Flour Chlorination. II. Effects on Water Binding¹

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ABSTRACT

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The effects of commercial levels of flour chlorination on some physicochemical characteristics of the flour were studied. At temperatures of 90°C and above, swelling power and solubility of the high-starch fractions from the air classifier increased as a result of chlorination. Although chlorination did not alter the differential scanning calorimetry gelatinization phase transition in either simple (flour-water) or complex batter systems compared to unchlorinated counterparts, at 90°C starch granules in batters containing chlorinated flour exhibited a greater loss of birefringence and swelled more than starch granules in batters made with untreated flours. Pulsed nuclear magnetic resonance studies showed that at

the same moisture content, the characteristics of the T_2 relaxation curves were affected by chlorine dose. Chlorination levels of 2 or 4 oz/cwt altered the distribution of molecular species of water in flours having a moisture content of 16%. Water activities of batters made with unchlorinated and chlorinated flours were identical until the temperature reached 80° C. At that temperature, batters containing chlorinated flour exhibited higher water activity than batters containing untreated flour. Greater mobility of water in systems containing chlorinated flour might account for the greater starch gelatinization in those samples.

The importance of water in a food system is well known. In fact, the functionality of a food is often related to its water-binding characteristics (Labuza 1977). The improving effects of chlorination on cake flour may be related to the effects of chlorine on the water-binding capacity of the flour, which in turn affects such characteristics as starch gelatinization, swelling, and solubilization.

The most critical factor in cake quality is starch gelatinization (Farrand 1959, Gough et al 1978, Howard et al 1968, Shepherd and

0009-0352/82/06050007/\$03.00/0 @1982 American Association of Cereal Chemists, Inc. Yoell 1976). Yet, starches washed from untreated flours and flours chlorinated to normal levels do not differ in starch gelatinization characteristics as determined microscopically (Cauvain et al 1977, Kulp et al 1972). Neither do the gelatinization phase transitions (by differential scanning calorimetry) of starch in chlorinated and untreated cake batters differ (Jacobsberg and Daniels 1974). However, in a study of starch chlorinated to pH 2.5–2.9, Miller et al (1973) found that, although granules of treated and of control samples did not appear to differ during the initial phase of gelatinization, chlorination suppressed starch granule deformation when the starch suspension was heated above 70° C.

No extensive work has been reported on the effects of commercial levels of chlorination on flour's water-binding characteristics. We report the effects of chlorination on patterns of flour swelling and solubility (a consequence of water binding), gelatinization phase transitions in flour-water and batter systems, and bound water and water activity in flour-water and batter systems.

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MATERIALS AND METHODS

Materials

Untreated pin-milled cake flour (Mennel Milling Co., Fostoria, OH) with protein (N \times 5.7) and ash contents of 8.3 and 0.3%, respectively, was used. The chlorine treatments and a description of the subsequent air classified fractions were given in a previous paper (Huang et al 1982).

Methods

Moisture Content. Moisture content of the flour and flour fractions was determined according to methods of the AACC (1972).

Swelling Power and Solubility. Swelling power and solubility of the high-starch fraction (fraction E) were measured according to the method of Leach et al (1959) using a starch concentration of 1%.

Differential Scanning Calorimetry (DSC). A Perkin-Elmer model DSC-2 differential scanning calorimeter (Perkin-Elmer Corporation, Norwalk, CT) with an Intracooler II system was used in measuring bound water and starch gelatinization phase transitions in simple systems (flour and water) as well as batter systems. Samples were prepared by accurately weighing the flour and other ingredients, eg, sugar (130%) and high-ratio shortening (27.9%) in proportions suggested by Kissell (1959), into dry screwcap vials. Double distilled water was added so that the flour to water ratio was 1:1 (dry basis). The sample mixture was then stirred into a smooth slurry, and the vials were sealed to prevent moisture loss. Portions of the mixed sample were transferred into previously weighed aluminum DSC pans (Perkin-Elmer part 219-0062), immediately sealed with a pan sealer (Perkin-Elmer part 219-0061), and then reweighed. Samples were placed in the DSC sampling block; an empty pan was used as reference.

Bound Water. Bound water, as determined on the calorimeter, is the amount of unfreezable water in the sample. Samples were cooled down to 220° K and then heated to 300° K at a rate of 10° C/min, a scanning rate of 5 mcal/sec, and a chart speed of 40 mm/min. The area of the endotherm was determined with a planimeter. Deionized water was used as the standard. The amount of bound water in the sample was calculated as grams of bound water per gram of flour; a gram-molecule of sucrose was assumed to bind 108 g of water as a result of hydration (Skovholt and Bailey 1935). That value, expressed as a percent, was derived by the following formulas:

Free water (g) =
$$\frac{K \times R \times A}{\triangle H_w \times S}$$

where K = calibrated instrument constant in cm⁻¹, R = range sensitivity in mcal/sec, A = peak area in cm², $\triangle Hw = \text{transition}$ energy of water in cal/g, and S = recorder chart speed in mm/min;

and

Bound water (%) =
$$\frac{\text{Total water (g) - free water (g)}}{\text{Flour (g)}} \times 100$$

Starch Gelatinization Phase Transition. Starch gelatinization phase transitions were determined in the calorimeter using a heating rate of 10° C/min, a scanning rate of 1 mcal/sec, and a chart speed of 20 mm/min.

Pulsed Nuclear Magnetic Resonance (NMR). Proton relaxation studies were done on unfractionated, treated, and control flours at 150 MHz using a Nicolet NMR Spectrometer. Longitudinal (T_1) and transverse (T_2) relaxations were determined at 30° C, using the Carr-Purcell-Meiboom-Gill (CPMG) technique (Farrar and Becker 1971, Meiboom and Gill 1958). T_1 was determined from the free decay signal amplitude and T_2 from the spin-echo signal amplitudes.

Gel Permeation Chromatography. A Sepharose 4B Cl column (Pharmacia, Uppsala, Sweden) was prepared by dispersing the gel in 0.01N NaOH containing 0.02% NaN₃. The V_o and V_t of the

column were determined with blue dextran and glucose. The elution profiles of commerical dextran (Pharmacia, Uppsala, Sweden) of known molecular weights were determined. For the unknowns, a sample containing 10 mg of carbohydrate was applied to the column and eluted with 0.01 N NaOH containing 0.02% NaN₃ at a flow rate of 20 ml/hr. Five-milliliter fractions were then collected. Total carbohydrate in the fractions was determined by the phenol-sulfuric acid method (Dubois et al 1956).

Thin-Layer Chromatography. The low molecular weight peaks from the Sepharose 4B Cl column were tentatively identified by thin-layer chromatography on silica gel (Whatman pk 5F, 20×20 , PKG/22) using chloroform-acetic acid-water (55:45:5, v/v). Spots were visualized by spraying the plate with 0.7% resorcinol in isopropanol and 20% $\rm H_2SO_4$ (ratio 10:1) and then heating at 118° C for 15 min.⁴

Water Activity. Water activity (Aw) of flours and batters was measured with a Beckman Water Activity Meter (Beckman, Cedar Grove, NJ), using a 1–100% rh module. The sensor was placed in a laboratory oven to measure water activities at high temperatures (35–80°C). Saturated KNO3 was used to standardize the instrument. Equilibration times were at least 1 hr before recording Aw.

Light Microscopy. Samples were heated in the DSC to predetermined temperatures, the pan was opened, and a portion of the sample placed on a glass slide in distilled water. Photomicrographs were taken on a Reichert (Austria) light microscope with brightfield and polarized light.

RESULTS AND DISCUSSION

Swelling and Solubilization

Gough et al (1978) hypothesized that the material adhering to starch granules and removed during wet fractionation may play a key role in affecting starch swelling and solubility patterns. Therefore, we determined the swelling and solubility patterns of the high-starch E fractions from the air-classified flours instead of studying the starch after wet fractionation.

Swelling power data are presented in Fig. 1. As expected, swelling power increased with temperature. In addition, our data show that, although chlorine treatment had little effect on the swelling patterns of the 0-4-oz/cwt E fractions from 60 to 80° C, at 90° C the swelling power increased as a result of chlorination. At

⁴Ponte. 1981. Personal communication.

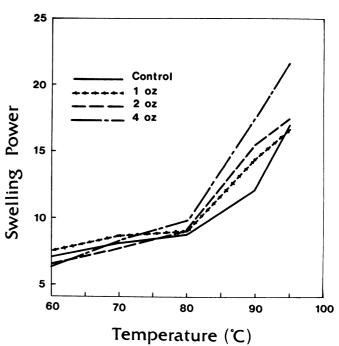


Fig. 1. Swelling power of untreated and treated E fractions.

that temperature the 1-, 2-, and 4-oz/cwt treated fractions showed significantly greater swelling (P < 0.05) than did the control.

In our study, unusual solubility patterns were obtained for E fractions of control and chlorinated flours (Fig. 2). At 60 and 70° C, the control and the 1- and 2-oz chlorine-treated samples showed higher solubility than did the 4-oz treated sample. Thinking that the increased protein solubility in those samples might be responsible for the high solubility observed at the low temperatures, we determined the nitrogen content in the solubles. At 60° C, protein solubility increased with chlorine dose; ie, more nitrogen was solubilized from the 4-oz sample than from the lower-chlorine treated flours (Table I). If protein solubility were responsible for the high solubles content, then one would expect the highest total solubility for the 4-oz chlorine treatment. That was not the case. Therefore, other factors in the flour contributed to the high solubility at low temperatures.

In further studies, we determined the composition of the solubles obtained on heating the E fraction to 60° C. Values for total carbohydrates (Table I), as determined by the method of Dubois et al (1956), paralleled the solubility patterns observed at 60° C (Fig. 2). In other words, the solubles obtained from the 1-oz/cwt treated E fractions contained the most carbohydrate, whereas the solubles from the 4-oz/cwt treated flour contained the least. Solubles from the E fractions of the control and 1-oz/cwt treated flours showed a carbohydrate peak eluting near the V_t of a Sepharose 4B Cl column (Fig. 3a), that corresponded to a molecular weight of about 10,000 (Fig. 3b). Conversely, the elution pattern of solubles from the E fractions of the 4-oz/cwt flour consisted of three carbohydrate peaks; the major portion of the eluate had a molecular weight greater than 40,000. These data suggested that the carbohydrates in

TABLE I
Nitrogen-Solubility Index and Total Carbohydrates
in the Solubles^a at 60°C

Chlorine Level (oz/cwt)	Nitrogen-Solubility Index ^b	Total CHO (mg/ml)
0	8.56	5.60
1	11.53	6.95
$\frac{1}{2}$	12.30	6.24
4	14.10	3.65

^aSolubles in E fractions were determined according to Leach et al (1959). ^bMiller's (1959) method.

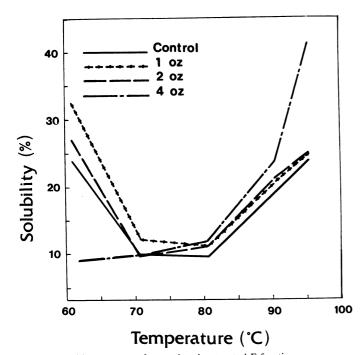


Fig. 2. Solubility patterns of treated and untreated E fractions.

the control and 1-oz treated samples were degraded to a larger extent than were those in the 4-oz treated sample.

According to Elton and Ewart (1964) and Jones and Dimler (1966), air-classification concentrates nongluten proteins such as albumins and globulins in the high-starch fraction. Because many enzymes are albumins, we determined α-amylase activity in both treated and untreated flours by the Phadebas method (Barnes and Blakeney 1974). Wheat α -amylase is thermostable at 70° C. In fact, a short heat treatment at 70°C is used in α-amylase purification procedures. The 1-oz/cwt chlorine-treated E fraction exhibited the highest α-amylase activity, whereas the 4-oz/cwt chlorine-treated E fraction had the lowest enzyme activity: 7.32 $mU/g,\,10.98\ mU/g,$ and 1.46 mU/g for control, 1- and 4-oz samples, respectively. Thin-layer chromatography (not pictured) showed that the low molecular weight sugars were primarily maltose and glucose. Therefore, increased amylase activity (mostly α -amylase) was responsible for the high solubility observed for the control and for the 1- and 2-oz treated flours at 60 and 70° C. The higher enzyme activity in those samples compared to the 4-oz/cwt sample was a function of pH. The pH of the control (pH 5.7), 1-oz (pH 5.1), and 2-oz (pH 4.5) samples fall within the optimum range for α -amylase activity, whereas the pH of the 4-oz sample (pH 3.6) does not. When the pH of the treated flours was adjusted to that of the control, no significant differences were observed among control and chlorine-treated samples with respect to swelling power or solubility at 60 or 70° C.

The solubility of the chlorine-treated flours at 90 and 95° C was significantly higher (P < 0.05) than that of the control flour (Fig.

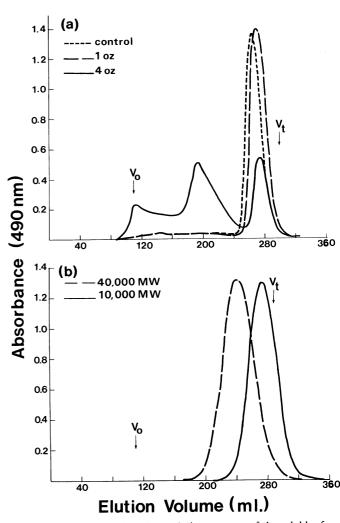


Fig. 3. a, Sepharose 4B Cl column elution patterns of the solubles from unchlorinated and chlorinated E fractions heated to 60°C. Dextran standards are shown in b.

2). However, no differences were observed between 1- and 2-oz treated flours. The high starch fraction from the 4-oz treated flour exhibited the highest solubility at those temperatures, even when the pH was adjusted to that of the control flour. That implicated other factors besides pH, such as depolymerization of flour components, as being responsible for the higher solubility of that sample.

Gelatinization Phase Transitions

The swelling and solubility patterns determined in excess water, as described above, are unlike those found in batter systems in which water is limited. In addition, the E fraction is normally blended with other flour fractions for producing good-quality cake flour. Therefore, we studied the heating characteristics of chlorinated (pH 4.8) and unchlorinated cake flours (not airclassified fractions) using a DSC. Before sample preparation for the DSC, baking tests were performed on treated and untreated cake flours using the lean formula described by Kissell (1959). The optimum water level was obtained when the ratio of flour to water was 1:1.

No apparent differences were seen in the gelatinization phase transitions for untreated and treated flours in both simple flourwater (Fig. 4) and batter (Fig. 5) systems. For the simple system, transition temperature (T_i) was 51° C, peak temperature 64° C, and termination temperature 89° C. Adding sugar and shortening to the system increased T_i to 76° C (Fig. 5). According to studies of Spies and Hoseney (1981) and Jacobsberg and Daniels (1974), sugar delays starch gelatinization; the higher the concentration of sugar, the higher will be the temperature for starch gelatinization. Our DSC data agree with those of Jacobsberg and Daniels (1974), who found no effect of chlorination on the gelatinization phase transition.

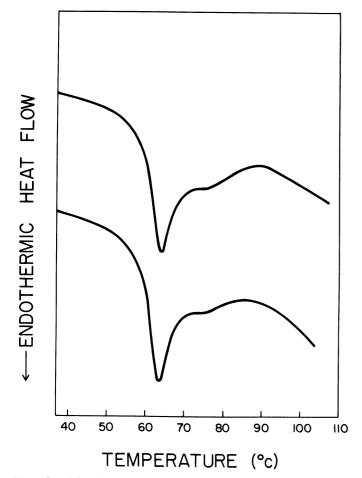


Fig. 4. Gelatinization phase transitions of untreated (top curve) and treated (bottom curve) flours; flour to water ratio was 1:1.

The gelatinization phase transition as affected by the amount of water present in the system was investigated by Donovan (1979) and Ghiasi et al (1982). Ghiasi et al (1982) showed that in low-water systems, the peak temperature in DSC thermograms did not coincide with the complete loss of birefringence. Therefore, we studied the birefringence and swelling of the starch in batter samples that had been heated in the calorimeter. For batters made with chlorinated or untreated flours, no differences in loss of starch birefringence were observed at the T_i of the DSC thermogram (Fig. 6a and b). At 90°C, however, starch granules from batters containing chlorinated flour showed extensive swelling and loss of birefringence (Fig. 6e and f), whereas starch in batters containing untreated flour did not (Fig. 6c and d). At 97°C a complete loss of starch birefringence occurred in treated and untreated flours (not pictured).

Several authors (Farrand 1959, Frazier et al 1974, Russo and Doe 1970) indicated that no major difference between treated and untreated flours is observed until heating proceeds to within the final few minutes of the baking period, when the temperature in batters reaches about 90° C. Mizukoshi et al (1979) showed that during cake baking the temperature in the cake batter does not reach 90° C and above until the last few minutes of baking. Our microscopical data indicate that starch in chlorine-treated flours gelatinizes (loses its birefringence) earlier and swells to a greater extent than starch in untreated flour, thus providing the cake with a structure that has enough strength to prevent collapse after it is removed from the oven.

Water Binding by Chlorinated and Unchlorinated Flours

Because starch swelling involves absorption of water, differences between chlorinated and unchlorinated flours may be related to differences in the binding of water by the flour. Therefore, we conducted a series of experiments to determine bound water and water activity in chlorinated and unchlorinated flours.

Reports on the bound water content in flour-water mixtures are confusing because of the diversity of definitions for bound water. Theoretically, bound water is that water equivalent to a monolayer as calculated by Brunauer, Emmet, and Teller (1938), the BET monolayer. Using density methods, Gur-Arieh et al (1967) determined that the BET monolayer for a low-protein fraction of

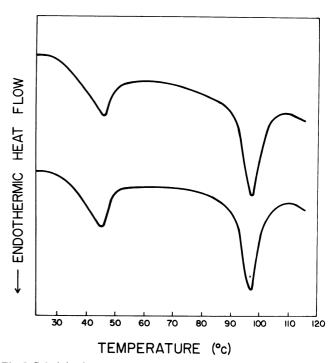


Fig. 5. Gelatinization phase transitions of untreated (top curve) and treated (bottom curve) flours in batter systems (flour-sugar-shortening-water = 1:1.3:.279:1).

hard wheat flour was 0.07 g of water per gram of solids. Additional adsorbed water up to approximately 0.26 g water per gram of solids is considered to be caused by the building up of hydration layers. In our work, bound water refers to the BET monolayer plus the hydration layers. Unbound or free water is the water content above about 0.26 g of water per gram of solids (Gur-Arieh et al 1967).

To determine the effect of flour chlorination on water content and condition, we held untreated and chlorinated flours over water in a desiccator for 15 hr at room temperature and then measured their water activities. During that 15-hr period, unchlorinated flours adsorbed more water and showed a higher water activity than did chlorinated flours (Table II). Similar equilibration of high-protein and high-starch fractions from unchlorinated and chlorinated flours suggested that the major effect on Aw was related to changes in the water activity of the starch fraction (Table III).

Aw measurements do not provide information on the existence of different water species (bound versus free) in the flours, so pulsed NMR studies were conducted on the samples listed in Table II. NMR T₂ relaxation times provide information on the strength or degree of water binding by a flour (Leung et al 1979). A direct plot of the amplitude and pulse spacings (msec) produces relaxation curves such as that shown in Fig. 7. At first glance, the simple exponential decay might lead one to believe that the system is monophasic (ie, only water molecules of one molecular species are present). However, we cannot ascertain the true complexity of the system from a direct plot of the data. To determine whether more than one T₂ is present (ie, whether the system is monophasic or biphasic), we plotted the data on semilog paper (Zimmerman and Brittin 1957, Zimmerman and Lasater 1958). Those data are shown in Fig. 8.

Flour treatment did affect the T2 relaxation curves (Fig. 8). The

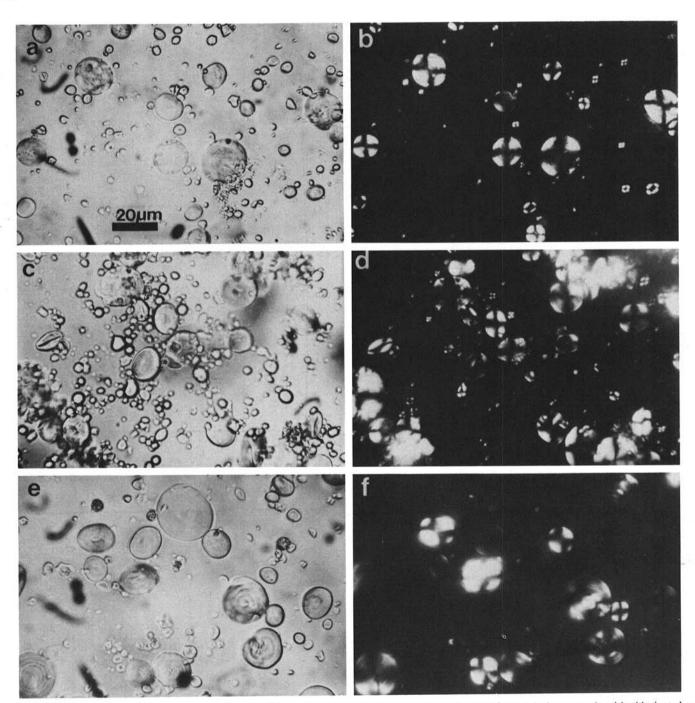


Fig. 6. Light photomicrographs of starch granules from batters made with chlorinated or unchlorinated flour. a,b, batter made with chlorinated or unchlorinated flour, and heated in the differential scanning calorimeter (DSC) to 76°C; c,d, batter made with unchlorinated flour and heated in the DSC to 90°C; e,f, batter made with chlorinated flour and heated in the DSC to 90°C.

control, 2- and 4-oz chlorinated flours showed a nonexponential decay, implying the existence of two or more species of water molecules: a tightly bound fraction with a short T2, and a more mobile fraction having a longer relaxation time. The 1-oz chlorinetreated sample showed simple exponential decay indicative of a monophasic system. One might expect that the biphasic character of the control curve was caused by the fact that more water was adsorbed by that sample during the 15-hr holding period in the desiccator (Table II). Although T2 values increase with increasing moisture content (Leung et al 1976), moisture content cannot explain the biphasic character of the 2- and 4-oz chlorinated flours because all treated flours had the same moisture content, which was lower than that of the control (Table II). Furthermore, at the same moisture content, the characteristics of the relaxation curves were affected by chlorine dose. These data show that, even at moisture contents of 16%, chlorination alters the distribution of water in the

TABLE II Moisture Content and Water Activity of Untreated and Chlorinated Flour^a

Flour	Moisture Content (%)	Water Activity at 25° C	
Control	17.74	0.83	
Chlorinated			
1 oz/cwt	15.55	0.77	
2 oz/cwt	15.43	0.76	
4 oz/cwt	15.72	0.76	

^{*}Samples held in a desiccator over water for 15 hr.

TABLE III
Moisture Content and Water Activity of High Protein
and High Starch Fractions from the Air Classification
of Chlorinated and Unchlorinated Flours

	Moisture Content (%)		Water Activity at 25°C	
Flour Fractiona	Control	Chlorinated ^b	Control	ntrol Chlorinated ^b
High protein (B)	17.61	16.65	0.85	0.81
High starch (E)	17.06	16.18	0.82	0.77

^aLetter in parenthesis refers to fraction obtained from the air classifier (Huang et al 1982).

^b2 oz/cwt.

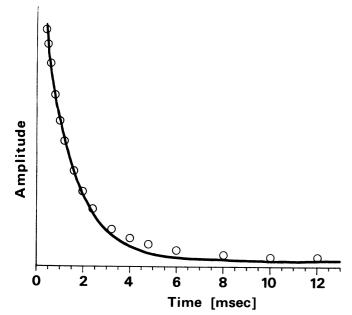


Fig. 7. Direct plot of amplitude and pulse spacings.

flour. Apparently chlorine treatments of at least 2 oz/cwt are necessary to alter the water distribution.

Because of the difficulties in obtaining useful data on batter systems using pulsed NMR, we used the DSC to determine the effect of chlorination and batter ingredients on the amount of bound water in systems having a flour to water ratio of 1:1. The DSC definition of bound water is the unfreezable water at -50° C, which includes the monolayer and hydration layers. The condition of water in flours containing less than 20% moisture cannot be ascertained using DSC. We found that flour chlorination had no effect on the unfreezable water in simple flour-water systems or in batters. However, unfreezable water was significantly affected

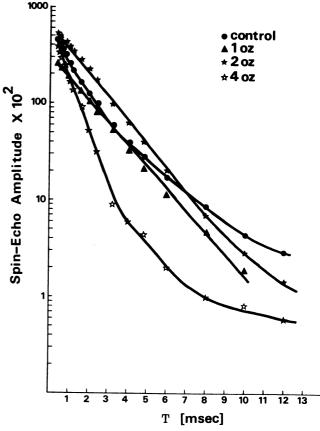


Fig. 8. T₂ relaxation curves for untreated and chlorinated flours.

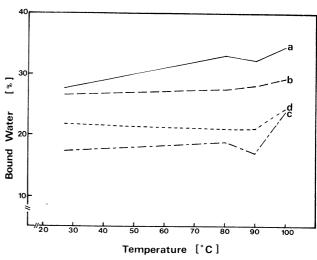


Fig. 9. Unfreezable water content of batters heated to various temperatures in the DSC: **a**, flour and water; **b**, flour, water, and shortening; **c**, flour, water, and sugar; **d**, batter (flour, water, shortening, and sugar).

TABLE IV
Effect of Flour Chlorination on Water Activity of Batters

	Water Activity ^a		
emperature (°C)	Control	Chlorinated	
25	0.90	0.90	
55	0.90	0.90	
75	0.88	0.88	
80	0.84	0.87	

^aStandard deviation of the instrument readings = 0.01.

(P < 0.01) by adding sugar and shortening to the system (Fig. 9). Simple flour-water systems gave the highest bound water at all temperatures. Adding shortening lowered the unfreezable water content. Hydrophobic interaction between shortening and flour components probably inhibited water binding. The lowest value for unfreezable water was obtained when sugar was added to the system (Fig. 9). Adsorption of sucrose in the amorphous regions of starch granules, as suggested by Spies and Hoseney (1981), could be responsible for the decreased level of unfreezable water in batters containing sucrose.

Batter samples were heated in the DSC to various temperatures, and unfreezable water was then determined. In the simple flourwater system, unfreezable water increased as temperature was increased except at 90° C when unfreezable water content dropped (Fig. 9). That same phenomenon was observed when sugar was added to the system. The reason for this decrease was not apparent.

Generally, the water above 1 g of water per gram of solids is considered free water because the Aw is very high, but the free water can be bonded tightly in a food system (Labuza 1977). We determined the water activity of batters made with unchlorinated and chlorinated flours and found no differences in Aw up to a temperature of 80° C (Table IV). At that temperature, the water in batters made with chlorinated flour was slightly more reactive than the water in batters made with unchlorinated flour. Although the differences in Aw are small, such small changes in Aw can dramatically affect the quality of foods by changing the activation energy for chemical reactions (Labuza 1970). Thus, a higher water activity in batters made with chlorinated flour might explain the greater starch gelatinization and swelling that we observed in those systems compared with the unchlorinated counterpart (Fig. 6).

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