically search for and display the maximum excitation and emission wavelengths of an unknown sample.

Accordingly glutens were scanned first to determine the excitation wavelength that gave the maximum emission, which was found to be 410 nm. Maximum emission wavelength was also determined for each sample and was found to be 462 nm for most samples. Accordingly, the fluorescence values reported herein were determined at an excitation wavelength of 410 nm and an emission wavelength of 462 nm. Excitation and emission slit widths were set at 10 and 5 nm, respectively, and a fixed scale of two. Fluorescence values reported are averages of five replicates determined over 30 days. In each assay, the fluorescence value was read after the position of the sample was changed at least five times to minimize sampling error.

[1]Publication no. 141 of the Food Science Department, University of Manitoba, Winnipeg, MB, Canada R3T 2N2, with financial assistance of the Natural Sciences and Engineering Research Council of Canada.

[2]Present address: 89-134 Portsmouth Blvd., Winnipeg, MB, Canada R3P 1B6.

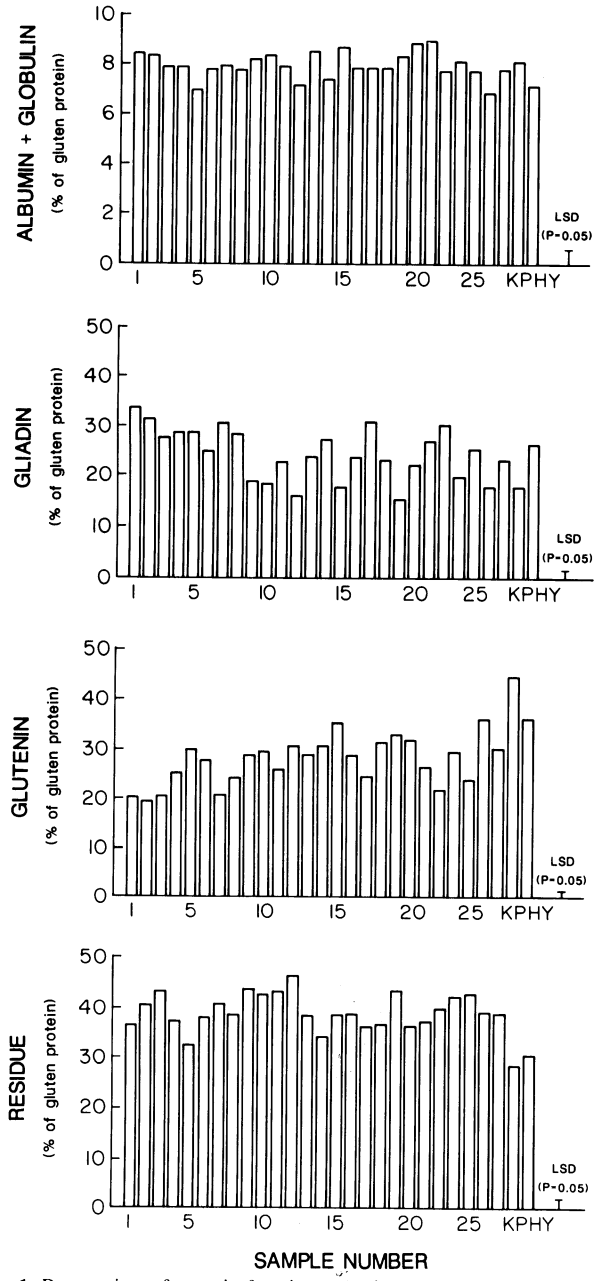

Fig. 1. Proportion of protein fractions obtained by the modified Osborne fractionation procedure. Results shown in order for samples 1–27 (Wadhawan and Bushuk 1989) except sample 24. (LSD = least significant difference).

Fluorescence of the acetic acid-soluble fraction of gluten was obtained at an excitation wavelength of 333 nm and emission wavelength of 422 nm, slit widths of 10 nm on both excitation and emission, and a fixed scale of one. Fifty milligrams (dry protein basis) of freeze-dried acetic acid (HAc)-soluble fractions were redissolved in 5 ml of 0.05N HAc for fluorescence measurements. *N*-Acetyl-tryptophanamide (Sigma, St. Louis, MO) was used as standard (at a concentration of $10^{-4}M$ in HAc). For the temperature study, each solution was equilibrated for 10 min at the desired temperature.

RESULTS AND DISCUSSION

Solubility Characteristics

Results of solubility fractionations have been proven useful in predicting breadmaking quality of wheat varieties (Orth and Bushuk 1972, Orth and O'Brien 1976). Also, it is common knowledge that the solubility of proteins in selected solvents is altered drastically upon denaturation. Since loss of gluten vitality during processing and/or storage is considered to be a type of protein denaturation, solubility techniques were adopted for the present study.

The proportion of the albumin-globulin fraction was essentially the same for all gluten samples that were fractionated (Fig. 1). The proportion of gliadin varied widely from 15.3 to 36.0% (Fig. 1) and decreased significantly upon devitalization (Fig. 2). The proportion of soluble glutenin also varied widely among the samples (Fig. 1). Glutenin content of the laboratory-prepared samples was higher than that of commercial samples.

A substantial decrease in the proportion of the soluble glutenin fraction was observed upon devitalization by heating (Fig. 2). The effect was more pronounced for this fraction than for gliadin. The results agree in general with those obtained by other workers (Booth et al 1980, Jeanjean et al 1980, Pence et al 1953). These workers studied the effect of elevated temperature on wet gluten.

TABLE I
Relationship Between Vitality (Loaf Volume of 5:95 Blend Gluten-Flour) and Some Properties of Commercial and Laboratory-Prepared Glutens

Parameter	*r*
Osborne solubility fractions ($n = 26$)	
Gliadins	0.08
Glutenins	0.43
Residue	−0.75**[a]
Acetic acid solubility ($n = 8$)	
Soluble protein	0.88**
Residue	−0.64*
Fluorescence	
Dry gluten ($n = 15$)	−0.74**
Soluble gluten ($n = 8$)	−0.98**

[a]*, ** Significant at 95 and 99%, respectively.

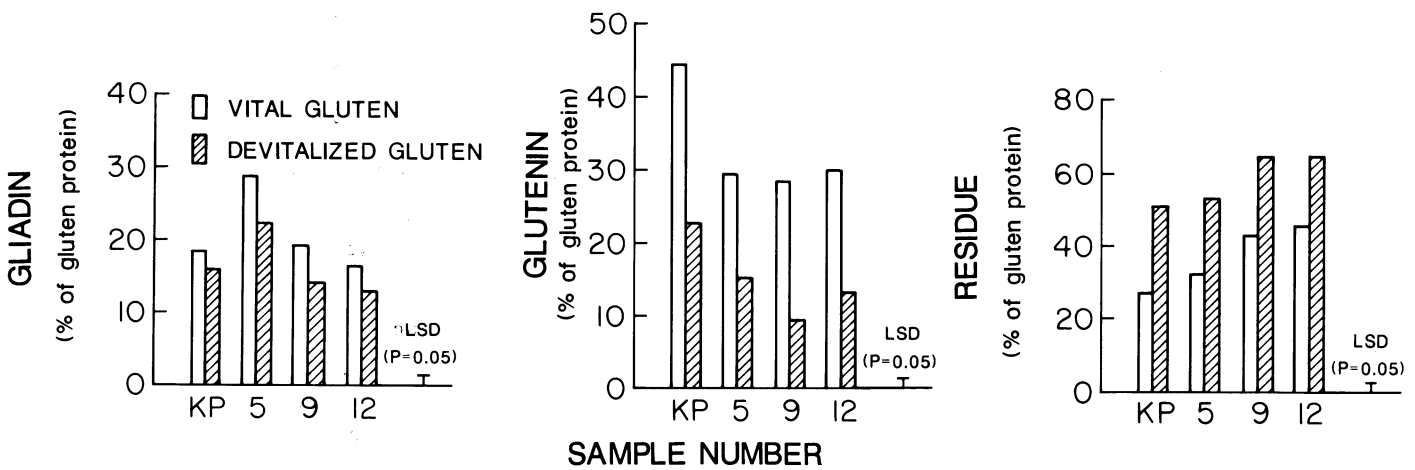

Fig. 2. Effect of devitalization on the proportion of three Osborne fractions of one laboratory-prepared and three commercial glutens. (LSD = least significant difference).

In the present study, the dry glutens were heated at higher temperature and for a longer period of time.

Statistical analysis of the solubility fractionation data showed no relationship between vitality (i.e., loaf volume of 5:95 gluten-flour blends), and the proportion of the albumin/globulin, gliadin, or glutenin fractions. On the other hand, the negative correlation between loaf volume and proportion of residue protein (Fig. 1) was highly significant ($r = -0.75$, $P < 0.01$; Table I). This finding is consistent with the fact that devitalization by heating led to a decrease in the proportions of both gliadin and soluble glutenin and a parallel increase in the proportion of residue protein (Fig. 2).

Glutens of good vitality contained a much higher proportion of protein that was soluble in HAc solution (Table II). For the eight samples examined, the range of solubility extended from 67.5% for gluten of good vitality down to 49.5% for gluten of low vitality. The amount of protein that was soluble in HAc solution was highly significantly correlated with vitality ($r = 0.88$, $P < 0.01$; Table I). Accordingly, the proportion of gluten protein that dissolves in dilute HAc solution under standardized conditions could be a simple test of vitality. Unfortunately, the test has a large experimental error (± 5.5%) and therefore should be used under rigorously controlled conditions. The results of the two solubility fractionations are mutually consistent.

TABLE II
Acetic Acid Soluble and Insoluble (residue) Protein (as a Percentage of Total Protein) of Selected Gluten Samples

Sample	Soluble Protein (%)	Insoluble Protein (%)
01	60.1	39.5
05	67.5	42.5
09	49.5	50.5
11	55.4	44.6
12	54.9	45.1
16	59.3	40.7
26	66.0	34.0
27	66.9	33.1
KP	68.1	31.9
HY	64.8	35.2
DG	42.1	57.9

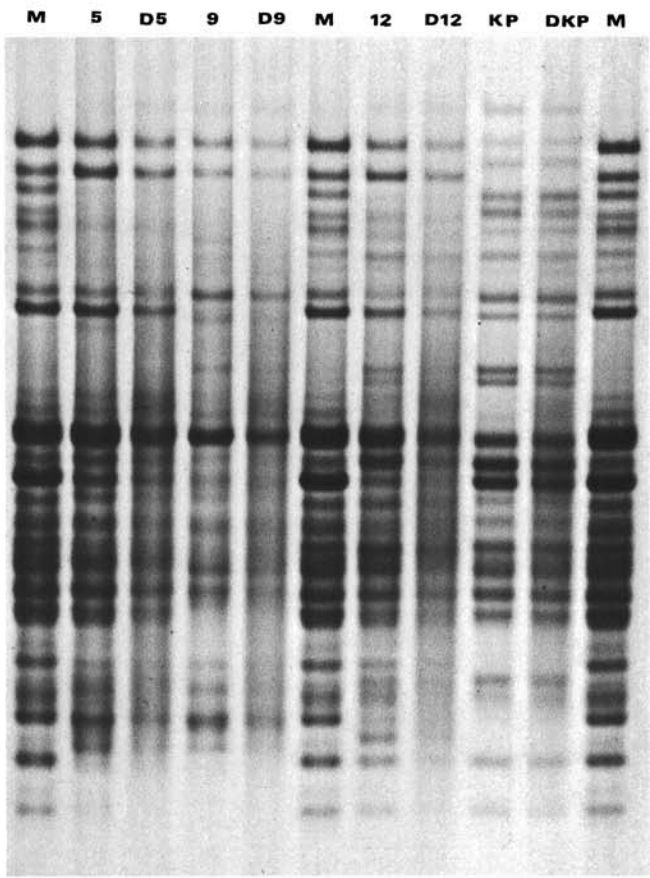

Fig. 3. Gliadin polyacrylamide gel electrophoregrams for selected vital (**5, 9, 12,** and **KP**) and devitalized (**D5, D9, D12,** and **DKP**) glutens and flour of cv. Marquis used as the reference (**M**).

PAGE and SDS-PAGE Results

Four gluten samples and their corresponding devitalized forms were analyzed by PAGE (Fig. 3). Marquis wheat gliadin was used as the standard reference. Upon devitalization, the intensity of all bands decreased. Schofield et al (1983) reported that upon heating (wet gluten, 5 min at 100°C) the ω-gliadins were the only ones that remained soluble in HAc solution and therefore could

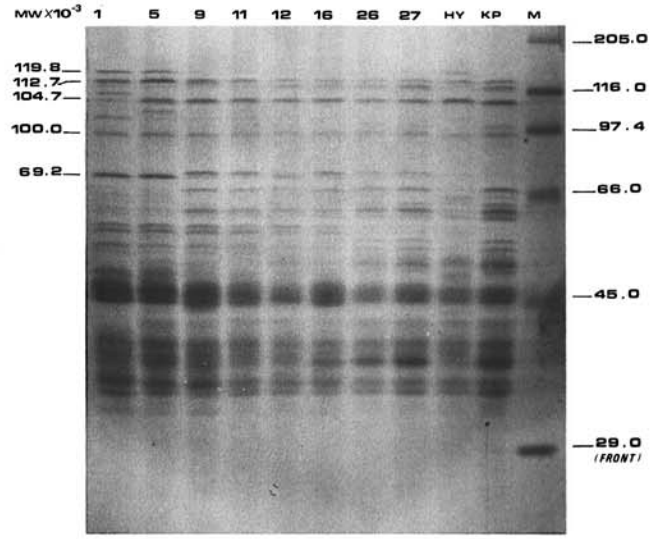

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoregrams of the reduced glutenins of eight selected commercial glutens, two laboratory-prepared glutens (**HY** and **KP**) and molecular weight markers (**M**).

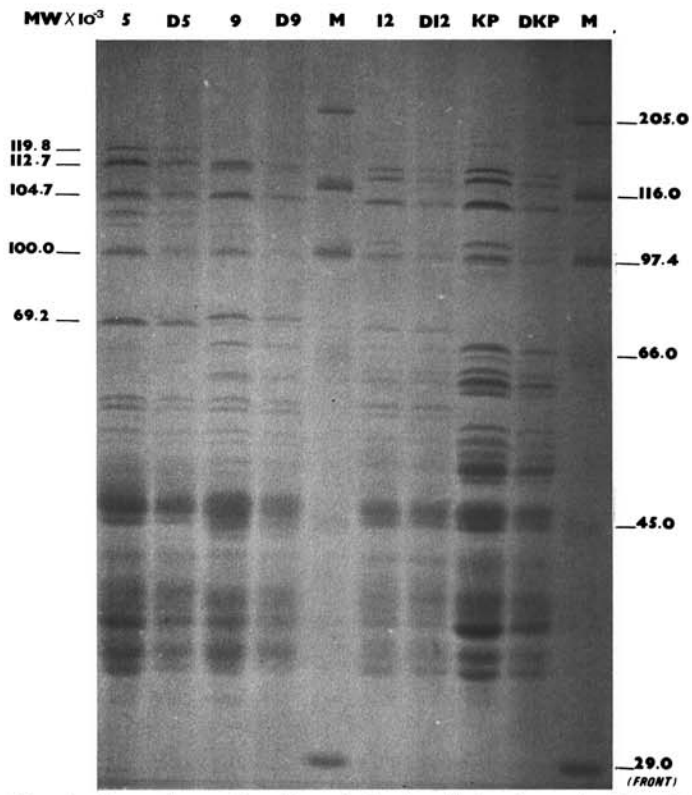

Fig. 5. Comparison of sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of reduced glutenins of vital and devitalized (prefix D) gluten samples.

be analyzed by PAGE. The difference in the results of the two studies is probably due to the different methods of devitalization. In the present study, the magnitude of the decrease in band intensity appears to be sample specific; the greatest decrease was for samples 9 and 12 and the least for the laboratory-prepared gluten (sample KP). Also, it was noted that the intensity of bands for samples 9 and 12 was somewhat lower than that of the bands for samples 5 and KP. These results suggest that samples 9 and 12 had already suffered some devitalization during manufacture. The fact that samples 9 and 12 showed the greatest change on devitalization, and that sample KP showed the least change, suggests that prior heat devitalization sensitizes the gliadin proteins to subsequent further devitalization by elevated temperature.

SDS-PAGE electrophoregrams (Fig. 4) showed that the intensity of all bands decreased upon devitalization (Fig. 5). The decrease of band intensity appears to be greater for the commercial glutens than for the laboratory-prepared glutens. Taken together, the PAGE and SDS-PAGE results indicate a decrease in solubility/extractability of the proteins upon devitalization. The two techniques should be useful in fundamental studies of thermal properties of gluten.

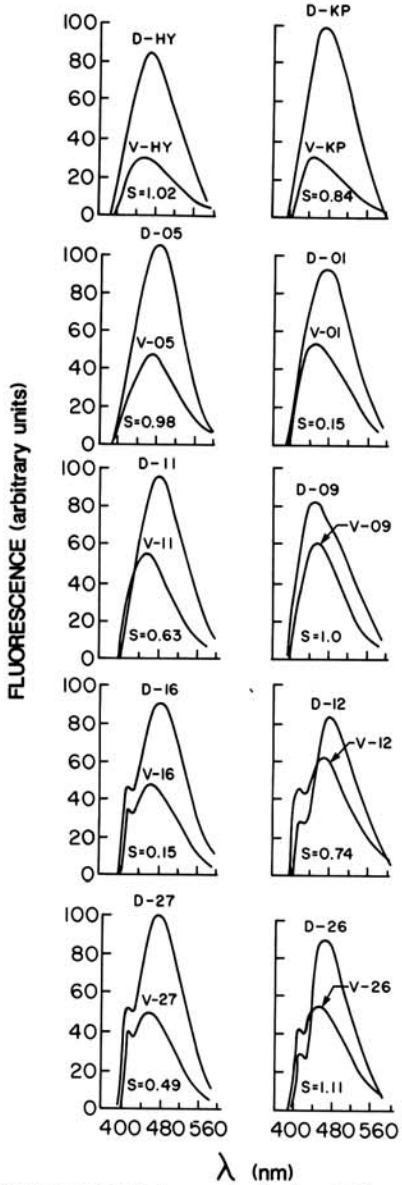

Fig. 6. Fluorescence emission spectra for dry vital (prefix V) and devitalized (prefix D) gluten samples. S = standard deviation.

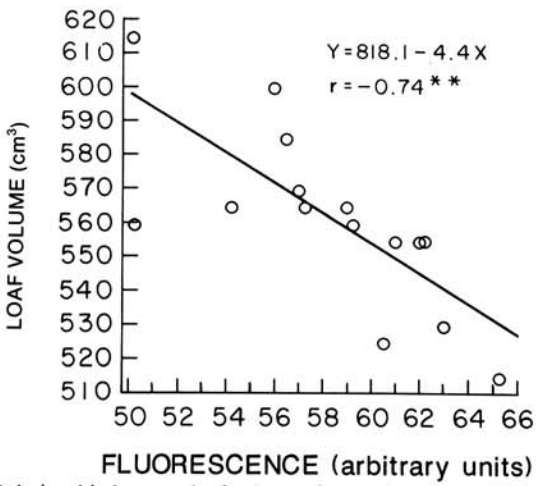

Fig. 7. Relationship between loaf volume of bread from gluten-flour (5:95) blends and peak fluorescence value of dry gluten.

Intrinsic Fluorescence Results

Vital and devitalized gluten samples were examined by fluorescence spectroscopy because it has been reported that the technique can be used to detect and measure thermal denaturation of some proteins, for example thermolysin (Khan et al 1980) and soybean 7S globulin (Yamagishi et al 1982). Commercial gluten samples of lower vitality gave higher intrinsic fluorescence values than samples of higher vitality (Fig. 6). A highly significant

TABLE III
Fluorescence Values for Dry Gluten Samples

Sample	Vital Gluten	Devitalized Gluten	% Increase Upon Devitalization
01	54.2	90.4	66.8
04	60.5	97.5	61.2
05	50.2	111.0	121.1
07	61.0	110.0	80.3
08	62.2	95.0	52.7
09	63.0	88.1	39.8
11	57.0	88.4	55.1
12	65.2	90.0	38.0
13	50.2	118.0	135.1
16	58.9	87.9	49.2
19	62.0	111.0	79.0
22	57.2	99.1	73.2
23	59.2	125.0	111.1
26	56.6	107.0	89.0
27	56.0	110.0	96.4
KP	31.3	96.3	207.7
HY	30.7	81.3	164.8

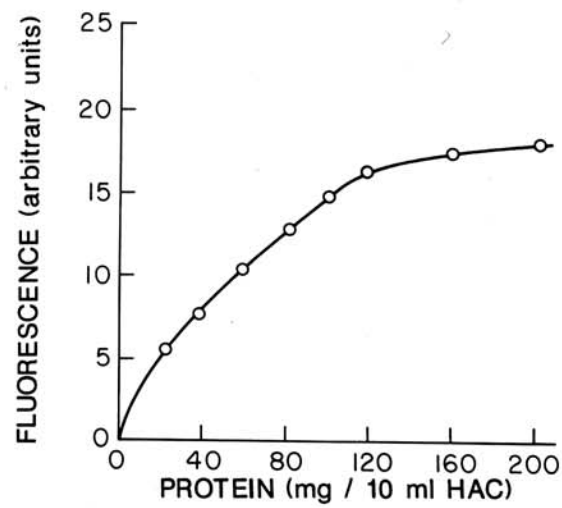

Fig. 8. Effect of the amount of protein dissolved in acetic acid (**HAc**) upon the fluorescence value of the solution.

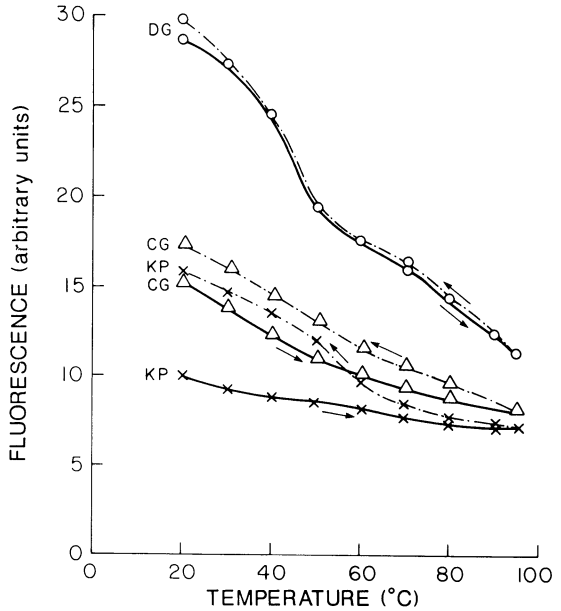

Fig. 9. Effect of heating and cooling (20–100°C) of acetic acid soluble gluten protein upon its fluorescence value. **KP** = Katepwa gluten, **CG** = commercial gluten, **DG** = devitalized gluten. Arrows indicate direction of temperature change.

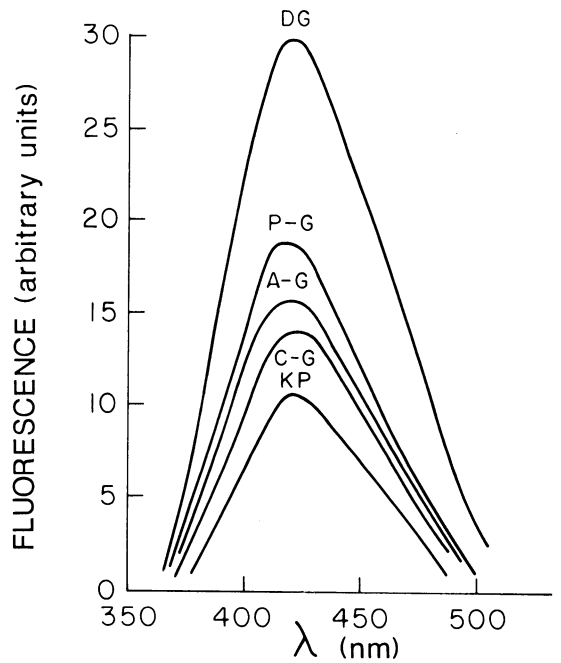

Fig. 10. Fluorescence spectra of gluten proteins dissolved in acetic acid. **DG** = devitalized, **P-G** = poor vitality gluten, **A-G** = average vitality gluten, **G-G** = good vitality gluten, and **KP** = Katepwa gluten.

negative correlation was obtained between vitality and fluorescence value for the 15 gluten samples that were analyzed (Fig. 7). Fluorescence increased on devitalization (Table III). The two laboratory-prepared glutens had similar fluorescence values that were lower than those of the commercial glutens. The marked increase in fluorescence during devitalization may be explained by the presumed unfolding of the protein at high temperatures, which results in exposure of buried hydrophobic groups that fluoresce. The fundamental aspects of fluorescence of gluten proteins warrant investigation.

Fluorescence of Gluten Extracts

Fluorescence can also be measured on proteins in solution. In the present study gluten extracts with 0.05*N* HAc solution were examined by fluorescence spectroscopy. Fluorescence

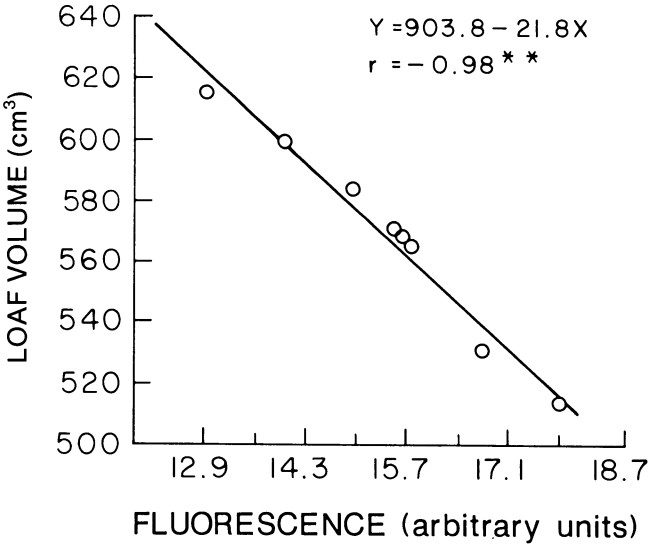

Fig. 11. Relationship between loaf volume of breads from gluten-flour blends (5:95) and fluorescence value of acetic acid soluble gluten proteins.

increased markedly with protein concentration and eventually leveled off to a plateau at approximately 10 mg/ml (Fig. 8). Accordingly this concentration was adopted for comparison of gluten samples. Also, it was observed that fluorescence decreased with increasing temperature (Fig. 9). The rate of decrease with temperature was greater for devitalized gluten than for vital gluten. The largest difference between samples of vital gluten was at the lowest temperature used in the measurements.

With regard to the effect of temperature on fluorescence, it was observed that the fluorescence at a specific temperature was higher if the temperature was reached by cooling than if it was reached by heating. This observation is generally consistent with the expected hysteresis effect in the unfolding and refolding of proteins with increasing and decreasing temperature.

Comparative fluorescence values of HAc extracts of three selected commercial glutens, one laboratory-prepared gluten, and one sample of devitalized gluten are presented in Figure 10. A highly significant negative correlation was obtained between vitality and fluorescence values of the HAc extracts for the eight samples examined here ($r = -0.98$, $P < 0.01$; Fig. 11). Analysis of additional samples would be required to increase the number of data values for this statistical analysis.

CONCLUSION

This study confirmed previous findings that gluten vitality for breadmaking is directly related to solubility in diluted HAc solution. Electrophoresis can be used to detect (and perhaps quantitate) the loss of vitality during thermal denaturation. Additionally it was shown that either of the two fluorescence techniques (i.e., direct measurement on dry vital gluten or measurement on the HAc extract) can differentiate glutens in terms of vitality as measured by the baking test. Of the two techniques, the one based on dry gluten could be a useful practical test of gluten vitality applicable to routine quality control in the manufacture of vital gluten.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 46-13, approved October 1976. The Association: St. Paul, MN.

BOOTH, M. R., BOTTOMLEY, R. C., ELLIS, J. R. S., MALLOCH, G., SCHOFIELD, J. D., and TIMMS, M. F. 1980. The effect of heat on gluten's physico-chemical properties and baking quality. Ann. Technol. Agric. 29:399.

CHEN, C. H., and BUSHUK, W. 1970. Nature of proteins in triticale and its parental species. I. Solubility characteristics and amino acid

composition of endosperm proteins. Can. J. Plant Sci. 50:9.
JEANJEAN, M. F., DAMIDAUX, R., and FEILLET, P. 1980. Effect of heat treatment on protein solubility and viscoelastic properties of wheat gluten. Cereal Chem. 57:325.
KHAN, S. M., DARNALL, D. W., and BIRNBAUM, E. R. 1980. Tyrosine fluorescence as a measure of denaturation in thermolysin. Biochim. Biophys. Acta. 624:1.
NG, P. K. W., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weight of glutenin subunits by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Cereal Chem. 64:324.
ORTH, R. A., and BUSHUK, W. 1972. A comparative study of the protein of wheats of diverse baking qualities. Cereal Chem. 49:268.
ORTH, R. A., and O'BRIEN, L. 1976. A new biochemical test of dough strength of wheat flour. J. Aust. Inst. Agric. Sci. 42:122.
PENCE, J. W., MOHAMMAD, A., and MECHAM, D. K. 1953. Heat denaturation of gluten. Cereal Chem. 30:115.
SAPIRSTEIN, H. D., and BUSHUK, W. 1985. Computer-aided analysis of gliadin electrophoregrams. I. Improvement of precision of relative mobility determination by using a three reference band standardization. Cereal Chem. 62:372.
SCHOFIELD, J. D., BOTTOMLEY, R. C., TIMMS, M. F., and BOOTH, M. R. 1983. The effect of heat on wheat gluten and the involvement of sulphydryl-disulphide interchange reactions. J. Cereal Sci. 1:241.
WADHAWAN, C., and BUSHUK, W. 1989. Studies on vitality of gluten. I. Physical, chemical, and technological characteristics. Cereal Chem. 66:456.
YAMAGISHI, T., EBINA, F., and YAMAUCHI, F. 1982. Analysis of the state of aromatic amino acid residues in heated soybean 7S globulin by absorption derivative spectrophotometry and spectrofluorimetry. Agric. Biol. Chem. 46:2441.

[Received September 14, 1988. Revision received May 24, 1989. Accepted May 30, 1989.]