Denaturation of Wheat Endosperm Proteins During Drying

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ABSTRACT

Wheat seeds (*Triticum aestivum*) of different moisture contents were dried at different temperatures and times. Drying decreased the subsequent loaf volume and the solubility of flour proteins. This loss of solubility resulted from sulfhydryl-disulfide interchange reactions. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis performed on flour and dough protein extracts treated or untreated with mercaptoethanol showed a decrease of certain polypeptides as a consequence of drying. This decrease was observed mainly in the glutenin fraction. The results indicate that two types of polypeptides, which differ in molecular weight and disulfide bonds, were among the protein species most affected by the thermal treatment. The loss of baking quality correlates with the alterations observed in the proteins.

Hot air drying is a widely used procedure to allow storing of wheat harvested at a moisture content higher than the adequate limit for conservation. However, during this process the seed can suffer alterations that affect both its viability and its baking quality. As a result, the heat-damaged wheat may not be apt for baking or for use as seed (Hook 1980). Denaturation of wheat germ proteins due to drying of the seeds, which is related to their viability, was studied previously and the results published elsewhere (Lupano and Añón 1986). Regarding alterations in the endosperm, several studies concern the effect of temperature on gluten proteins (Booth et al 1980, Anno 1981, Schofield et al 1983). The glutenin fraction was found to be more sensitive to heat than the gliadin fraction; these changes involve disulfide bonds. Nevertheless, there are still many aspects to be solved in this matter, and in-depth analyses of the alterations produced in flour proteins as a consequence of seed drying could not be found in the literature.

The aim of the present work was to analyze the changes undergone by flour proteins during the drying process, to compare the results obtained with those found in the literature on heated gluten, and to correlate these alterations with loss of industrial quality.

MATERIALS AND METHODS

Wheat Sample

The sample used in this study was hard wheat *Triticum aestivum* L. Marcos Juarez-INTA grown in Pergamino, Argentina, during 1982–1983.

Drying

Grain was dried by placing a layer about two seeds thick on a cotton net and into a ventilated oven. Temperatures were determined by means of copper-constantan thermocouples placed above the grain layer, at the grain surface, and also within the grain.

Table I shows the moisture content before and after drying, the air temperature, the final grain temperature, and the drying time. Reference to the various moisture-temperature drying regimes throughout this paper correspond to those given in Table I. Seeds without any treatment were used as control.

Moisture Content

Different moisture levels were obtained by direct addition of distilled water. Seeds were immersed in water at 4 or 10° C until they reached the desired moisture level. Then the grains were dried with a cotton cloth to remove the superficial water and kept at room temperature for 1 hr 30 min before drying.

Moisture content was determined by drying the whole seeds in an oven at 130° C for 2 hr. Values obtained were corrected to the AOAC method (AOAC 1980, Giner and Calvelo 1987).

Flour Extraction

Seeds were milled in a Brabender Junior mill. The flour extraction rate was about 50%.

Preparation of Doughs and Baking

A small-scale microbaking technique was used, according to the official method of the Ministerio de Agricultura de la Nación, adapted to to small samples. Doughs were prepared by mixing 10 g of flour and other ingredients (Table II) for 1.5 or 2 min. The fermenting time was 75 min. During fermenting (30° C and 80% relative humidity), doughs were degassed two times (at 15 and 30 min after mixing began), and at 45 min the loaves were molded. Loaves were baked in an electric oven with wet atmosphere for 15 min at 210° C. Breads were allowed to cool, and then volumes were determined by rapeseed displacement.

Zeleny Test

Assays were carried out according to the method of Zeleny (Zeleny 1947), measuring the sedimentation volume of 3.2 g of flour.

Protein Extraction

Endosperms were cut and manually separated from the seeds. Samples of 15 endosperms were placed into centrifuge tubes (30-ml capacity). Proteins were extracted with 2% sodium dodecyl sulfate (SDS)-0.1 *M* tris-HCl, pH 8 (Bottomley et al 1982), at a solvent-to-endosperm ratio of 20:1. The samples were exposed to the solvent and stirred occasionally for 24 hr. After extraction, the samples were centrifuged at 17,000 × *g* for 1 hr and 10 min. All treatments were carried out between 0 and 5° C. The supernatants were stored at −60° C.

Extraction of flour and dough proteins was performed as described for endosperm. Solvent-to-flour ratio was 25:1, and solvent-to-dough ratio was 15.6:1. Dough was prepared by mixing flour and distilled water at a ratio of 1:0.6.

Protein Content

The soluble protein content was determined according to the method of Lowry et al (1951), using bovine serum albumin as a standard. The standard solutions were prepared under the same conditions as the samples. Total flour protein content was determined using Kjeldahl Buchi equipment. The conversion factor was N × 5.7. Both determinations were performed in duplicate.

Electrophoresis

Slab SDS-polyacrylamide gel electrophoresis (PAGE) of the protein extracts was performed according to the Laemmli

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discontinuous buffer system (Laemmli 1970, Laemmli and Favre 1973) at a gel concentration of 12.6%, using a Pharmacia gel electrophoresis apparatus GE-2/4. Gel slabs were fixed in a solution of isopropanol, acetic acid, and water (25:10:65) and stained with 0.02% Coomassie Brilliant Blue R-250 in 7% acetic acid. The gels were scanned in a Shimadzu dual wavelength TLC scanner CS-910 (sample wavelength 570 nm and reference wavelength 395 nm) attached to a C-R 1A chromatopac Shimadzu integrator.

The molecular weights of the proteins of the different bands were estimated by the use of a Pharmacia protein molecular weight calibration kit. The standard proteins used and their molecular

TABLE I
Drying Conditions of the Seeds[a]

Treatment	Moisture Content Before Drying[b] (%)	Moisture Content After Drying[b] (%)	Drying Time (min)	Air Temperature (°C)	Final Temperature of Seeds (°C)
Ia	22.2	16.2	25	110	53
Ib	22.5	17.5	20	125	56
Ic	22.1	16.7	15	140	62
IIa	27.9	18.4	40	110	59
IIb	27.5	16.0	35	125	57[c]
IIc	27.9	15.9	30	140	63
IIIa	35.4	16.2	65	110	60
IIIb	34.8	15.9	55	125	61
IIIc	34.8	16.6	45	140	70

[a]Seeds without treatment were used as control.
[b]Expressed as grams of water per 100 g dry matter.
[c]The temperature of seeds of lot IIb was less than that of lot IIa because the thermocouple was positioned differently within the grain.

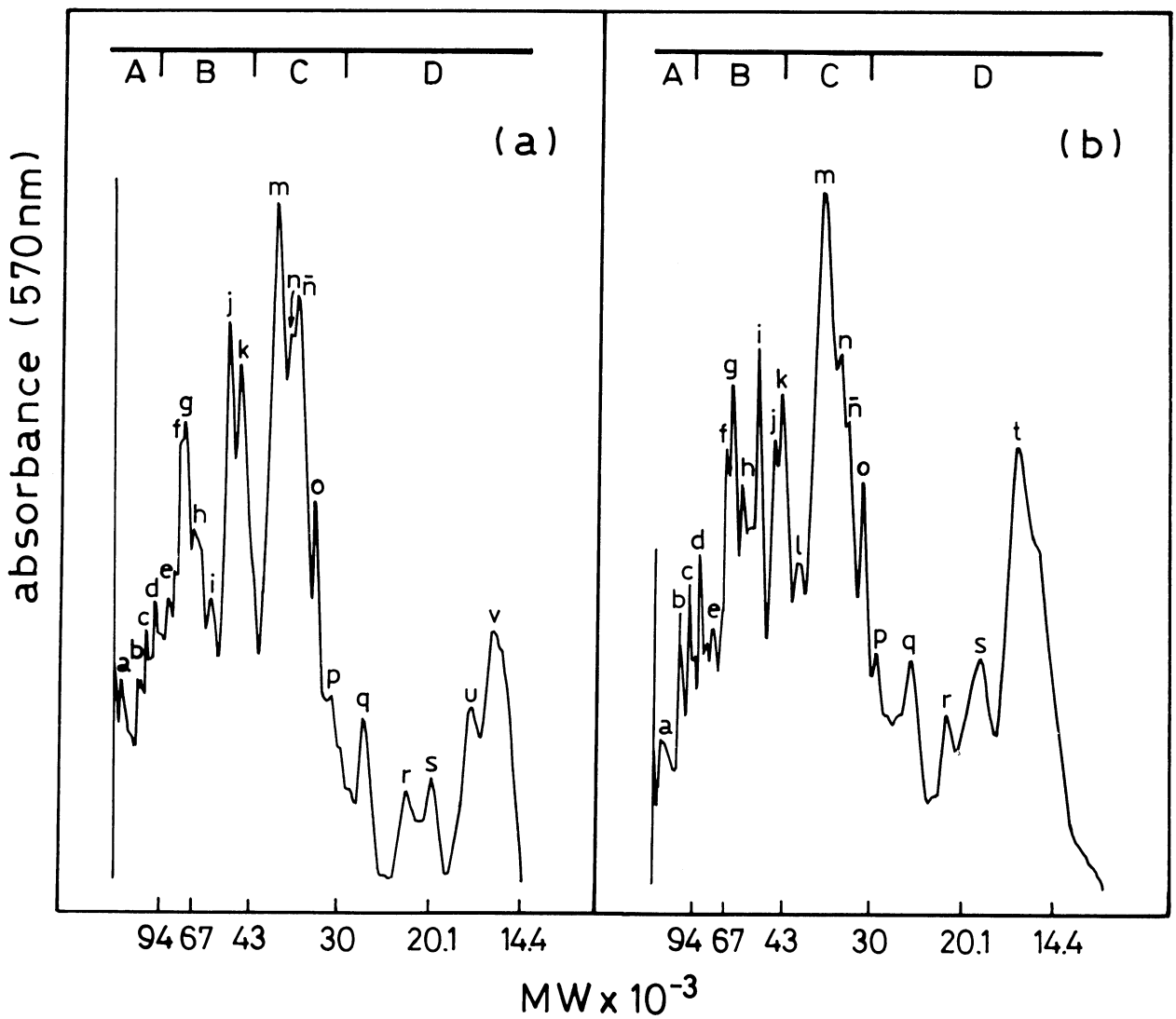

Fig. 1. Densitometric tracings of the electrophoretic patterns of endosperm proteins from control wheat seeds. On the left (**a**), the sample was treated with sodium dodecyl sulfate (SDS); the right (**b**) sample was treated with SDS and mercaptoethanol. Molecular weights of the peaks: **a** = 121,000, **b** = 103,000, **c** = 97,000, **d** = 89,000, **e** = 79,000, **f** = 70,000, **g** = 67,000, **h** = 62,000, **i** = 54,000, **j** = 47,000, **k** = 43,000, **l** = 40,000, **m** = 37,000, **n** = 34,000, **ñ** = 33,000, **o** = 31,000, **p** = 30,000, **q** = 26,000, **r** = 22,000, **s** = 20,000, **t** = 16,000, **u** = 17,000, **v** = 15,000.

weights were as follows: phosphorylase-b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400).

Samples were treated with sample buffer (0.01*M* tris-0.001*M* ethylenediaminetetraacetic acid-1% SDS, pH 8, with or without mercaptoethanol [ME]), at a sample-to-buffer ratio of 3:1 for endosperm, and 2:1 for flour and dough. The samples were heated at 100°C in a boiling water bath for 5 min immediately before electrophoresis.

RESULTS AND DISCUSSION

Assays of Baking Quality

Table III shows the loaf volumes obtained by small-scale test baking in terms of percentage of the control. More severe seed drying conditions resulted in smaller loaf volumes. When dough leavening was decreased, loaf shape became more irregular.

Results of the Zeleny test are also shown in Table III. This method reflects the flour protein quality and consists of determining the sedimentation volume of a suspension of flour in a lactic acid solution. The sedimentation volume is higher when the flour protein quality is better. Data in Table III show that the sedimentation volume was smaller for flour obtained from heated seeds.

Solubility of Flour Proteins

Content of total protein of flour from control seeds was determined by the Kjeldahl method; the value was 9.65% (wet basis). The determination of soluble protein yielded a value of 8.95% (wet basis), which represents 92.7% of the total protein content. These values are in agreement with those obtained by Bottomley et al (1982), who reported a yield of 95% for the used extraction solution.

In this study, the soluble protein contents of seeds subjected to different drying treatments (determined on two extractions and expressed as a percentage of the total protein content) were 93.7–97.0 for treatment Ib; 88.0–93.7, Ic; 95.0–98.7, IIa; 87.0–87.3, IIIb; and 79.0–79.7, IIIc. An analysis of variance of these data showed that there are differences at a level of significance of 1% in protein solubility among lots of seeds subjected to different thermal treatments ($F_{0.01} = 11.4$; $F_{\text{solubility}} = 17.15$). As can be readily seen, the solubility of the flour proteins decreased as the drying conditions became more severe, and this might result from the formation of aggregates during seed drying. Because these proteins became insoluble in an SDS-containing medium, these aggregates could have developed through formation of covalent bonds. Working with heated gluten, Schofield et al (1983) found that during thermal treatment, sulfhydryl-disulfide interchange reactions could be produced that were probably facilitated by unfolding of the proteins when the temperature rose. According to the results of the present work, this hypothesis could be extended to heating of seeds.

SDS-PAGE of Endosperm Proteins from Control Seeds

Figure 1 shows typical electrophoretic patterns of endosperm proteins of control seeds and the molecular weights of the peaks. Figure 1a corresponds to an SDS-treated sample and Figure 1b to a sample treated with SDS and ME.

By knowing the range of molecular weights of the different endosperm protein fractions, these diagrams can be analyzed by regions. Thus, gliadins would be located in the regions of molecular weights between 30,000 and 45,000 (region C of Fig. 1)(Bietz and Wall 1972, Sexson and Wu 1972, Ewart 1973), except for the ω-gliadins, which have higher molecular weights (between 75,000 and 79,000; Fig. 1, region A); whereas the glutenin subunits with molecular weights between 11,000 and 133,000 (Bietz and Wall 1972) would account for all regions of Figure 1. Albumins and globulins should also be present in these electrophoretic patterns, even though they normally account for only 10–20% of the endosperm proteins (Simmonds 1978), and would be located, at least partially, in the region of molecular weights lower than 30,000 (Booth et al 1980), which corresponds to zone D of Figure 1.

The differences between the patterns of Figures 1a and b appear mainly in zones B and C, the result of the presence of interchain disulfide bonds. These differences should correspond to the glutenin subunits, because gliadins only have intrachain disulfide bonds (Wall 1979). These electrophoretic patterns are very similar to the diagrams for flour and dough proteins.

Changes in Electrophoretic Patterns due to Seed Drying

Protein extracts of flour and dough, from both control seeds and seeds dried under some of the conditions listed in Table I, were used for the electrophoretic runs. Because the electrophoretic patterns for flour and dough are very similar, and the same differences due to thermal treatment were found with both types of samples (Fig. 2), they are considered together in the results obtained.

Electrophoretic patterns corresponding to flour from control seeds and from seeds drying under two of the conditions listed in Table I (lots Ib and IIIb) can be seen in Figure 3. The upper part includes electrophoretic patterns corresponding to samples treated with SDS and ME, and the lower part corresponds to samples treated with SDS only.

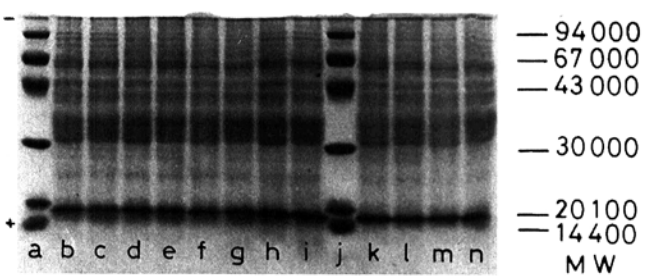

Fig. 2. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoretic patterns of wheat flour and dough proteins from seeds dried under different conditions: **a,j** = marker proteins; **b,c** = dough, control; **d,e** = dough, Ic; **f,g** = dough, IIIc; **h,i** = flour, control; **k,l** = flour, Ic; **m,n** = flour, IIIc. **b,d,f,h,k,m** = samples treated with SDS; **c,e,g,i,l,n** = samples treated with SDS and mercaptoethanol. **MW** = molecular weight.

TABLE II
Bread Formula

Ingredients[a]	%
Flour	100
Yeast	3
Salt	1
Water	variable
Sugar	2.5
Malted flour	0.1

[a] On flour basis.

TABLE III
Assays of Baking Quality

Drying Condition	Control	Ia	Ib	Ic	IIa	IIb	IIc	IIIa	IIIb	IIIc
Loaf volume, %[a]	100	92.6	92.6	88.9	88.9	92.6	85.2	92.6	85.2	74.1
Zeleny test volume, ml	24	22	21	21	19	20	18	19	18	18

[a] Results are expressed as percentages of control loaf volume.

A decrease of some of the peaks in the region of molecular weights higher than 70,000 was observed in samples obtained from heated seeds. In the region of molecular weights between 45,000 and 70,000, a peak that decreases in heated seed samples treated with SDS and ME can be observed (peak 3, Fig. 3). This decrease was not observed—at least to the same extent—in the electrophoretic patterns of samples treated with SDS only. In these patterns this band was much less intense, as can be seen in the diagrams corresponding to the control seeds where the effect of drying is not superimposed. This would be caused by the presence of subunits linked by disulfide bonds that are soluble in the extraction medium and, upon treating the samples with ME, undergo separation and appear in the gel in an already existing band. It should be kept in mind that peak 3 in these patterns is formed by several peaks of similar molecular weights, as can be seen in the diagrams of Figure 1. As a consequence of the thermal treatment of the seeds, these proteins would form compounds that are insoluble in the extraction medium.

In order to accentuate the differences observed, the relative heights of some of the peaks (peaks 1, 2, and 3 in Fig. 3) were measured with reference to a peak that did not show variations upon heating (asterisked peak in Fig. 3). These measurements were made on flour and dough protein samples, either treated or untreated with ME (Table IV). Values corresponding to peaks 1 and 2 were similar in untreated samples or those treated with ME, so they were considered together in Table IV and in the statistical treatment. An analysis of variance showed that there are differences at a level of significance of 1% in the relative height of peaks 1 and 2, and in the relative height of peak 3 in samples treated with ME, among lots of seeds subjected to different thermal treatments ($F_{\text{peak 1}} = 9.35$, $F_{\text{peak 2}} = 10.66$, $F_{\text{peak 3 without ME}} = 1.36$, $F_{\text{peak 3 with ME}} = 20.99$, $F_{0.01} = 3.51$ for peaks 1 and 2, $F_{0.01} = 4.34$ for peak 3 with and without ME). Results obtained are listed in Table IV, where a decrease of the three peaks (except in peak 3 in samples treated only with SDS) can be seen as the drying conditions become more severe. Peak 3 decreases in samples treated with ME,

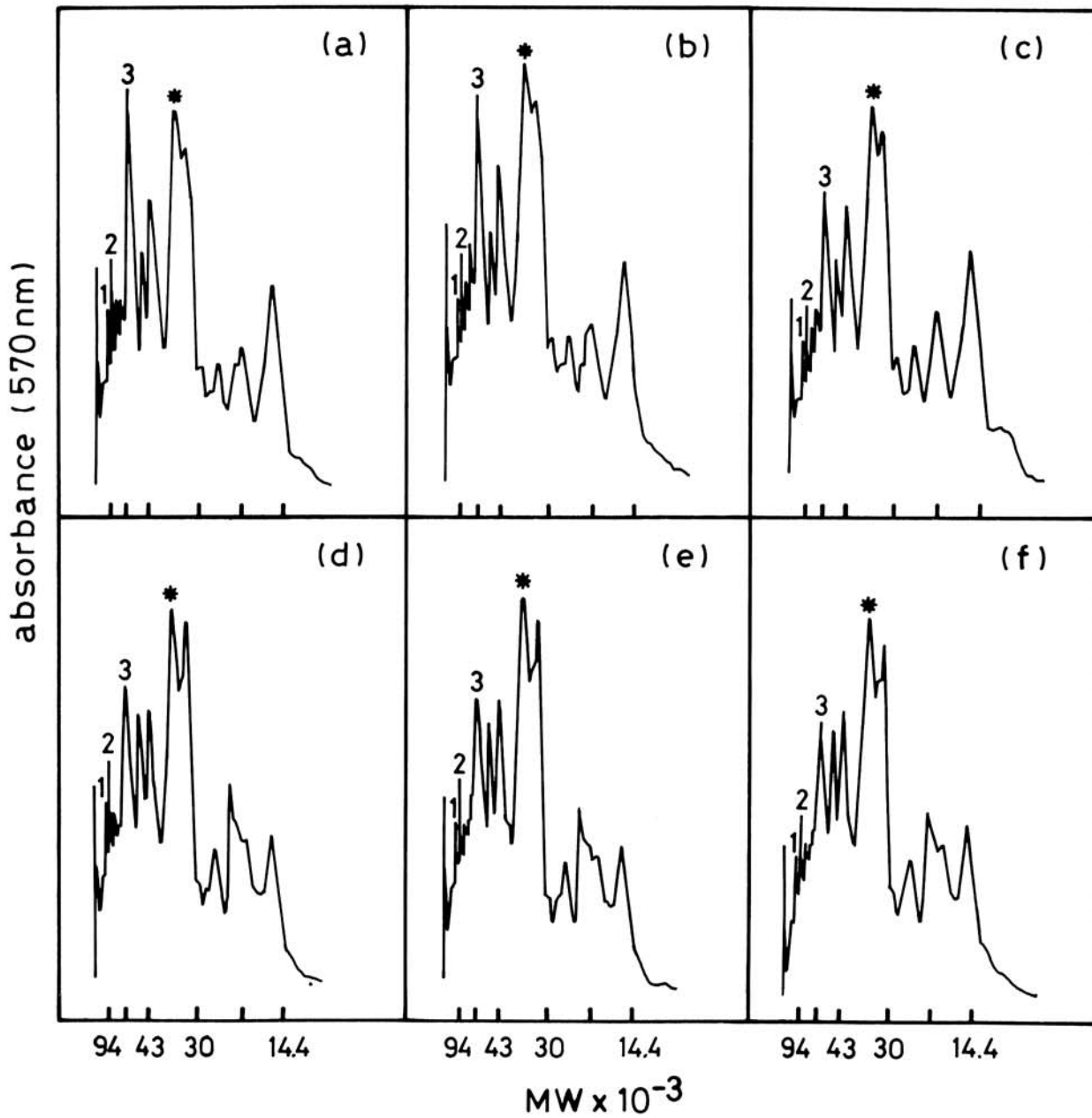

Fig. 3. Densitometric tracings of the electrophoretic patterns of wheat flour proteins from seeds dried under different conditions. Upper part: samples were treated with sodium dodecyl sulfate and mercaptoethanol. Lower part: samples were treated with sodium dodecyl sulfate only. **a,d** = control; **b,e** = Ib; **c,f** = IIIb. Reference to the various moisture-temperature drying regimes correspond to those given in Table I.

TABLE IV
Relative Heights of Electrophoretic Peaks

Peak	Control	Drying Condition				
		Ib	Ic	IIa	IIIb	IIIc
1[a]						
Mean	0.399	0.365	0.313	0.421	0.273	0.181
SD	0.084	0.084	0.032	0.084	0.093	0.082
n	8	8	7	8	8	7
2[a]						
Mean	0.554	0.478	0.426	0.541	0.384	0.287
SD	0.091	0.098	0.051	0.084	0.084	0.090
n	8	8	7	8	8	7
3 SDS treated[b]						
Mean	0.860	0.750	0.717	0.828	0.700	0.730
SD	0.115	0.105	0.031	0.062	0.124	0.155
n	4	4	3	4	4	4
SDS + ME[b]						
Mean	1.028	0.930	0.910	0.965	0.735	0.690
SD	0.105	0.029	0.014	0.053	0.035	0.044
n	4	4	4	4	4	3

[a]These values correspond to flour and dough proteins, either treated or untreated with ME.

[b]These values correspond to flour and dough proteins. SDS = sodium dodecyl sulfate, ME = mercaptoethanol.

in which it reaches values similar to those obtained in samples treated with SDS only.

On the basis of these results it can be concluded that alterations of endosperm proteins may take place during drying of the seeds. These alterations are noted by a loss of solubility in an SDS-containing medium, and the changes involve sulfhydryl-disulfide interchange reactions. Among proteins that have a direct effect on baking quality, glutenin was the most affected fraction. Similar studies on the effect of seed drying were not found in the literature, but our results can be compared with those of other authors (Booth et al 1980, Schofield et al 1983), who worked with heated gluten and obtained results similar to ours. The electrophoretic studies performed on samples either treated with ME or not, shows that two types of protein species were among the proteins most affected by the thermal treatment: a type of high molecular weight that is not derived from chains bound by disulfide bonds, and another type of lower molecular weight that had been part of higher molecular weight proteins by means of disulfide bonds.

Correlations Between the Alterations of Proteins and Loaf Volume

Figure 4a shows the correlation between the loaf volume and the solubility of the flour proteins in the solution used for the extraction, whereas Figure 4b shows the correlation between the loaf volume and the relative heights of peaks 1, 2, and 3 in the electrophoretic diagrams. The correlation coefficients were 0.946 for protein solubility, 0.861 for peak 1, 0.898 for peak 2, and 0.900 and 0.632 for peak 3 in samples treated with ME and untreated, respectively. The results indicate that there is a linear correlation between the decrease in solubility of the flour proteins and the loss of baking quality, and between the latter and the dissappearance of some bands in the electrophoretic patterns. In the case of peak 3, the correlation, which was the highest, was only observed in samples treated with ME, whereas the decrease in the region of high molecular weights (peaks 1 and 2), was equivalent in samples either treated or untreated with ME.

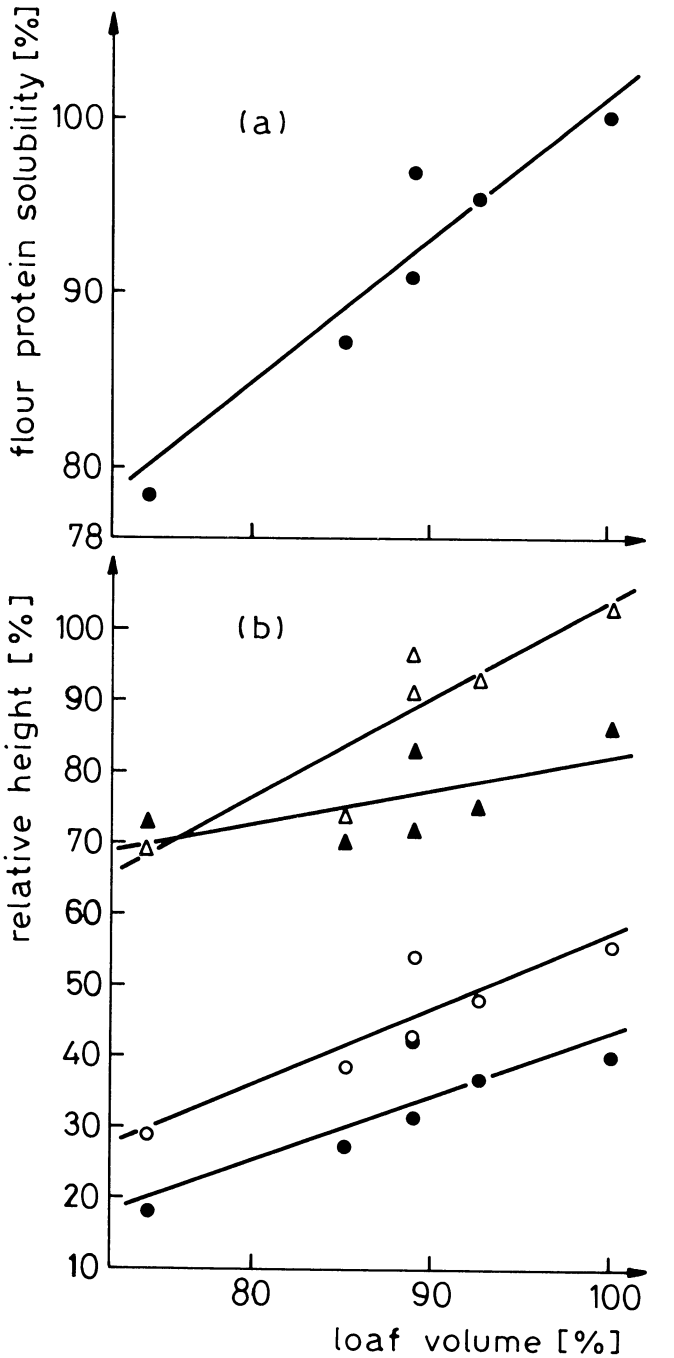

Fig. 4. (a) Correlation between loaf volume and solubility of flour proteins. **(b)** Correlation between loaf volume and relative height of peaks 1, 2, and 3 of Fig. 3. • = peak 1; o = peak 2; Δ = peak 3 (samples treated with mercaptoethanol); ▲ = peak 3 (samples not treated with mercaptoethanol).

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