

Frozen and Unfrozen Water Contents of Wheat Flours and Their Components

Alma Delia Roman-Gutierrez,¹ Stéphane Guilbert,¹ and Bernard Cuq^{1,2}

ABSTRACT

Cereal Chem. 79(4):471–475

The properties of frozen and unfrozen water in two different wheat flours (hard and soft), and in their main components (gluten, starch, damaged starch, water-soluble and water-insoluble pentosans), were described using modulated differential scanning calorimetry (DSC). As a reference, enthalpy values of crystallization (298 J/g) and melting (335 J/g) of pure water were determined from the total heat flow curves. The separation of thermal events between the reversing and nonreversing heat flows with modulated DSC was not effective due to disturbances in the modulated temperature scan. For wheat flours and their components, linear regres-

sions described well the changes in frozen water content calculated from enthalpies of freezing ($R^2 = 0.970\text{--}0.982$) or melting ($R^2 = 0.783\text{--}0.996$). The unfrozen water content (UFWC) calculated for the hard wheat flour (29–31%, db) was close to that calculated for the soft wheat flour (30–32%). The UFWC of wheat gluten (38–47%), starch (38–42%), damaged starch (37–40%), water-soluble pentosans (51%), and water-insoluble pentosans (40–44%) were higher than the corresponding values for the flours. The simple summation of the contributions of each component cannot be used to estimate the overall behavior of flours.

Many studies on water interactions in food systems have centered on the determination of properties of the water fraction known as bound water (Leung and Steinberg 1979). Bound water is formed at low water contents and results from a high density of interactions between water molecules and food components. Bound water also results from kinetically retarded diffusion of water molecules in a metastable glassy solid state (Levine and Slade 1989). Even if no clear definitions of bound and free water are available, this concept is useful. Whatever the physical properties considered (monolayer sorption, nuclear magnetic resonance [NMR] mobility, electron spin resonance [ESR], state diagram, etc.), the characteristics of bound water differ largely from those of free (or unbound) water that exists at higher moisture contents. The amounts of bound and free water depend strongly on the measurement method used. Measurements of unfrozen water content by differential scanning calorimetry (DSC) have been used as molecular-level probes for the physical state of water and its mobility in food systems (Given 1991; Marcus 1995; Roos 1995). The determination of frozen water content (FWC) by DSC is related to the amount of water that freezes during cooling. The FWC is usually determined from the melting enthalpy of ice at $\leq 0^\circ\text{C}$ (Ollivon 1991; Li et al 1998). Unfrozen water does not freeze at subzero temperatures and cannot be detected by DSC. The unfrozen water content (UFWC) is indirectly deduced by difference between total and frozen water contents. Due to the melting point depression at low moisture content, and the dependence of melting enthalpy on temperature, UFWC is slightly underestimated if calculated from measurements at only one moisture content (Reid et al 1993). One preferred way to arrive at an accurate value for UFWC is to calculate the x -axis intercept of a plot of enthalpy versus total moisture content (Schenz et al 1991).

The description of interactions between water and wheat flour components (starches, proteins, pentosans) is a key factor in understanding the physicochemical events occurring during the processing of cereal products. Depending on moisture content and characteristics of wheat components, water acts as directly bound to components or as a free water phase in dough (Hlynka 1959; Larsen 1964). Bound water equals the amount of water necessary to fully hydrate and plasticize (Slade et al 1989) the wheat components and contributes directly to the supramolecular organization of dough structure. Free water is partly responsible for the flow and mobility properties of dough. The bound water content in flour has been estimated at 27–32% (db) from sorption equilibrium determinations (Baker

et al 1946a; Bushuk and Mehrotra 1977b; Leung and Steinberg 1979). The amount of unfrozen water in wheat flour has been determined to be 30–35% (Davies and Webb 1969; Bushuk and Mehrotra 1977b; Kaletunc and Breslauer 1996). Bushuk and Mehrotra (1977b) indicated that the UFWC of wheat flour seemed not to depend on flour strength, protein content, amount of damaged starch, dough mixing-time, added chemicals (salt or ascorbic acid), or conditioners including pentosans. Above the UFWC, formation of a free water phase induces drastic changes in dough properties (Hlynka 1959; Eliasson and Larsson 1993; Sahi 1994). Using ultracentrifugation, it is possible to separate a liquid phase from a dough at >35% moisture content (db) (Baker et al 1946b; MacRitchie 1976). There must be >50–54% moisture to form a continuous free water phase in dough (MacRitchie 1976).

From NMR studies, the content of bound water in dough was estimated at 23–35% (Toledo et al 1968; Leung and Steinberg 1979; Leung et al 1983; Richardson et al 1986; Belton et al 1995; Li et al 1998; Ruan et al 1999). Using NMR relaxation methods, D'Avignon et al (1991) found that hard wheat flour has slightly less low mobility bound water than does soft wheat flour.

For wheat flour components, only limited data for FWC and UFWC are available in the literature. The bound water capacity of wheat starch was measured as 29% by sorption equilibrium and as 30% by NMR or DSC (Leung and Steinberg 1979). The bound water capacity of wheat gluten was measured as 37% by sorption equilibrium and NMR (Leung and Steinberg 1979) and as 22–39% by DSC (Slade et al 1989; Cherian and Chinachoti 1997). However, no extensive study has been published on the characterization of the water-binding properties of wheat flours and their main components. The present study was an attempt to describe the properties of unfrozen water in two different wheat flours (hard and soft) through the behavior of their main components (gluten, starch, damaged starch, water-soluble and water-insoluble pentosans). The bound properties of water were estimated by measurements of frozen and unfrozen water contents using modulated DSC.

MATERIALS AND METHODS

Commercial soft and hard wheat flours, industrial wheat gluten (Manito from Eurogerm, Dijon, France), industrial wheat starch (Roquette, Lestrem, France), water-insoluble wheat pentosans (INRA, Montpellier, France), and water-soluble pentosans (CH-8033020, Leuven Bioproducts, Belgium) were used. Damaged starch was obtained by grinding wheat starch with a mortar grinder (RM 100, Fisher Scientific, France) for 30 min. All materials were stored at 4°C in hermetic cans before experiments. The contents of water, damaged starch, ash, and lipids were determined according to Approved Methods 44-15, 76-31, 08-12 and 76-12, respectively (AACC 2000). Protein contents were determined according to the Dumas method

¹ Laboratoire Technologie des Céréales et des Agro-Polymères, ENSAM—INRA Montpellier, 2 place Pierre Viala, 34060 Montpellier, Cedex 1, France

² Corresponding author. E-mail: cuq@ensam.inra.fr

(NA 2000, Fisons Instrument). The contents of water-soluble and total arabinoxylans were determined by colorimetric and chromatographic methods (Rouau and Surget 1994). Calculations estimated the contents of water-insoluble arabinoxylans (difference between total and water-soluble arabinoxylans) and starch (difference with the other components). The biochemical composition of the wheat flours and their components is shown in Table I.

Hydration of samples was done by addition of a predetermined quantity of water (0–60 g of water/100 g of material) from a microsyringe to 1.5 g of material. Material and water were agitated (Vortex Engineering, 5804, EP 900, Scientec) at speed 3 for 15 sec, and then mixed with a spatula until a homogeneous mixture was obtained. Hydrated samples were stored for 30 min in a closed tube at 25°C before centrifugation at 5,000 rpm for 20 min at 20°C (rotor JLA-16250, Avanti Beckman). The samples were then stored for 24 hr at 25°C before analysis.

The FWC and UFWC of the hydrated samples were determined using DSC with a modulation extension device (DSC 2920, TA Instruments, New Castle, DE). Cooling was performed with a refrigerated cooling system (TA Instruments). The purge gas was nitrogen. The temperature calibration of the DSC instrument was performed using TA Instruments software with indium (melting point 156.6°C, TA Instruments) or water (melting point 0°C). Heat capacity was calibrated with sapphire (aluminum oxide). Hydrated samples (5–25 mg) were deposited and hermetically sealed in aluminum DSC pans (TA Instruments). The pans were weighed (Sartorius microbalance, R2 210 type) with 0.1-mg accuracy. The reference pan was filled with air. The DSC was operated in modu-

lation mode; the scan rate was 5°C/min, over an appropriate temperature range: 1) equilibration at 30°C, modulation $\pm 0.796^\circ\text{C}$ every 60 sec, isothermal for 5 min; 2) cooling at $-5^\circ\text{C}/\text{min}$ to -40°C ; 3) equilibration at -40°C for 5 min; 4) heating at $5^\circ\text{C}/\text{min}$ to 40°C . TA Instruments software was used to record and analyze the thermograms. Total, reversing, and nonreversing heat flows were recorded during the temperature scans. Both enthalpies of melting (heating mode) and crystallization (cooling mode) were used to calculate samples FWC.

RESULTS AND DISCUSSION

The composition of the selected flours and flour components is presented in Table I. The principal composition differences between the two selected flours are the higher contents of proteins, damaged starch, and arabinoxylans for the hard wheat flour. It is important to note that the selected fractions of flour components are not pure fractions and that they were not obtained from the two selected flours. There is 16% starch in the gluten fraction and 52% of non-arabinoxylan components (starch) in the insoluble pentosan fraction. Nevertheless, we considered that the functional properties of these fractions could be attributed to their major component.

Typical modulated DSC curves for pure water are shown in Fig. 1. During cooling, a well-defined exothermic peak is discerned between -10 and -15°C in the total and nonreversing heat flow curves. In the reversing heat flow curve, a smaller exothermic peak is detected in the same temperature range. These peaks are associated with the freezing or crystallization of pure water during cooling from 20°C to -40°C , at $-5^\circ\text{C}/\text{min}$. During subsequent heating, a large endothermic peak is detected between -2 and 5°C in the total and nonreversing heat flow curves (Fig. 1). A small endothermic peak is also observed in the reversing heat flow curve in the same temperature range. These endothermic peaks are associated with ice melting during heating.

DSC experiments were first conducted with various weights of pure water (2–10 mg) to determine enthalpies for water crystallization and ice melting. The measured values of enthalpy were 298 J/g for water crystallization and 335 J/g for ice melting, as determined from total heat flow. This enthalpy of ice melting is consistent with the heat of fusion of pure water and ice at 0°C (334 J/g) as reported by Miles et al (1983). As expected, the sum of the enthalpies (for water crystallization and ice melting) measured in the reversing and

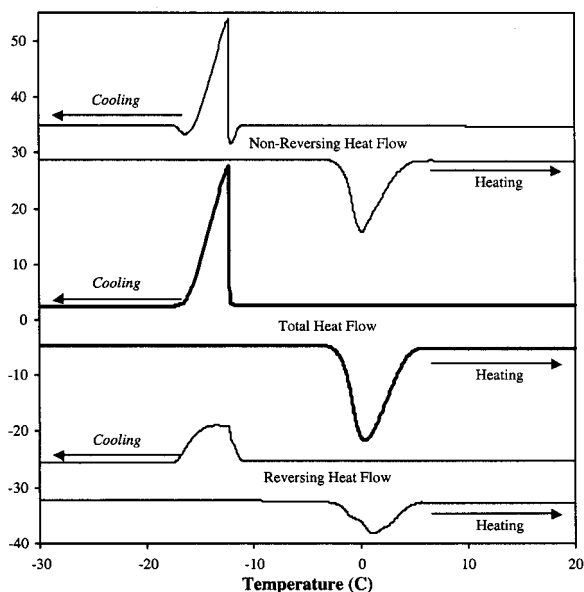


Fig. 1. Total, nonreversing, and reversing heat flow thermograms for pure water during cooling and subsequent heating using modulated differential scanning calorimetry (DSC).

TABLE II
Average Values of Enthalpy for Water Crystallization
on Cooling and Ice Melting on Heating^{a,b}

	Water Crystallization ΔH (J/g)	Ice Melting ΔH (J/g)
Total heat flow	298 (13)	338 (12)
Reversing heat flow	103 (41)	62 (22)
Nonreversing heat flow	182 (26)	275 (13)

^a Measured using modulated differential scanning calorimetry.

^b Values in parentheses are standard deviations.

TABLE I
Biochemical Composition of Wheat Flours and Their Components^a

	Chemical Composition (% db)							
	Water Content (% db)	Protein	Starch	Damaged Starch	Total Arabinoxylans	Soluble Arabinoxylans	Ash	Lipids
Soft wheat flour	14.8	10.5	86.5	3.65	1.51	0.49	0.44	1.25
Hard wheat flour	15.3	12.9	84.0	6.57	1.76	0.45	0.47	1.28
Gluten	14.8	79.8	15.6	3.28	0.65	0.20	0.47	0.29
Starch	14.0	0.22	98.9	1.73	0	0	0.12	0.03
Damaged starch	13.7	0.21	98.9	22.0	0	0	0.13	0.03
Insoluble pentosans	15.0	5.23	51.7	2.85	37.8	0	3.78	...
Soluble pentosans	10.6	...	1.0	0.21	...	99.0

^a Content of glucans and residual resistant starch formed during sample preparation.

nonreversing heat flow curves is close to the value measured in the total heat flow curve (Table II). Water crystallization and ice melting are observed in all three heat flow curves. Ice melting would only have to be detected in the reversing heat flow curve. Water crystallization on cooling is a nonequilibrium phase transition and normally would only have to be detected in the nonreversing heat flow curve (Gallagher 1997; Lai and Lii 1999). Practical difficulties due to large modulation range made reliable measurement of reversing and nonreversing heat flows extremely difficult. Disturbances in the modulated temperature profiles were responsible for the poor separation of thermal events between heat flow curves. Accurate determinations of FWC were only obtained using freezing and melting enthalpies measured from the total heat flow curve (Table II).

The FWC in wheat flours and their components was determined at different moisture contents. Typical DSC scans, measured during cooling and subsequent heating, are shown in Figs. 2 and 3 (for the hard wheat flour). Crystallization and melting of frozen water in hard wheat flour were only observed at moisture contents >30–33% db. Similar DSC scans were observed for the soft wheat flour and the flour components (data not presented). The melting temperatures observed for wheat flour and its components increased from –15 to 0°C as a function of moisture content. The melting temperature depression for free water in food materials is commonly reported (Roos 1995; Kaletunç and Breslauer 1996). For high molecular weight carbohydrates, the melting temperature of ice in contact with a maximally freeze-concentrated solution (T_m') nearly coincides with glass transition temperature of the maximally freeze-concentrated matrix (T_g') (Levine and Slade 1986; Reid et al 1993). Starch, amylopectin, amylose, gluten, glutenin, and hemicellulose share similar values for T_g' (–10°C ± 5) (Levine and Slade 1989, 1994; Roos and Karel 1991; Liu and Lelievre 1992; Kokini et al 1994).

As expected, the exothermic and endothermic peak areas for water crystallization and ice melting increased as the water content in the wheat flours and their components increased (Figs. 2 and 3). This indicated that the amount of frozen water increased with water content. FWC was calculated directly from measured enthalpies using the average enthalpies of pure water as a reference (Table III). For the wheat flours and their components, linear regressions described the changes in FWC calculated from freezing ($R^2 = 0.970–0.982$) or melting ($R^2 = 0.783–0.996$) enthalpies. The discrepancy observed for the water-soluble pentosans was mainly due to the dispersion of experimental points and not to nonlinear behavior.

The distribution of water molecules between frozen and unfrozen fractions was estimated from the values of the slopes of the regression lines (Table III). Standard deviations of the slopes (0.02–0.09) were calculated using analysis of variance (Excel software). For the selected wheat flours, the slopes were 0.67–0.83. Higher values of the slopes were found for the wheat flour components: gluten (1.02–1.34), starch (1.12–1.33), damaged starch (0.88–1.01), and insoluble pentosans (0.85–0.94), but for not water-soluble pentosans (0.51). It should be noted that lower values for the slopes were observed when the enthalpy values were calculated from ice melting rather than from water crystallization. Slopes differing from 1 could indicate that increasing water content induced structural reorganization of the materials. Such reorganizations could increase the surface area of the hydrophilic sites able to interact with the added water (slope < 1) or in new configurations decreasing the density of molecular interactions with water (slope > 1). Hydration of flour particles seemed thus to favor increased interactions with water due to increased plasticization and swelling of the initial in situ structure (slope < 1). On the other hand, the initial structures of wheat gluten and starch seemed to be reorganized during hydration to the

TABLE III
Linear Regressions of Changes in Frozen Water Content as a Function of Moisture Content^{a,b}

	Crystallization			Melting		
	<i>a</i>	<i>b</i>	<i>R</i> ²	<i>a</i>	<i>b</i>	<i>R</i> ²
Hard wheat flour	0.80 (0.05)	–24.8 (2.9)	0.974	0.67 (0.04)	–20.1 (2.6)	0.973
Soft wheat flour	0.83 (0.05)	–25.8 (3.1)	0.970	0.67 (0.04)	–20.1 (2.6)	0.971
Gluten	1.34 (0.09)	–62.7 (6.1)	0.979	1.02 (0.05)	–38.5 (3.1)	0.978
Starch	1.33 (0.08)	–56.2 (5.1)	0.979	1.12 (0.05)	–42.9 (3.3)	0.984
Damaged starch	1.01 (0.05)	–40.0 (2.9)	0.982	0.88 (0.02)	–32.7 (1.1)	0.996
Soluble pentosans	0.51 (0.07)	–26.1 (4.9)	0.783
Insoluble pentosans	0.94 (0.07)	–41.1 (4.4)	0.970	0.85 (0.04)	–33.5 (2.4)	0.986

^a $W_F = a W_T + b$, where W_F is frozen water content (% db) and W_T is total water content. Values in parentheses are standard deviations.

^b Measured from differential scanning calorimetry (DSC) of total heat flow curves.

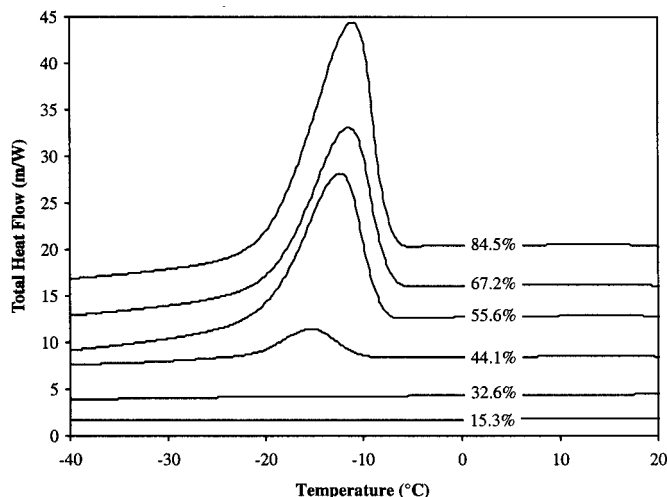


Fig. 2. Influence of water content (% db) on total heat flow thermogram measured during cooling of hard wheat flour using modulated differential scanning calorimetry (DSC).

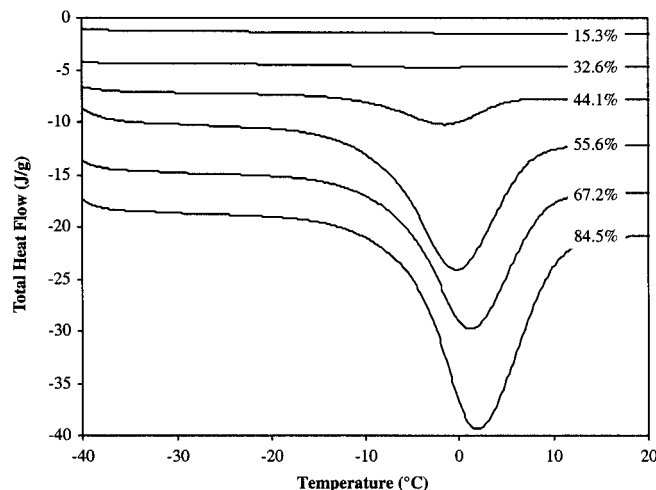


Fig. 3. Influence of water content (% db) on total heat flow thermogram measured during heating of hard wheat flour using modulated differential scanning calorimetry (DSC).

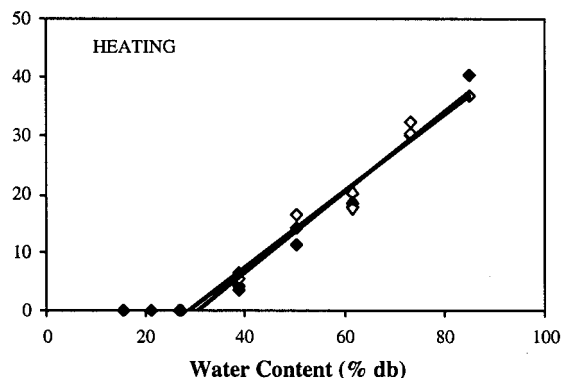


Fig. 4. Influence of total water content on frozen water content for hard wheat flour (◆) and soft wheat flour (◇) estimated from melting enthalpy measured during heating from differential scanning calorimetry (DSC) total heat flow curve.

TABLE IV
Unfrozen Water Content of Wheat Flours and Their Components^a

	Crystallization		Melting	
	(%, db)	(%, wb)	(%, db)	(%, wb)
Hard wheat flour	31 (6)	25	30 (6)	24
Soft wheat flour	31 (6)	23	30 (6)	23
Gluten	47 (8)	32	38 (5)	28
Starch	42 (7)	30	38 (5)	28
Damaged starch	40 (5)	28	37 (2)	27
Soluble pentosans	51 (2)	34
Insoluble pentosans	44 (8)	30	39 (5)	28

^a Measured from differential scanning calorimetry (DSC) of total heat flow curves. Values in parentheses are standard deviations.

detriment of interaction with water molecules (slope > 1). For the selected wheat materials, not all of the added water above the unfrozen water content (UFWC) appeared to behave as frozen water. Bushuk and Mehrotra (1977a) also noted that only two-thirds of the added water above the UFWC behaved as free water in flour. On the other hand, Davies and Webb (1969) reported that all the added water above the UFWC behaved as free water in flour.

The values of UFWC for wheat flours and their components were determined from the *x*-axis intercepts of the regressions lines constructed to describe the changes in FWC as a function of total water content (Figs. 4 and 5). Average values and standard deviations of UFWC are shown in Table IV. Values of standard deviations of UFWC were estimated from the values of error type for the equation parameters by analysis of variance. Calculated values of standard deviation were 2.1–8.4%, except for the soluble pentosans (20%). Any localized nonuniform distribution of water in these quite hydrophilic and viscous soluble pentosans will lead to poor reproducibility. Only nonsignificant differences were observed between values of UFWC calculated from freezing or melting enthalpies, except for wheat gluten. The ice melting method has a lower standard deviation and appears to be a better way to measure UFW than the water crystallization method. The UFWC calculated for the hard wheat flour (30%) was similar to that calculated for the soft wheat flour (30–31%). For the wheat flour components, the UFWC of gluten (38–47%), starch (38–42%) and water-insoluble pentosans (40–44%) were higher than those calculated for the flours. The water-soluble pentosans were characterized by an apparent relatively high value of UFWC (51%). No significant difference was observed between the UFWC for starch and damaged starch. Even though gluten proteins have a partly hydrophobic structure, the gluten UFWC value was not significantly different from those measured for starches, which are characterized by highly hydrophilic behavior. As a consequence, the description of UFWC properties of wheat components cannot be derived directly from knowledge of their biochemical structures. In addition, the calculated values

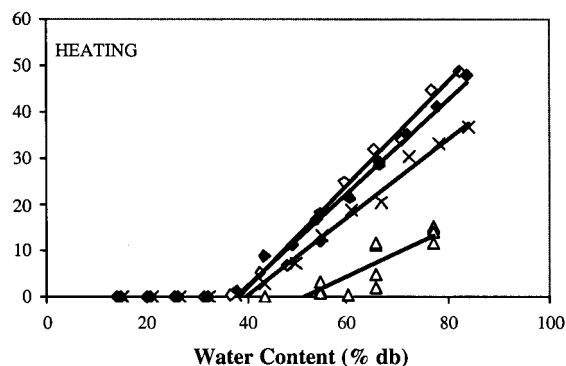


Fig. 5. Influence of total water content on frozen water content for wheat gluten (◆), wheat starch (◇), insoluble pentosans (×), and soluble pentosans (△) estimated from melting enthalpy measured during heating from differential scanning calorimetry (DSC) total heat flow curve.

of UFWC for wheat flour components (37–51%) were higher than those calculated for the selected flours (30–31%). Thus, it was not possible to calculate or predict the UFWC of wheat flours from the behavior of their components. The simple summation of the contributions of each component cannot be used to estimate the overall behavior of flours. Specific structural configurations of components in wheat flour (involving the exposure of hydrophilic or hydrophobic sites) and interactions between components could be responsible for this result. Indeed, using DSC data for pure components to describe complex systems (such as wheat flour) ignores interactions between polymeric components and potential alteration of properties of individual components which might occur as a result of a purification process.

The present values of UFWC are in agreement with much of the previously published data. The UFWC of hard wheat flour was estimated as 30% from a state diagram (Kaletunç and Breslauer 1996). A lower value of UFWC for wheat gluten (22%) was proposed by Cherian and Chinachoti (1997) from DSC measurements. In addition, our calculated values of UFWC for wheat components are close to values of the unfrozen water content in the maximally freeze-concentrated solute matrix (W_g') (Levine and Slade 1986) determined from state diagrams (Roos 1995). Most high molecular weight biopolymers (including starch, amylopectin, amylose, gluten, glutenin, gliadin, and hemicellulose) appear to share similar values for W_g' (25–43%) (Flink 1983; Levine and Slade 1986; Slade et al 1989; Levine and Slade 1994; Madeka and Kokini 1994; Kokini et al 1994; Roos 1995).

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[Received July 9, 2001. Accepted January 23, 2002.]