Physical Analysis of Isolated Gluten Model Systems Heated in an Experimental Conventional-Microwave Oven[1]

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ABSTRACT

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Water loss rates and temperature profiles for isolated gluten model systems heated by conventional and microwave methods were determined before and after hexane extraction of gluten powder. Lipid contents, phosphorus, and sulfur contents were determined. Surface phosphorus-to-sulfur (P:S) and osmium-to-sulfur (Os:S) ratios were determined by energy dispersive X-ray microanalysis. Wide-angle X-ray diffraction was also determined for nonhexane-extracted and hexane-extracted powders. Free and bound lipids, Os:S ratios, and P:S ratios (used as an index of surface lipid and phospholipid content) were not changed by extraction. Wide-angle X-ray diffraction showed major peaks between 4.39 and 4.54Å and 9.01 and 9.10Å that were not changed by extraction. Water loss rates for rehydrated gluten samples heated at 200° C showed a local maximum as surface temperatures approached 100° C, followed by increasing rates of water loss as internal temperatures stabilized at 100° C. The local water loss rate maximum was absent in samples heated by microwave radiation, and water loss rates increased until a constant or falling rate period was reached. Surface temperatures and internal temperatures increased at similar rates and stabilized at 100° C. These patterns were similar for both nonhexane-extracted and hexane-extracted glutens.

Quality differences between cereal-based formulated foods heated by conventional and microwave methods are well known (Martin and Tsen 1981, Evans et al 1984). The underlying reasons for these differences are less well understood, but recent studies with model systems have contributed to better understanding of the heating processes and their consequences.

Wei et al (1985 a,b), using a Darcy-law approach, modelled heat transfer and water movement in liquid and vapor states during heating of porous materials by convection and by microwave heating modes. Mass transport in the convective heating mode was found to be mainly capillary driven; in the microwave mode, it was found to be mainly temperature driven. The importance of thermophysical properties, including dielectric and structural properties of the system, in determining temperature profiles and mass flux was also demonstrated.

Dielectric properties of a material are affected by, among other factors, the physical state of the material (e.g., different properties of ice and water [Mudgett 1986], or monoglyceride polymorphs [Crowe and Smyth 1950]). In a mixed system, dielectric properties can be either additive or interactive (Mudgett 1986).

Isolated gluten is inhomogenous in its composition and probably in the distribution of the component protein, lipids, and carbohydrates, and each of these components could interact with microwave energy either additively or interactively. The surface distribution of these components may also influence the extent of the reflection and absorption of microwaves as they enter the material, thus contributing to the subsequent attenuation of energy as the waves proceed through the material. In addition, the lipid fraction has been found to make important contributions to the functionality of flour in dough systems (Chung 1986) and may also contribute to the structure of gluten at the molecular level, which in turn may be related to its interaction with microwave energy as well as providing the capillary system through which liquid and vapor move.

An additional question about heating by microwave energy is whether any structures unique to microwave heating are formed. In isolated starch systems, the structures characteristic of the gelatinization sequence were similar for both microwave and conductive heating, but the stage of gelatinization varied depending on heating conditions (Zylema et al 1985, Goebel et al 1984).

The objective of the present study was to study the response of rehydrated isolated gluten systems, before and after hexane extraction, to microwave and conventional heating, and to relate the physical properties of the glutens to their response to heating modes. The two heating methods were adjusted so that heating times were equalized.

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MATERIALS AND METHODS

Commercially available gluten powder (Ogilvie Mills) was used in all experiments. Protein content and free lipids by petroleum ether extraction were determined by AACC methods (AACC 1983), moisture, lipids by acid hydrolysis, and ash by AOAC methods (AOAC 1970).

Hexane Extraction

Gluten powder and hexane in a ratio of 1:2.5 were slurried at room temperature for 24 hr, rinsed with fresh hexane, air-dried, and stored over anhydrous calcium sulfate until used. Nonhexane-extracted and hexane-extracted powders were also rehydrated (40 g powder and 60 ml water), freeze-dried, and ground to pass through an 80 mesh (177 μm) screen. Portions of nonhexane-extracted and hexane-extracted powders were also freeze-dried to serve as controls for the rehydration experiments.

Scanning Electron Microscopy and Energy Dispersive X-ray Microanalysis

Gluten powders before and after hexane extraction were dusted on stubs coated with double-stick tape or liquid carbon. Stubs were stored in a desiccator over anhydrous calcium sulfate. Some samples used for energy dispersive X-ray microanalysis (EDAX) were exposed to osmium tetroxide for 15 min in a desiccator. Samples were carbon coated alone or in combination with Ni and examined at 6 kV in a Philips model 500 scanning electron microscope. K shell electron counts of S and P and M shell counts of Os were measured at 12 and 6 kV for gluten and starch surfaces. P counts were normalized to S (Grider et al 1983, Nasir 1976).

Mineral Analysis

P content of ashed samples before and after hexane extraction was determined by inductively coupled plasma analysis (ICP), using an Applied Research Laboratories spectrometer (model QA 137). S content was determined by atomic emission spectroscopy (Leco Furnace, model S-132).

Wide-Angle X-ray Diffraction

Wide-angle X-ray diffraction measurements were made using a flat plate holder and Cu Kα radiation source with a Siemens D500 diffractometer. Measurements were made on the dry powder before and after hexane extraction. In addition, nonhexane-extracted and hexane-extracted powders were rehydrated (40 g powder and 60 ml water), freeze-dried, and then examined and compared to comparable freeze-dried powders that had not been rehydrated.

Heating Studies

Forty grams of gluten powder were mixed with 60 ml of glass-distilled water, stirred, kneaded by hand for 75 sec, and formed into balls approximately 8 cm in diameter and 4 cm high.

Hydrated samples were heated in a hybrid environmental oven equipped for conventional and microwave heating (Hung 1980). Still air was used. A schematic diagram of the oven is shown in Figure 1.

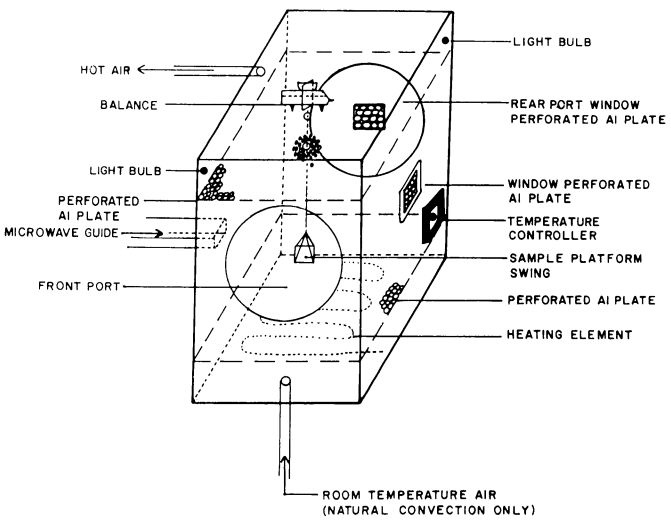

Fig. 1. Schematic diagram of oven cavity of conventional-microwave oven.

In conventional heating trials, samples were heated at 200° C for 25 min. Microwave heating trials used 2,450 MHz and 70 watts power, measured as the difference between transmitted and reflected power, for 25 min. This power level most closely approximated the structural development under conventional heating conditions and permitted monitoring of water loss and temperatures.

Water loss was measured gravimetrically. Temperature measurements were made using a fiber optic probe (Luxtron, model 1000A) positioned either at the surface of the gluten ball or 1 cm below the surface.

Data Analysis

Analysis of variance was used to analyze the effects of hexane extraction and differences among structures on P:S and Os:S ratios as determined by EDAX and effects of hexane extraction on P content as determined by ICP.

RESULTS AND DISCUSSION

Characterization of Gluten Before and After Hexane Extraction

The results of proximate analysis of the gluten used in this study before and after hexane extraction are shown in Table I. The lipid content as measured by acid hydrolysis (more tightly bound) and petroleum ether (free) extraction methods was decreased somewhat by hexane extraction.

The visual appearance of the powders was unchanged with hexane extraction; the powders were somewhat more free-flowing after hexane extraction.

Scanning electron micrographs of particles from dry gluten powders before and after hexane extraction and of nonhexane-extracted and hexane powders that had been freeze-dried or rehydrated and freeze-dried are shown in Figure 2. Starch granules that had separated from the main matrix during preparation of the sample for scanning electron microscopic examination are shown in Figure 3.

The electron micrographs show particles with differing surface textures. Some of the gluten structures were smooth and uninterrupted, appearing as a continuous sheet over the rounded shape of the particle. Some particles had cleaved surfaces with open pores distributed throughout.

Hexane extraction did not affect these features. Freeze-drying of the dried powders also did not affect these features (Fig. 2A and B vs. C and D). Rehydration with subsequent freeze-drying did result in changes in the gluten powders. Some air cells formed around the starch granules, and some were scattered through the matrix. Hexane extraction did not change the appearance of the rehydrated material.

Starch granules found in the gluten powders were often imbedded within gluten fragments. Occasionally, some granules appeared apart from gluten particles, with attached material on their surfaces (Fig. 3). The attached material may be similar to the

TABLE I
Proximate Analysis of Gluten Powders

Analysis	Before Extraction (g/100 g)	After Extraction (g/100 g)
Moisture	6.00	6.00
Protein	83.60	84.20
Fat		
acid hydrolysis	5.95	4.55
petroleum ether	1.10	0.55
Ash	0.60	0.70
Carbohydrates (by difference)	3.85	3.20

adhering material described by Kulp and Lorenz (1981). Most of the granules were small (10–15 μm in diameter). Hexane extraction did not alter the appearance of the starch granules.

The structures identified for measurement of the surface distribution of minerals by EDAX were gluten particle surfaces (GS, Fig. 2); gluten wisps, structures attached to gluten surfaces (GW, Fig. 2); starch surfaces (SS, Fig. 3); and starch wisps, structures attached to starch surfaces (SW, Fig. 3). Areas in which the wisps did not lie over starch or gluten surfaces were used for analysis of the wisps.

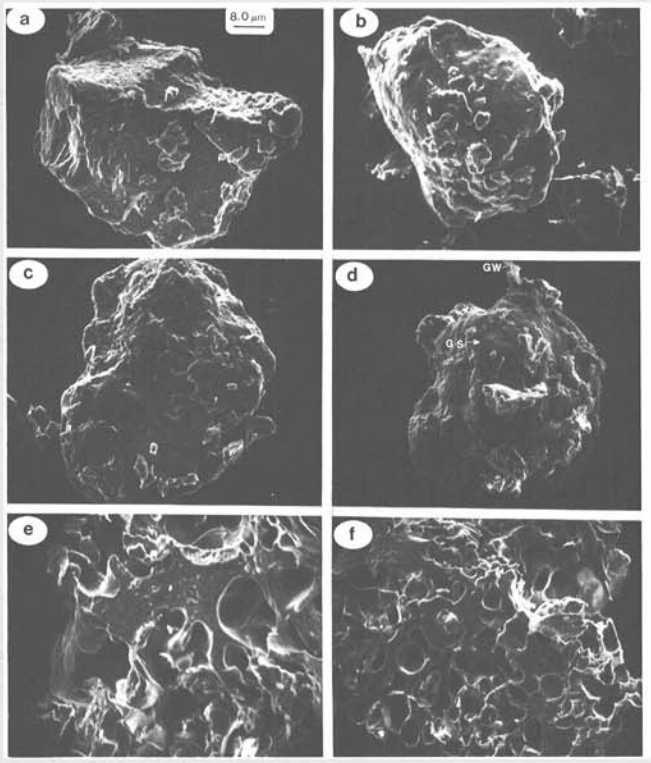

Fig. 2. Scanning electron micrographs of gluten powder. **A,** unextracted gluten powder; **B,** hexane-extracted gluten powder; **C,** freeze-dried gluten powder; **D,** freeze-dried hexane-extracted gluten powder; **E,** rehydrated, freeze-dried gluten powder; **F,** rehydrated, freeze-dried hexane-extracted gluten powder. **GS** = gluten surface; **GW** = gluten wisp.

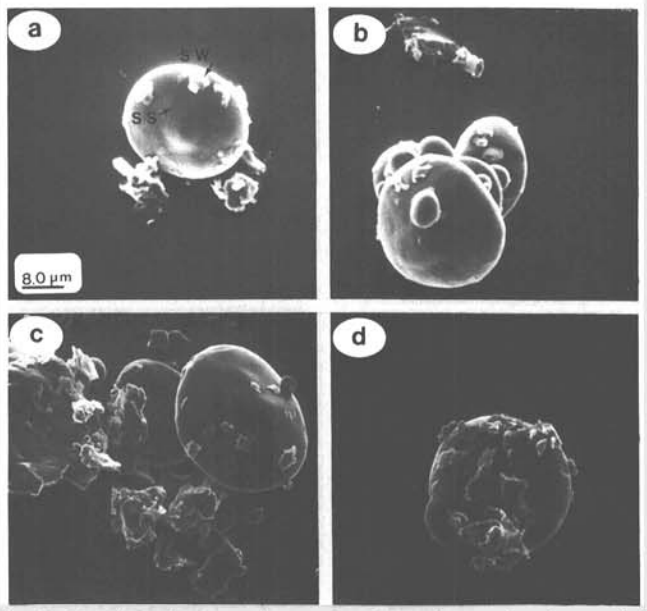

Fig. 3. Scanning electron micrographs of starch granules. **A,** starch in unextracted gluten powder; **B,** starch in hexane-extracted gluten powder; **C,** starch in freeze-dried gluten powder; **D,** starch in freeze-dried hexane-extracted gluten powder. **SS** = starch surface; **SW** = starch wisp.

Os:S and P:S ratios based on EDAX measurements are summarized in Table II. Os:S ratios were used as an indicator of the surface distribution of total lipid and P:S ratios as a measure of phospholipid surface distribution.

Os:S ratios were decreased significantly ($P < 0.01$) by hexane extraction. This decrease paralleled the decrease in lipids shown by proximate analysis for the total sample. Before extraction, Os:S ratios for starch wisps tended to be higher than those for gluten wisps and gluten surfaces. After extraction, starch wisps and gluten wisps had similar ratios, and both were higher than that for gluten surfaces. S counts for starch surfaces were too low to permit calculation of Os:S ratios.

No significant differences ($P > 0.05$) due to hexane extraction or among gluten surfaces, gluten wisps, and starch wisps were found when P:S ratios were measured at 12 kV. When P:S measurements were made at 6 kV so that beam penetration was reduced and variability among individual measurements was somewhat reduced, differences among structures were significant, with starch wisps having the highest ratios. Extraction did not change the ratios significantly.

P and S contents for the whole sample as measured by ICP before and after hexane extraction were not significantly different and averaged 1.77 mg/g for P and 8.22 mg/g for S. The P:S ratio of 0.2153 for the whole sample was in the upper range of those for the surface P:S ratios. Batten and Lott (1986) used EDAX to measure P, K, and Mg contents in aleurone structures. If some of the aleurone material were present in gluten, P:S ratios would be less representative of phospholipids.

Bekes et al (1983) reported that hexane extraction removed some but not all of the nonpolar and polar lipids from hand washed glutens. Phospholipids were found to be low initially and primarily in the bound lipid fraction, and hexane extraction resulted in only minor reductions. Their results are consistent with the finding in this study that Os:S but not P:S ratios were reduced by hexane extraction.

Wide-angle X-ray diffraction scans for gluten powders before and after hexane extraction are shown in Figure 4. All of the powders showed a major peak in the vicinity of 4.39–4.54Å and a less intense peak at 9.01–9.10Å. Traub et al (1957) attributed spacings at 5 and 10Å in gluten powders to protein, but lipids as well as starch can show spacings in these regions (Chapman 1965, Zobel 1964). Regardless of the sources of diffraction in this region, it appeared to be only slightly altered by hexane extraction or rehydration followed by freeze-drying. Conformational changes and differences in crystal type formed during extraction and rehydration could, however, contribute to the changes (Chapman 1965, Zobel 1964, Hinrichs et al 1987).

The results of this part of the present study are supportive of the electron spin resonance studies in our laboratory that showed that

TABLE II
Osmium-to-Sulfur (Os:S) and Phosphorus-to-Sulfur (P:S) Ratios in Gluten Powders Before and After Hexane Extraction as Measured by Energy Dispersive X-ray Analysis

	Unextracted Powder		Hexane-Extracted Powder	
Structure	**Mean**	***n***	**Mean**	***n***
Os:S[a]				
Gluten surfaces	0.347	5	0.177	4
Gluten wisps	0.824	3	0.327	4
Starch wisps	1.207	2	0.344	2
P:S (12 kV)				
Gluten surfaces	0.157	19	0.221	14
Gluten wisps	0.195	13	0.149	9
Starch wisps	0.204	9	0.118	6
P:S (6 kV)				
Gluten surfaces	0.146	11	0.132	13
Gluten wisps	0.182	15	0.188	12
Starch wisps	0.224	10	0.0982	11

[a]Standard deviation based on error term of analysis of variance for Os:S = 0.2345, 14 df; P:S (12 kV) = 0.1414, 64 df; P:S (6 kV) = 0.0877, 66 df.

hydrophobic-hydrophilic environments, as measured by the distribution of the probe TEMPO in fluid environments, change after hexane extraction of the gluten (Pearce et al 1988). The fraction of the probe in more hydrophobic environments decreased after hexane extraction.

Heating Studies of Rehydrated Gluten Samples

Typical water loss rates, interior temperatures, and surface temperatures for gluten balls heated at 200° C by the conventional method are shown in Figure 5. Comparable plots for microwave heating are shown in Figure 6. These curves show the major characteristics of water loss rates and temperature profiles. Individual heating runs differed somewhat in the numerical value of water loss rates.

Total water losses for rehydrated unextracted and extracted gluten heated by the conventional method were 11.6 and 13.3 g, respectively, and for the samples heated by microwave energy, 9.9 and 13.2 g, respectively.

Water loss rates for gluten balls heated at 200° C by the conventional method were characterized by an initial increase to a local maximum early in the heating period followed by a relatively long period of decreasing water loss rates before a second period of rapidly increasing rates began. At the local maximum (3–6 min) the dough had relaxed (Fig. 7), and surface temperatures were above 100° C but the internal temperatures remained low. Surface evaporation may account for the initial rapid loss of water. Heat transfer from the oven to the gluten ball at high temperature maintained high surface temperature. After this time, the surface temperatures continued to increase above 100° C, indicating that the evaporation front had moved into the sample.

Water loss rates then decreased until the samples reached internal temperatures in the range of 60° C. At this point water loss rates began a period of rapid increase. Expansion was accelerated and the internal temperatures continued to increase. As the internal temperatures approached 100° C, the increase in water loss

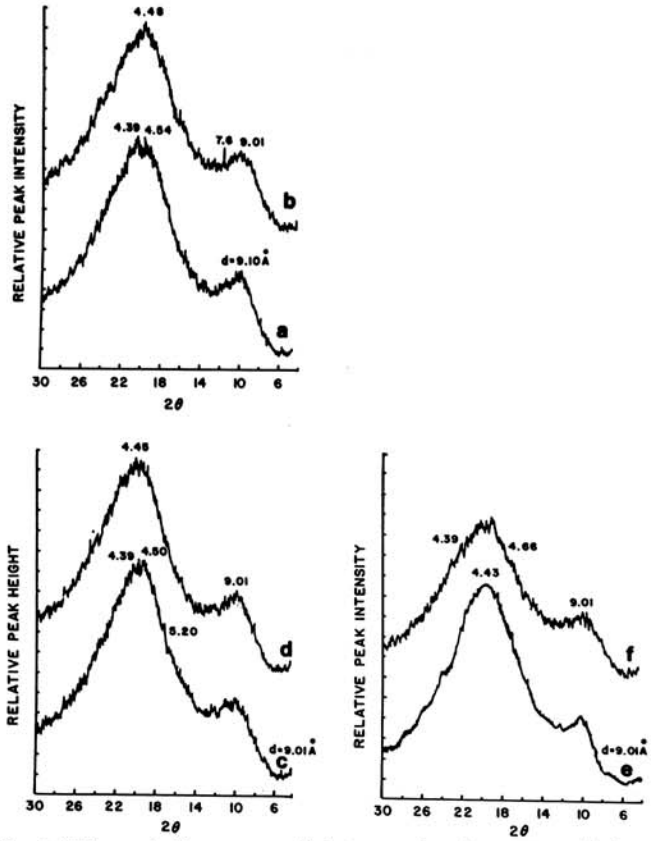

Fig. 4. Wide-angle X-ray scans of gluten powder. **A,** unextracted gluten powder; **B,** hexane-extracted gluten powder; **C,** freeze-dried gluten powder; **D,** freeze-dried hexane-extracted gluten powder; **E,** rehydrated, freeze-dried gluten powder; **F,** rehydrated, hexane-extracted, freeze-dried gluten powder.

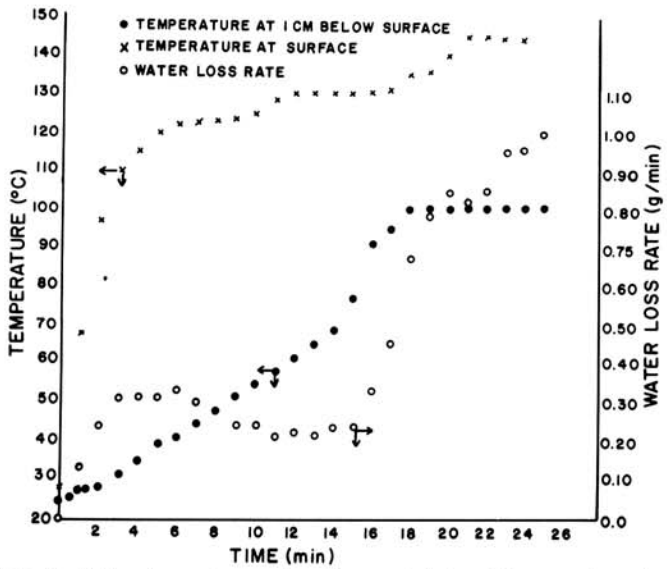

Fig. 5. Water loss rates and surface and internal temperatures in rehydrated unextracted gluten sample heated at 200° C.

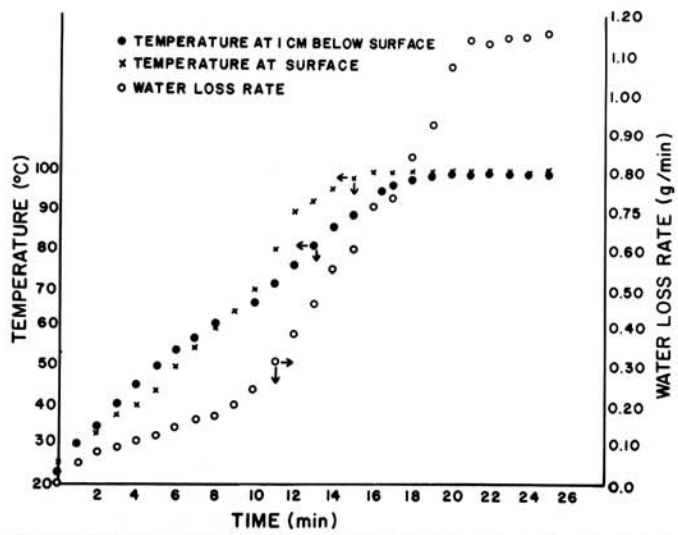

Fig. 6. Water loss rates, surface and internal temperatures in rehydrated unextracted gluten sample heated by microwave irradiation, 2450 MHz and 70 watts power.

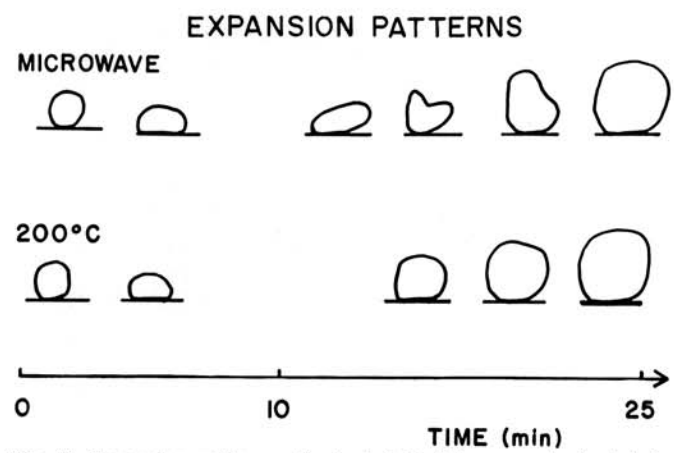

Fig. 7. Expansion patterns of rehydrated gluten sample heated by microwave irradiation (**A**) and at 200° C (**B**). Direction of microwave beam is from right to left.

rates began to slow. In some samples, water loss rates declined and a falling rate period occurred. Interior temperatures of 100° C were maintained. Baking was terminated at this point although internal structure was not developed sufficiently to prevent collapse. In full formulation products, "doneness" coincides with the beginning of the constant rate period (Davis and Gordon 1982). Before collapse, the gluten balls heated by either method had a smooth, spherical shape with diameter of approximately 13 cm.

Water loss rates for gluten balls heated by microwave (Fig. 6) showed a different pattern than those heated at 200° C by conventional methods. The extended initial local maximum shown by conventionally heated samples was absent in microwave heated samples. Instead, the rate of water loss increased slowly during the comparable initial heating period. Surface and interior temperatures were similar to each other. The gluten ball relaxed but no expansion occurred (Fig. 7). The low surface temperatures reflected both the low ambient oven temperature and the cooling effects of surface evaporation.

Water loss rates began to increase more rapidly when the interior temperatures reached the 60–70° C range. This is the same temperature range in which acceleration of water loss rates was seen in conventional heating.

Water loss rates continued to increase after both the surface and interior reached 100° C but entered a constant rate period toward the end of the heating period. Expansion was asymmetrical initially, but as heating progressed, the gluten balls became more symmetrical.

The values for the bulk properties of water transport, water loss rates, and temperature profiles for hexane-extracted gluten samples were similar to those for nonhexane-extracted samples regardless of whether the samples were heated by conventional or microwave methods.

The effects of lipid extraction of flour on overall structural development have been extensively studied (Chung 1986). Similar effects may be present in the isolated gluten system that will affect structural development.

The release of water during heating depends on the bulk movement of water in liquid and gas forms within the developing capillary structure as thermal energy is convected through the porous structure that develops (Wei et al 1985 a,b). Thus, water loss rates depend on the availability of water and its freedom to move within the capillary structure that develops during heating. Water can behave as essentially bulk water or be bound to the protein, lipid, or starch components. Development of the capillary system also depends on these interactions.

The effect of lipid extraction of flour on overall structural development in dough systems has been extensively studied (Chung 1986), but the events at the molecular level that cause these differences are less clearly understood. In the present study of isolated gluten systems, hexane extraction resulted in a change in the total amount of lipids and the surface distribution of lipids. Measurements of crystallinity by wide-angle X-ray diffraction did not show major changes. Electron spin resonance measurements on similarly hexane-extracted gluten showed, however, that the distribution of the water-soluble, nonreactive probe, TEMPO, in hydrophilic and hydrophobic environments changed after hexane extraction (Pearce et al 1988). Even though these compositional and structural differences at the molecular level were present, they were not reflected in the more bulk water and heat transport properties nor in the overall morphology of the heated gluten system.

The development of a capillary system and the availability of water is also important in the release of water during microwave heating. In addition, absorption of energy from microwave radiation, which in turn relates to total energy balance within the sample, may be affected by the composition and structure of gluten.

Inhomogeneity in the distribution of components of the rehydrated gluten system may be present, which may affect the response to microwave energy. For example, Belton et al (1985) on the basis of nuclear magnetic resonance studies suggested the presence of two domains of water in gluten, one relatively immobile and the other showing considerable motional freedom. Pearce et al (1988) showed that the hydrophobic-hydrophilic environments experienced by a free radical probe in hydrated gluten systems changed with hot water bath heating to 95° C. In addition, binding of stearic acid in gluten-starch-water-stearic acid probe systems depended both on the ratio of gluten to starch and on the heating history of the combined system (Pearce et al 1987). Bekes et al (1983) reported differences in the binding of nonpolar and polar lipids to the various gluten protein fractions.

These inhomogeneities could result in sample differences in localized heating when microwave energy is used. Although the water loss rate curves showed the general pattern shown in Figure 6, differences in rates at any given time were found between individual runs. Localized heating differences could contribute to these differences in water loss rates.

The sequence of water loss rates in the prefalling rate period that was examined in this study depended on the heating method being used (conventional vs. microwave), but within each heating method, the characteristic pattern was not affected by structural changes at the molecular level that might have been introduced by hexane extraction. Therefore, even though we saw some differences with measurements that are more molecular in nature, the bulk water loss rates predominate in the prefalling rate period that this study considered.

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