Denaturation of Wheat Germ Proteins During Drying

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ABSTRACT

Wheat seeds (*Triticum aestivum*) of different moisture content were dried at different temperatures and times. Drying decreased the viability of the seeds, the heat of transition obtained by differential scanning calorimetry, and the solubility of germ proteins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed the disappearance of certain polypeptides after drying. These results indicate that the drying process causes a denaturation of the germ proteins. The results obtained by differential scanning calorimetry also suggest that the deterioration of wheat germ by high temperatures follows a first-order kinetics.

Drying with hot air is usually utilized to preserve grains by decreasing water content. Seeds can undergo different alterations during drying. Wheat that has been heated above a critical temperature (depending on its moisture content) shows damaged baking quality and germinative capacity. As a consequence, overheated wheat can be rendered useless both for growing and baking. Thus, drying temperature, time, and moisture content of the seeds should be considered when studying the detrimental effects of drying (Daudin 1983, Hook 1980, Multon 1982).

Data related to heat damage of wheat germ are not currently available, as most authors have carried out their studies on flour. The objective of this work was to study the changes in wheat germ dried under different conditions. The germinative capacity and the extent of protein denaturation were assessed after drying.

MATERIALS AND METHODS

Wheat Sample

Wheat used in this study was *Triticum aestivum* Marcos Juarez 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. The 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, and the drying time. Reference to the various moisture-temperature drying regimes throughout this paper corresponds to that given in Table I.

Moisture Content

Different moisture levels were obtained by direct addition of water. The grains were dried with a cotton cloth to remove the superficial water. Moisture content was determined by drying the seeds in an oven at 130° C for 2 hr; loss of water was expressed as grams of water per 100 g wet matter.

Germination

Assays were carried out at 20° C in sterile petri dishes. Twenty-five seeds were placed in each dish on three layers of filter paper, and distilled water was added. One hundred seeds were assayed for each drying condition. The percentage of germinated seeds was determined after three days.

Protein Extraction

Germs were cut and manually separated from the treated seeds. Samples of 25 germs were placed into centrifuge tubes. Proteins were extracted with 5% NaCl at a solvent to solid ratio of 25:1, and the germs were ground with a pestle. The samples were exposed to the solvent and stirred occasionally for 3 hr and 20 min. After extraction, the samples were centrifuged at 12,000 × g for 20 min. All treatments were carried out between 0–4° C.

Protein Content

The protein content was determined according to the method of Lowry et al (1951), using soluble bovine serum albumin as a standard.

TABLE I
Drying Conditions of the Seeds

Treatment	Moisture Content[a] Before Drying (%)	Moisture Content[a] After Drying (%)	Drying Time (min)	Air Temperature (°C)
I a	16.9	12.3	25	110
I b	17.1	13.4	20	125
I c	16.9	12.7	15	140
II a	20.9	14.1	40	110
II b	20.6	12.1	35	125
II c	20.9	12.2	30	140
III a	25.5	12.3	65	110
III b	25.1	12.1	55	125
III c	25.1	12.7	45	140

[a]Expressed as grams of water per 100 g wet matter.

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Electrophoresis

Slab sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the protein extracts was performed according to the Laemmli 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-R1A 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 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); α-lactalbumin (14,400).

Differential Scanning Calorimetry

A DuPont model 910 system calorimeter calibrated with indium was used. A Hewlett-Packard 7046 B recorder was attached to the thermal analyzer. All samples used for differential scanning calorimetry (DSC) had approximately the same moisture content (about 12%).

Heat of Transition

Germs were cut and manually separated from the seeds. Samples of 10 germs (approximately 6 mg) were transferred to weighed aluminum DSC nonhermetic pans. The pans were reweighed and sealed, and good contact between the sample and the bottom of the capsule was assured. The sample was placed in the calorimeter, using an empty pan as reference. The heating rate was 10°C/min.

After DSC analysis, the pans were punctured and the dry weight was determined by drying overnight at 100°C.

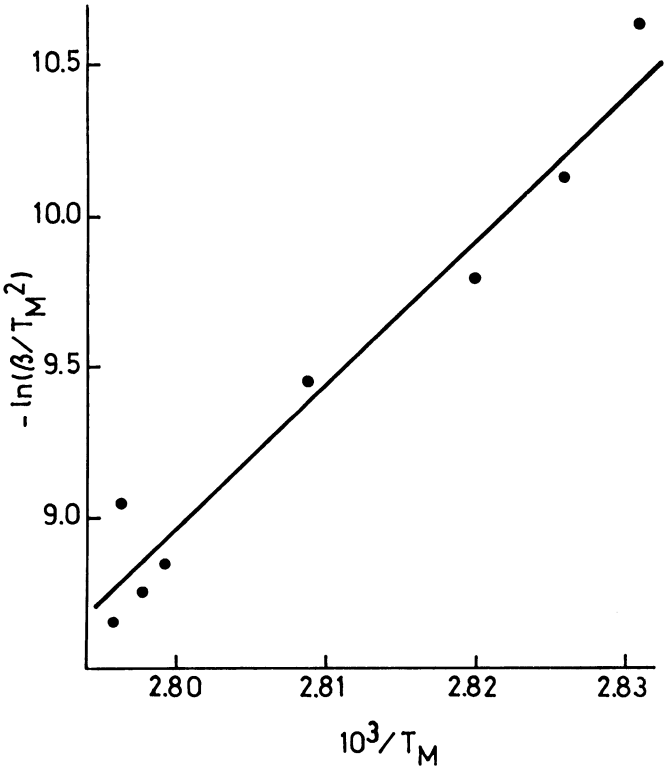

Fig. 1. Calculation of the activation energy (E_a) for the heat denaturation of wheat germ proteins using differential scanning calorimetry: β = heating rate (K/min), T_M = peak temperature (K).

The heat of transition, ΔH, was calculated from the equation:

$$\Delta H \text{ sample} = \Delta H \text{ indium} \frac{W \text{ indium}}{W \text{ sample}} \times \frac{A \text{ sample}}{A \text{ indium}} \times \frac{R \text{ sample}}{R \text{ indium}} \times \frac{S \text{ indium}}{S \text{ sample}} \quad (1)$$

where ΔH = heat of transition (J/g); W = weight (mg); A = area under the curve (mm^2); R = range of sensitivity (J/sec·cm); and S = recorder chart speed (mm/min). The area under the curve was determined with a Morphomat 34 Zeiss image analyzer.

Activation Energy

To calculate the activation energy (E_a), the samples (wheat germs in nonhermetic pans) were heated at eight different rates (β); (β = 3–22°C/min). The peak temperature (T_M) was determined for each run. The activation energy and the pre-exponential factor of the Arrhenius equation (Z) were calculated by plotting $-\ln(\beta/T_M^2)$ vs. $1/T_M$ (Fig. 1) according to the equation (Ozawa 1970):

$$\ln(\beta/T_M^2) = \ln\left(\frac{ZR}{E_a}\right) - \frac{E_a}{RT_M} \quad (2)$$

where β = heating rate ($K \cdot min^{-1}$); T_M = peak temperature (K); Z = pre-exponential factor of the Arrhenius equation (min^{-1}); R = gas constant ($cal \cdot mol^{-1} \cdot K^{-1}$). A least squares minimum linear regression was applied to 46 experimental points, selecting $-\ln(\beta/T_M^2)$ as the independent variable (Pravisani et al 1985). Each point of Figure 1 was the average of at least five runs. The maximum standard deviation was 0.0029 for $\ln(\beta/T_M^2)$ and 0.0040 for $10^3/T_M$.

The activation energy and pre-exponential factor obtained were confirmed by using an isothermal method. The procedure consists of predicting a convenient half-life for the reaction at a certain temperature, using the previously obtained kinetic values. The sample is aged for the predicted time at the proper temperature and then programmed in the DSC. Control germs were cut in two; one part was transferred to a hermetic pan, and the pans were then immersed in a water bath at different temperatures for a period of time equivalent to the half-life. The pans were heated in the DSC equipment (β = 10°C min^{-1}) and the areas under the curves were determined. The other part of each germ was heated in the DSC equipment under the same conditions, but without the isothermal treatment.

The temperature of the germ during drying was calculated indirectly by DSC. This was done by obtaining the rate constants from the ΔH values obtained (Table II) by assuming a first order reaction (Results and Discussion). With the known value of the activation energy, the temperature was calculated by means of the Arrhenius equation. These values were equivalent to those measured during the drying process.

TABLE II
Heat of Transition, Germ Temperature, Germination Assay, and Soluble Protein Content for Each Drying Condition

Drying Condition	Heat of Transition[a] (% ΔH)	Germ Temperature During Drying (°C)	Percentage of Germinated Seeds[a] (%)	Soluble Protein Content[a,b] (%)
I a	65	69.3	87	92
I b	59	70.3	75	86
I c	51	71.6	61	85
II a	55	69.0	77	91
II b	23	71.4	20	75
II c	8	73.1	2	72
III a	37	69.0	44	83
III b	6	71.9	4	76
III c	7	72.3	0	68

[a]Expressed as % with respect to control seeds.
[b]These values are the average of two extractions. The standard deviation was 4%.

RESULTS AND DISCUSSION

Calorimetric Studies

In DSC thermal curves of wheat germ, one endothermic peak at about 83° C was observed. This peak disappeared when the pan was reheated; thus, the transition would be the result of an irreversible process. The activation energy of this process was calculated according to the method of Ozawa. The value obtained was 98.3 kcal·mol^{-1}, with a correlation coefficient of 0.97, and the pre-exponential factor of the Arrhenius equation (Z) was 7.83 10^{60}·min^{-1} (Fig. 1).

The E_a values for thermal denaturation of proteins found in the literature range from 40.8 kcal·mol^{-1} (trypsin) to 129.0 kcal·mol^{-1} (ovalbumin) (Laidler and Bunting 1973). Therefore, the apparent E_a value obtained (98.3 kcal·mol^{-1}), and the characteristics of the thermal curve (peak temperature, irreversible transition) suggest that the recorder transition reflects the denaturation of germ proteins.

The heat of transition obtained for unheated wheat germ was 1.43 J·g^{-1} (dry matter) averaged from five determinations; the standard deviation was 0.27.

Assuming a kinetics of order one with respect to time, the half-life can be calculated and the isothermal method can be used to check the activation energy and frequency factor obtained (Duswalt 1974). The $\Delta H\%$ corresponding to germs subjected to isothermal treatment for a predicted half-life was 45.9 ± 3.9% of that of control germs. This result indicated that the prediction of the reaction order was correct, and the reaction kinetics was then verified.

The thermal curves corresponding to wheat germs of dried seeds in Table I showed a decrease in the peak areas, according to the drying conditions, with reference to control seeds (Fig. 2). Therefore, the ΔH values obtained for these germs were lower than those corresponding to unheated germs.

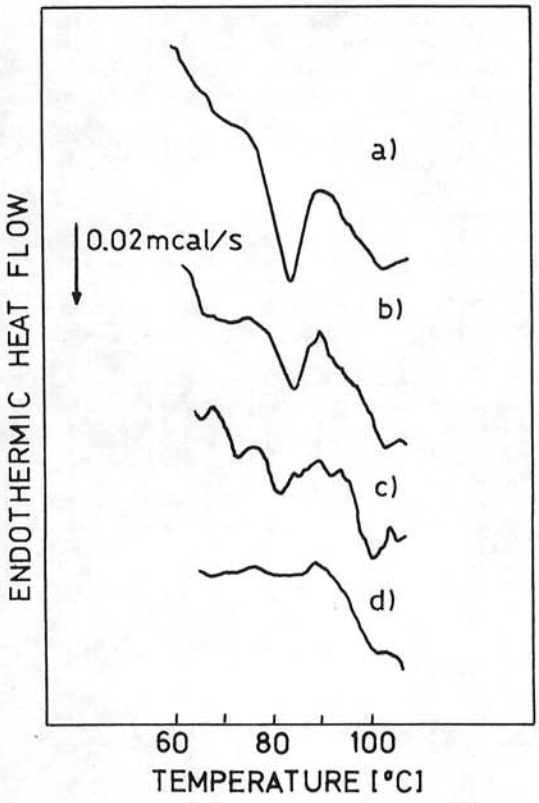

Fig. 2. Thermal curves of wheat germs from seeds dried in different conditions: **a** = Ia, **b** = IIIa, **c** = IIb, **d** = IIIc. Reference to the various moisture-temperature drying regimes corresponds to those given in Table I. Heating rate β = 10°C/min.

Table II shows the values of % ΔH obtained with respect to control seeds and the germ temperature. Seeds labeled with the same Roman numeral had the same initial moisture content and reached approximately the same moisture content after drying (Table I). Table II also shows that the values of ΔH decreased when the temperature of the germs increased.

Effects of Drying on Viability and Germ Proteins

Table II shows the percentage of germinated seeds after three days, and the percentage of soluble proteins of the germ for the different drying conditions. The soluble proteins were expressed in relation to the germ protein content (mg %) with control seeds taken as 100%.

As for % ΔH, the germination values and the protein solubility show a decrease with increasing drying temperature. These results agree with the assumption that the germ proteins become denatured during drying.

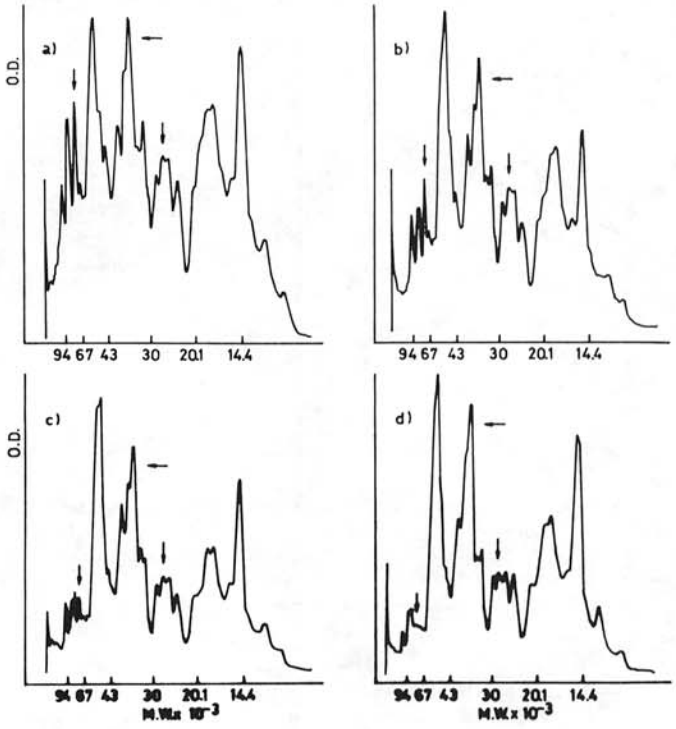

Fig. 3. Densitometric tracings of the electrophoretic patterns of wheat germ proteins from seeds dried under different conditions: **a** = control, **b** = Ib, **c** = IIb, **d** = IIIc. Reference to the various moisture-temperature drying regimes corresponds to those given in Table I.

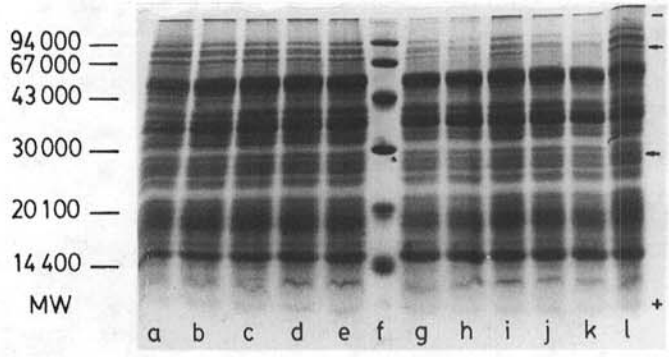

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of wheat germ proteins from seeds dried in different conditions: **a** = control, **b** = Ia, **c** = Ib, **d** = Ic, **e** = IIa, **f** = marker proteins, **g** = IIb, **h** = IIc, **i** = IIIa, **j** = IIIb, **k** = IIIc, **l** = control. Reference to the various moisture-temperature drying regimes corresponds to those given in Table I. The horizontal arrows correspond to the vertical arrows of Fig. 3.

TABLE III
Relative Heights of Electrophoretic Peaks[a]

Drying Condition	Relative Height of the High-Molecular-Weight Peak[b] (%)	Relative Height of the Low-Molecular-Weight Peak (%)
I a	66	91
I b	63	93
I c	51	83
II a	67	87
II b	32	74
II c	19	63
III a	47	83
III b	22	67
III c	15	63

[a]Expressed as % with respect to control seeds.
[b]These values are the average of two determinations.

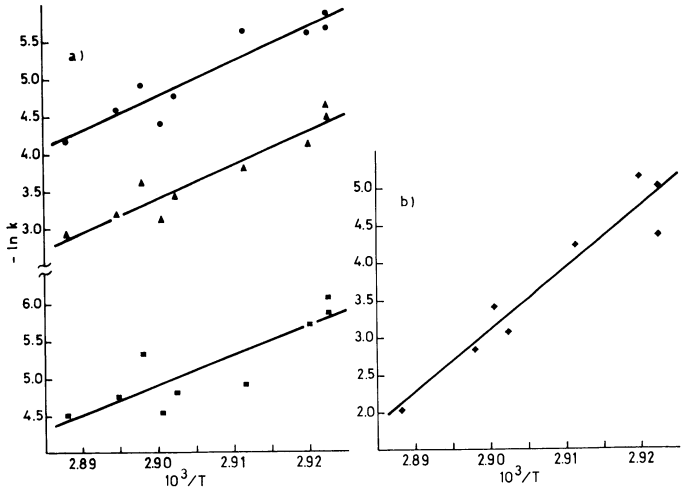

Fig. 5. Calculation of the activation energy (E_a) using the Arrhenius equation. **a**, Denaturation of wheat germ proteins determined by different methods: ● = electrophoresis (low-molecular-weight peak), ▲ = electrophoresis (high-molecular-weight peak), ■ = protein solubility. **b**, Germination: k = rate constant, T = temperature (K).

The electrophoretic patterns of germ proteins showed a decrease of some of the bands, according to the drying conditions of the seeds, in the zones of molecular weights 75,000 and 30,000 (Figs. 3 and 4). The relative heights of two of these peaks, taken as an example (vertical arrows in Fig. 3) with reference to another peak that did not change according to drying (horizontal arrow in Fig. 3), were determined. Table III shows these values as percentages of those corresponding to control seeds. A decrease of the polypeptide bands with increasing germ temperature can be observed; this is similar to the trends shown by % ΔH, germinative capacity, and solubility of the germ proteins. Decrease of the polypeptide bands could be caused by denaturation and subsequent insolubilization of proteins or by protein degradation.

Because the assumption of first order kinetics for the % ΔH was confirmed by the isothermal method, the thermal inactivation of the germination process, the insolubilization of germ proteins, and the disappearance of polypeptide bands were assumed to follow the same kinetics. All these phenomena point to a heat denaturation of germ proteins. In accordance with this, we propose a first order kinetics; the rate constants (k) were calculated from the following equation:

$$k = \frac{2.303}{t} \lg \cdot \frac{\% x_c}{\% x} \quad (3)$$

where t is the time (min), % x the property measured in the heated germs (dried seeds), and % x_c the property measured in the control seeds.

The activation energy was calculated from the slopes of the straight lines obtained by plotting $-\ln k$ as a function of $1/T$ (Arrhenius equation). The values obtained were 164.0 kcal·mol^{-1}, 78.0 kcal·mol^{-1}, and 88.5–90.8 kcal·mol^{-1} for the germination process, the insolubilization of germ proteins, and the disappearance of polypeptide bands, respectively (Fig. 5). The correlation coefficients were 0.96, 0.86, and 0.95–0.94, respectively.

The E_a of protein insolubilization, disappearance of polypeptide bands, and E_a obtained by DSC show similar values, which differ from that of the E_a of germination. This was expected, as we assumed that the peak observed in the thermal curve was caused by thermal denaturation of proteins, and protein denaturation usually results in a decrease of solubility (Neucere and Cherry 1982); besides, the insolubilization of proteins could determine the disappearance of polypeptide bands in the electrophoretic patterns.

CONCLUSIONS

The results obtained show that drying temperature is a very important parameter to maintain germ viability.

The deteriorating effect of high temperatures on wheat germ apparently follows a first order kinetics and induces decreases of germinative capacity and protein solubility. Certain proteins are selectively lost after the drying process due to denaturation and insolubilization. The E_a obtained confirms that the drying process at high temperatures produces denaturation of the germ proteins.

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