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Thermoanalytical characterization of high molecular weight glutenin subunits Water effect on their glass transition

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Abstract

The thermal behaviour of the high M_r glutenin subunits (a group of storage proteins in wheat grains) 1Dx5, 1Bx7, 1Dx2, 1Bx20, 1Dy12, 1Bx6 and 1Dy10, was investigated using Differential Scanning Calorimetry and Thermogravimetric Analysis.

Also included in these studies, for comparison, was a M_r 58 000 peptide (58 K) derived from the repetitive domain of subunit 1Dx5 and expressed in *Escherichia Coli*. The plasticizing effect of water on each protein was investigated.

When submitted to the calorimetric analysis, the high M_r glutenin subunits exhibited a glass transition phenomenon that was affected by the water content. As expected, the glass transition temperature decreased as the water content was increased.

Keywords: Glutenin; Calorimetry; Thermogravimetry; Glass-transition

1. Introduction

Gluten, the major storage protein fraction of cereal grains, is composed of two protein groups: gliadins and glutenins, which are respectively soluble and insoluble in 70–90% aqueous ethanol [1,2].

Gliadins are present as monomers and are responsible for the extensibility and cohesive properties of gluten, while the glutenins form high molecular weight polymers and contribute to the elasticity of gluten [3].

The combination of these two protein groups is, therefore, responsible for the viscoelastic properties that allow wheat doughs to be processed into bread and other foods. Both the gliadins and glutenins are mixtures of proteins that can be divided into groups. One such group of glutenin proteins, called the high molecular weight (high M_r) subunits of glutenin, appears to be particularly important in relation to determining the viscoelastic properties of gluten and the differences in this property between cultivars (cv) of good and poor breadmaking performance [4,5]. They have therefore been studied in detail to determine their structures and biophysical properties [6–9].

The gluten proteins, like all polymers, are characterized by a temperature dependent equilibrium between two phases, a semiliquid, prevalent at high temperature, and a glassy solid, prevalent at low temperature. This type of physical change has been called 'glass transition' [10]. The glass transition temperature (T_g) is the principal parameter for under-

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standing the mechanical properties of gluten proteins [11]. Details of this parameter should, therefore, provide useful information relevant for the comprehension of the rheological properties of wheat-flour-water based foods.

A powerful tool for studying the thermal behaviour of these proteins is the Thermal Analysis carried out by Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) techniques [11–14].

The first technique measures the change in weight caused by the loss of water and eventual product degradation, allowing the total amount of water present in the protein structure to be calculated. The second technique allows the observation of all phenomena that involve heat exchange. Recent studies have shown that the glass transition temperature can be influenced by the presence of a plasticizing agent such as water, increasing the mobility of polymeric chains and lowering the T_g value [3,12].

The aim of this work was to determine the thermal behaviour of different high M_r glutenin subunits and to correlate their glass transition temperatures with different water contents.

2. Experimental

2.1. Materials

High M_r glutenin subunits, 1Dx5 and 1Bx6 were obtained from bread wheat cv. Mercia, 1Dx2 and 1Dy12 from cv. Longbow, 1Bx7 from cv. Galatea, 1Dy10 from an isogenic Gabo/Olympic line, L 88-22 [15] and 1Bx20 from durum wheat cv. Bidi 17. The M_r 58 000 peptide, derived from the repetitive domain of subunit 1Dx5, was expressed in *Escherichia Coli* and isolated as described below.

2.2. Purification of the high M_r glutenin subunits from wheat

Proteins were extracted and purified according to the method of Shewry et al. [16] with minor modifications:

1. fractions collected after gel-permeation were dialysed against 1% (v/v) acetic acid prior to lyophilization;
2. the lyophilized fractions were dissolved in 50 mM glycine/acetate buffer, pH 4.6 containing 3 M urea and loaded onto a column (300 mm × 45 mm, packed bed volume 100 ml) containing CMC ion-exchange resin pre-equilibrated in the same buffer. Elution was with a 0–80 mM NaCl gradient (500 ml) followed by a 80–250 mM NaCl gradient (500 ml) in the same buffer.

Those fractions containing single subunits, as determined by SDS-PAGE, were pooled and dialysed exhaustively against 1% (v/v) acetic acid and then lyophilized.

2.3. Expression and purification of the M_r 58 000 repeat peptide

The plasmid construct of Buonocore et al. [17] was transferred to *E. Coli* strain BLR (DE3) pLysS (Novagen). Growth conditions, induction of expression and purification of the peptide were essentially as described with the following modifications:

1. 1 l cultures were grown in 2 l baffled flasks;
2. a final gel-permeation step on Sephacryl S-300 was introduced using the conditions described in [16].

2.4. Thermogravimetric analysis

TGA was carried out by using a Mettler TA 3000 system equipped with a TG 50 thermomicrobalance (10^{-3} mg) and TC-10 processor. The high M_r glutenin subunits were submitted to TGA employing the following procedure: all samples (6–8 mg) were accurately weighed and examined in the temperature range 25–150°C at a heating scan rate of 4°C/min. The water contents of the proteins are reported in Table 1.

2.5. Differential scanning calorimetry

The high M_r glutenin subunits were submitted to DSC analysis as-received, after addition of different known amounts of water as well as after removal of the water from the samples through lyophilization.

DSC was performed by using a Mettler TA 4000 system equipped with a DSC-30 cell and TC-11 processor. The sensitivity was 1.50 mW and the refer-

Table 1
Water contents of the as-received high M_r glutenin subunits and M_r 58 000 peptide, obtained by TGA

Compounds	1Dx5	1Bx7	1Dx2	1Bx20	1Dy12	1Bx6	1Dy10	58K
% of water	6.0	6.8	8.8	7.0	7.3	6.0	7.8	6.1

ence cell was empty. The sample weight was about 10 mg. After calibration of the calorimetric system as recommended by the manufacturer, fine temperature calibration was carried out in a narrow range using indium, stearic acid and water. Temperature and enthalpy were also checked using palmitic acid.

A first aliquot of each as-received protein was accurately weighed into an aluminium DSC pan (150 μl), sealed and analysed by employing the following procedure:

1. two subsequent scans in the temperature range −30 to +90°C, at a heating rate of 5°C/min;
2. an isothermal period at 5°C for 4 h;
3. two subsequent scans following the procedure reported above at point 1.

After these calorimetric scans, the pans were opened and the proteins were transferred into a preweighed DSC pan containing a known amount of water. This resulted in water contents of 13.8, 14.8, 17.0, 14.9, 16.5, 15.2, 16.5, 14.2% (wet basis), respectively, for the 1Dx5, 1Bx7, 1Dx2, 1Bx20, 1Dy12, 1Bx6, 1Dy10 subunits and the M_r 58 000 peptide. The eight samples were immediately submitted to two subsequent calorimetric scans and then stored for 12 h at room temperature (RT) prior to a final calorimetric scan.

A second fraction of each as-received protein was accurately weighed into a DSC pan containing a known amount of water. This resulted in water contents of 13.9, 13.3, 13.9, 15.3, 13.6, 14.0, 13.6 and 15.3% respectively for the 1Dx5, 1Bx7, 1Dx2, 1Bx20, 1Dy12, 1Bx6, 1Dy10 subunits and M_r 58 000 peptide. The pans were then sealed and stored for 24 h at room temperature. After this period, the samples were analysed as described previously.

A third portion of each as-received 1Dx2, 1Bx20, 1Dy12 and 1Dy10 glutenin subunit was accurately weighed into an aluminium pan (150 μl), lyophilized to give 3.0, 4.0, 2.0, 2.9% residual water, respectively, and analysed using the same procedure. It was not possible to examine the remaining dehydrated samples because of their hygroscopicity.

3. Results

3.1. Thermogravimetric analysis

TGA of the as-received samples showed different weight losses for each protein. The water contents (w/w) were 6.0, 6.8, 8.8, 7.0, 7.3, 6.0, 7.8 and 6.1% for 1Dx5, 1Bx7, 1Dx2, 1Bx20, 1Dy12, 1Bx6, 1Dy10 and M_r 58 000 peptide, respectively. These losses occurred in a single step as the proteins were heated to 120°C. As an example, the TG curve (curve a) and the DTG curve (curve b) of the glutenin subunit 1Dx5 are shown in Fig. 1.

3.2. Differential scanning calorimetry

Figs. 2 and 3 show the calorimetric curves of the high M_r glutenin subunits: 1Dx5 (A), 1Bx7 (B), 1Dx2 (C), 1Bx20 (D), 1Dy12 (E), 1Bx6 (F), 1Dy10 (G) and the M_r 58 000 peptide (H). Curves a–c in Fig. 2 (in each graph) refer to the calorimetric analysis of the as-received proteins. It is interesting to note that the thermal behaviour of the 1Bx6 sample was different from the others (compare curves a in Fig. 2). In fact, the 1Bx6 subunit (Fig. 2F, curve a) showed during the first scan a change in the curve slope and level, indicating a variation of the specific heat that appears again in the subsequent scan (Fig. 2F, curve b). In the initial scans, all the others proteins (Fig. 2A–E, G, H, curves a) showed, in addition, an endothermic peak, which disappeared during the subsequent scan (Fig. 2A–E, G, H, curves b) where a 'regular' shape in the specific heat variation was observed. After an isothermal period at 5°C for 4 h, the calorimetric scans of all the proteins showed a reproducible thermal behaviour (Fig. 2, curves c) as confirmed by a subsequent calorimetric heating scan (data not shown).

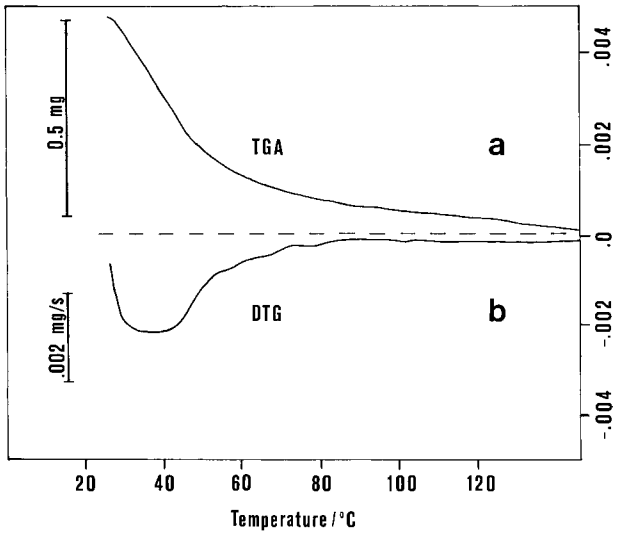

Fig. 1. Thermogravimetric analysis of the high M_r glutenin subunit 1Dx5 as-received: thermogram (TGA) curve a, differential thermogram (DTG) curve b.

Immediately following the addition of known amounts of water to the samples described above (to give total water contents of 13.8–17.0%), calorimetric analysis revealed an endothermic peak for each sample (Fig. 3A–H, curves a) followed by an exothermic peak. Again, the behaviour of the 1Bx6 subunit differed from the other compounds examined, in fact the exothermic peak was absent. These peaks disappeared in the subsequent scan, (Fig. 3, curves b) where only a change in the slope of the curves was evident.

Curves d reported in Fig. 2 refer to the samples prepared by adding to the as-received proteins known amounts of water, so to obtain a water contents similar to those previously obtained for the samples reported in the Fig. 3, and storing them for 24 h at room temperature in a sealed pan, in a way to allow the water to permeate into the substances. The calorimetric investigation of these samples revealed that the change in the heat capacity occurred at lower temperatures with respect to the as-received substances (compare Fig. 2, curves c–d). A comparison between the shape of the curve relative to subsequent scans (curves not-reported) shows the reproducibility of this process.

Instead, the change in heat capacity was shifted to a higher temperature (Fig. 2, curves e) when the as-received glutenin subunits were lyophilized prior to calorimetric analysis (the residual water contents after dehydration were: 3.0% for 1Dx2, 4.0% for 1Bx20, 2.0% for 1Dy12 and 2.9% for 1Dy10). Again, this process appeared to be reproducible, as confirmed by subsequent calorimetric scans (curves not-reported).

4. Discussion

The abrupt change in slope of the calorimetric curves of the HMW glutenin subunits (Figs. 2 and 3) can be attributed to the well-known glass transition typical for amorphous materials like biopolymers. The glass transition temperature (T_g), defined as the temperature at which a glassy polymer is converted into a softer rubbery one upon heating, can be readily calculated by extending the tangents of the curve immediately preceding and following the transition and then measuring the temperature at one-half the vertical distance between the two lines.

This behaviour was first observed by differential scanning calorimetry for gluten [18], and has since been reported for glutenins [10,19].

The first scan of the 1Dx5, 1Bx7, 1Dx2, 1Bx20, 1Dy12, 1Dy10 subunits and M_r 58 000 peptide (Fig. 2 A–E, G, H, curves a) showed an endothermic peak that

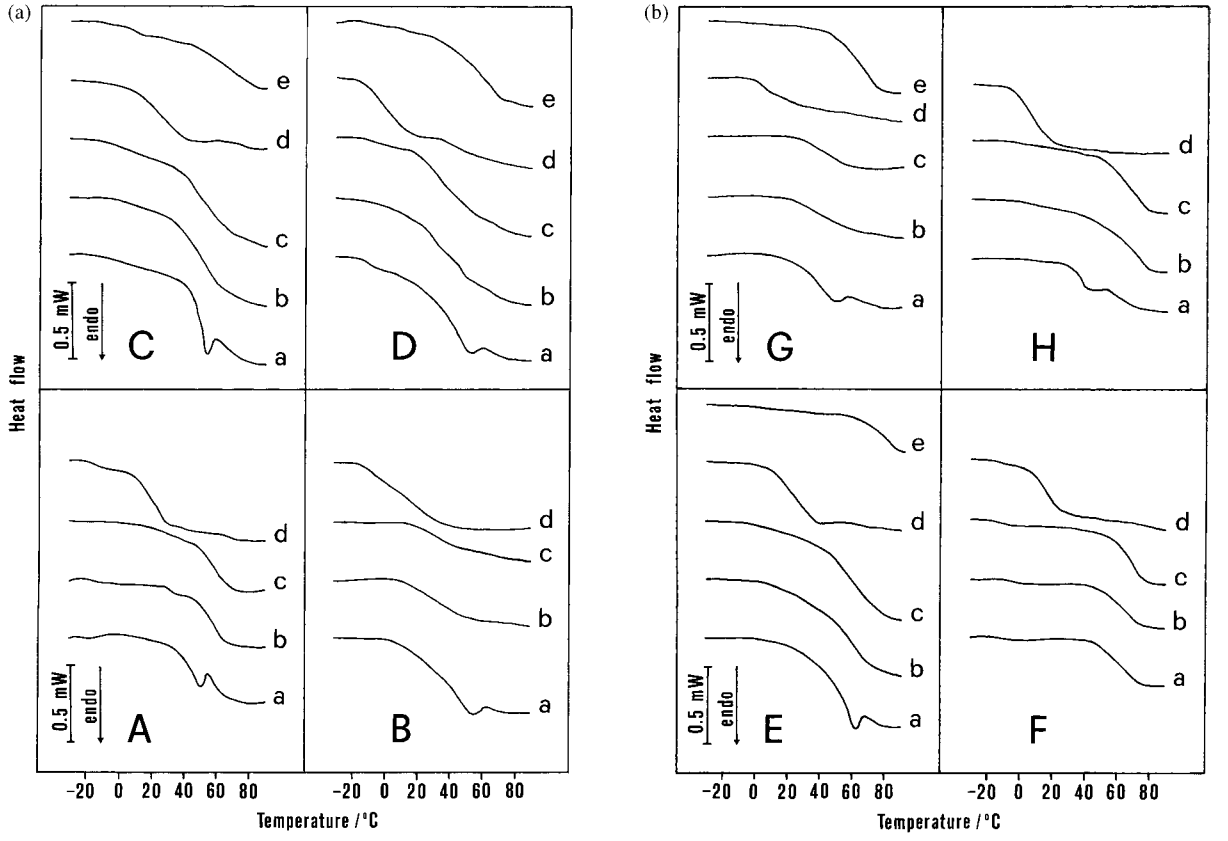

Fig. 2. Differential scanning calorimetry curves of the high M_r glutenin subunits and the M_r 58 000 peptide analysed as-received (curves a–c), after addition of water to the as-received glutenin (curves d) and after lyophilization (curves e): (A) 1Dx5: containing 6.0% (curves a–c) and 13.9% (curve d) of total water; (B) 1Bx7: containing 6.8% (curves a–c) and 13.3% (curve d) of total water; (C) 1Dx2: containing 8.8% (curves a–c), 13.9% (curve d) and 3.0% (curve e) of total water, (D) 1Bx20: containing 7.0% (curves a–c), 15.3% (curve d) and 4.0% (curve e) of total water, (E) 1Dy12: containing 7.3% (curves a–c), 13.6% (curve d) and 2.0% (curve e) of total water, (F) 1Bx6: containing 6.0% (curves a–c) and 14.0% (curve d) of total water, (G) 1Dy10: containing 7.8% (curves a–c) and 13.6% (curve d) and 2.9% of total water; (H) M_r 58 000 peptide: containing 6.1% (curves a–c), 15.3% (curve d).

overlaps the glass transition region. This peak, known as 'kinetic overshoot', is a quantitative measure of the relaxation enthalpy (ΔH) that occurs in a polymer during sub-T_g annealing [12,20] and was previously observed for gluten and glutenins [10,19]. Glasses exist in a non-equilibrium state and relax towards a lower energy and metastable state upon annealing [21] and this relaxation brings about the endothermic peak in the DSC heating scans. The area of the peak (ΔH) increases with increased annealing time until a limiting value that is the total enthalpy loss caused by the material relaxing to its lowest energy amorphous state at that specific temperature [22]. The rate at which a glass approaches this limiting value is a function of the temperature difference between the annealing temperature and T_g [20]. The farther the annealing temperature is below T_g, the slower the material anneals to a steady state [22]. The kinetic overshoot disappears in the subsequent scans (Fig. 2A–E, G, H, curves b and c) because heating beyond the T_g temperature, during the first scan, erases the changes produced by sub-T_g annealing in the polymer enthalpy [22]. This phenomenon (relaxation) doesn't appear when examining the 1Bx6 glutenin subunit by DSC (Fig. 2F, curve a).

The endothermic peak around 0°C, that appears in the first scan of all the samples examined immediately

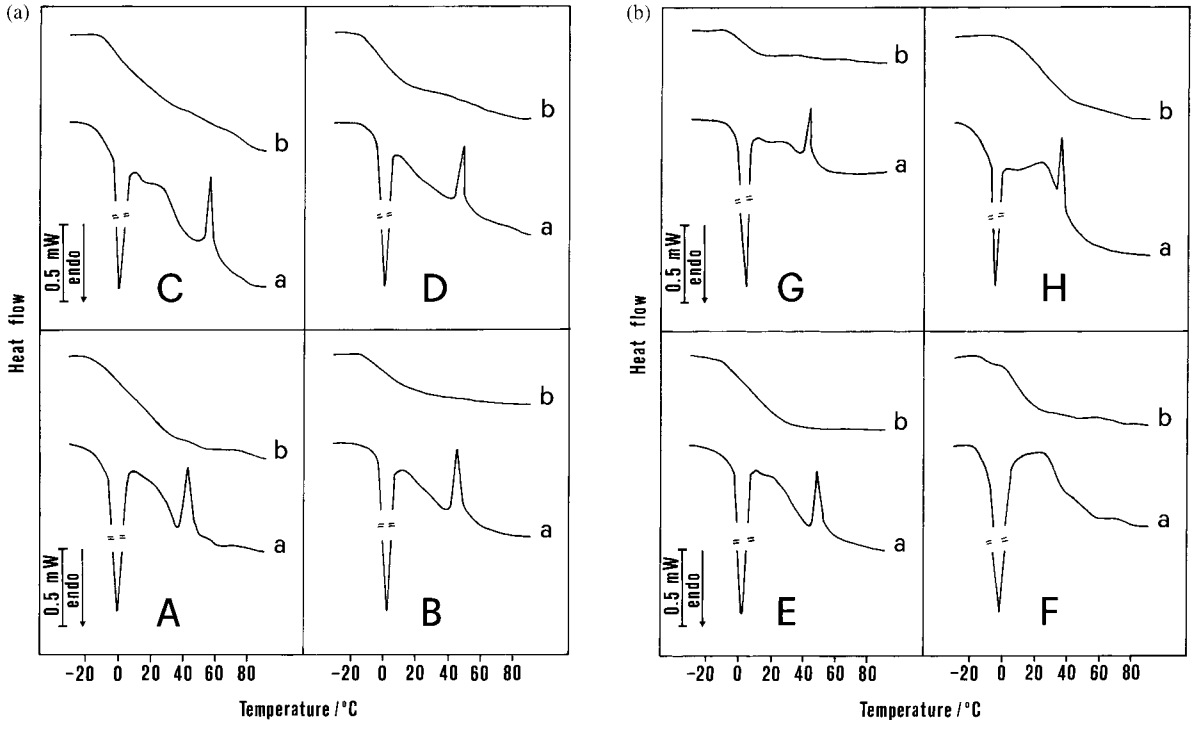

Fig. 3. Differential scanning calorimetry curves of the high M_r glutenin subunits and the M_r 58 000 peptide analysed after addition of water to the samples previously examined (see procedure reported in Section 3.3): (A) 1Dx5: containing 13.8% (curves a, b) of total water, (B) 1Bx7: containing 14.8% (curves a, b) of total water, (C) 1Dx2: containing 17.0% (curves a, b) of total water, (D) 1Bx20: containing 14.9% (curves a, b) of total water; (E) 1Dy12: containing 16.5% (curves a, b) of total water, (F) 1Bx6: containing 15.2% (curves a, b) of total water; (G) 1Dy10: containing 16.5% (curves a, b) of total water, (H) M_r 58 000 peptide: containing 14.2% (curves a, b) of total water.

after water addition (Fig. 3, curves a), is related to the melting of ice. This ice formed from water that did not permeate the protein and subsequently froze during the cooling stage prior to the heating scan. This peak disappears in the subsequent scans because the proteins are able to bind the added water during the heating of the samples in the hermetically sealed DSC pans. The exothermic peak that appears in Fig. 3 (A–E, G, H curves a), as reported previously [23,24] and which disappears in the subsequent calorimetric scan, can be related to a water-dependent cross-linking process that could involve next polymer chains. These links can be direct, such as hydrogen bonds between the functional groups of next-neighbouring chains, or mediated by interstitial water molecules bridging interchain gaps. Mobile water molecules would be displaced along polymer chains and would act as interchain zip-sliders moving from some embryonic structure [23,24]. Subunit 1Bx6 (Fig. 3F, curve a) again shows a different behaviour in fact it does not present this exothermic peak.

When water binds to the proteins, it produces a shift of the glass transition process towards lower temperatures (Fig. 3, curves b and Fig. 4). This effect, also observed for samples prepared by adding water to the as-received substances (Fig. 2, curves d), results from the plasticizing effect exerted by the water on the polymeric chain mobility, as reported in the literature for gluten and its components [3,11,12,25]. The water addition effect seems to be sufficiently independent of the sample preparation procedure and of its thermal history, as can be seen by comparing Fig. 2 (curves d) with Fig. 3 (curves b), where all the samples contain a similar amount of water.

The plasticizing effect of the water is confirmed by analysis of the lyophilized samples, where water subtraction produces a T_g shift toward higher temperatures (Fig. 2, curves e, and Fig. 4).

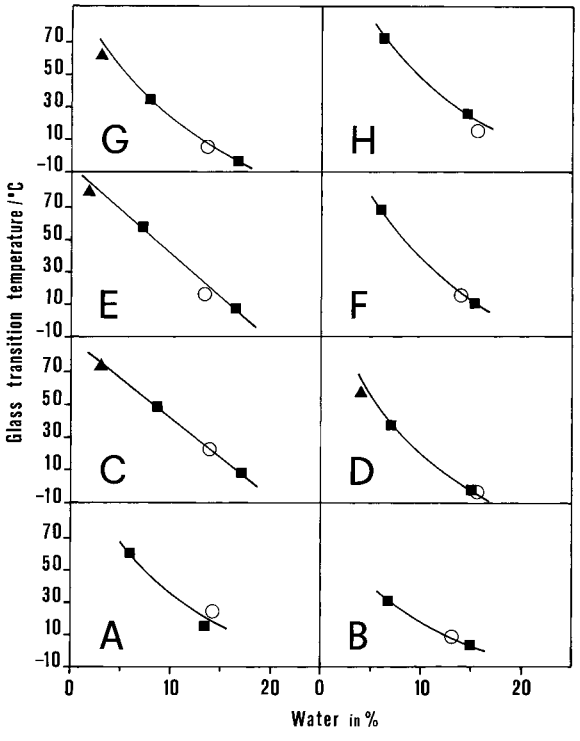

Fig. 4. Plots of the high M_r glutenin subunits and the M_r 58 000 peptide glass transition temperatures (T_g) referred to the percentage of total water content: (A) 1Dx5, (B) 1Bx7, (C) 1Dx2, (D) 1Bx20, (E) 1Dy12, (F) 1Bx6, (G) 1Dy10, (H) M_r 58 000 peptide. (■) Glutenin samples examined as-received and after addition of water to the same samples; (○) Samples examined only after addition of water to the as-received glutenins; (▲) Samples examined only after lyophilization of the as-received glutenins (low water content).

The experimental results coming from the calorimetric measurements are summarized in Fig. 4, where the correlation between the total water percentage and the T_g are reported.

From this figure, it appears evident that:

– for each individual sample, the glass transition temperature shifts towards lower or higher values when increasing or decreasing the water content, respectively;
– similar amounts of water produce different shifts of the glass transition temperature on the samples examined; this being related to the intrinsic mobility of the glutenin subunits polymeric chains.

5. Conclusions

From the results, we can infer that:

1. the high M_r glutenin subunits exhibit a different thermotropic behaviour. 1Dx5, 1Bx7, 1Dx2, 1Bx20, 1Dy12, 1Dy10 subunits and M_r 58 000 peptide show, during the first scan, an endothermic peak due to a relaxation process that superimposes on the glass transition. This relaxation phenomenon does not appear in studies on the 1Bx6 subunit;
2. since the second calorimetric scan, all the proteins present a similar behaviour, exhibiting a reproducible glass transition process because relaxation is not reversible, at least for short rest times;
3. T_g results are affected by the amount of water bound to the proteins. In fact, water addition causes a shift of the glass transition temperature towards lower temperatures, while water subtraction causes a T_g shift towards higher temperatures.

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References

[1] J.D. Schofield, M.R. Booth, in B.J.F. Hudson (Ed.), Developments in food proteins, Vol. 2, Applied Science Publishers, Barking, 1983, p. 14.
[2] P.R. Shewry, A.S. Tatham, J. Forde, M. Kreis, B.J. Miflin, J. Cereal Sci. 4 (1986) 97.
[3] G. Cherian, P. Chinachoti, Cereal Chem. 73 (1996) 618.
[4] P.I. Payne, P.A. Darris, C.N. Law, L.M. Holt, J.A. Blackman, Ann. Technol. Agric. 29 (1980) 309.
[5] P.I. Payne, Ann. Rev. Plant Physiol. 38 (1987) 141.
[6] A.S. Tatham, J.M. Field, J.N. Keen, P.J. Jackson, P.R. Shewry, J. Cereal Sci. 14 (1991) 111.
[7] J.M. Field, A.S. Tatham, P.R. Shewry, Biochem. J. 247 (1987) 215.
[8] A.S. Tatham, B.J. Miflin, P.R. Shewry, Cereal Chem. 62 (1985) 405.
[9] P.R. Shewry, N.G. Halford, A.S. Tatham, J. Cereal Sci. 15 (1992) 105.
[10] R.C. Hoseney, K. Zeleznak, C.S. Lai, Cereal Chem. 63 (1986) 285.

[11] M.T. Kalichevsky, J.M.V. Blanshard, Carbohydrate Polym. 19 (1992) 271.
[12] T.R. Noel, R. Parker, S.G. Ring, A.S. Tatham, Int. J. Biol. Macromol. 17 (1995) 81.
[13] R. Riesen, G. Widmann, Thermochim. Acta 226 (1993) 275.
[14] M.T. Kalichevsky, E.M. Jaroszkiewicz, J.M.V. Blanshard, Int. Biol. Macromol. 14 (1992) 257.
[15] G.J. Lawrence, F. MacRitchie, C.W. Wrigley, J. Cereal Sci. 7 (1988) 109.
[16] P.R. Shewry, J.M. Field, A.J. Faulks, B.J. Miflin, M.D. Dietler, E.J.L. Lew, D.D. Kasarada, Biochim. Biophys. Acta 788 (1984) 23.
[17] F. Buonocore, L. Bertini, C. Ronchi, F. Bekes, C. Caporale, D. Lafiandra, P. Gras, A.S. Tatham, J.A. Greenfield, N.G. Halford, P.R. Shewry, J. Cereal Sci. 27 (1998) 209.
[18] H. Levine, L. Slade, in: H. Faridi, J.M. Faubion (Eds.), Dough Rheology and Baked Product Texture: Theory and Practice, Van Nostrand Reinhold, New York, 1990, p. 157.
[19] A.M. Cocero, J.L. Kokini, J. Rheology 35 (1991) 257.
[20] S.E.B. Petrie, J. Polym. Sci. 10 (1972) 1255.
[21] G.B. McKenna, Comprehensive polymer science, in: G. Allen, J.C. Bevington (Eds.), Polymer Properties, Vol. 2, Pergamon Press, Oxford, 1988, p. 311.
[22] J.W. Lawton, Y.V. Wu, Cereal. Chem. 70 (1993) 471.
[23] A. Schiraldi, L. Piazza, M. Riva, Cereal. Chem. 73 (1996) 32.
[24] A. Schiraldi, L. Piazza, O. Brenna, E. Vittadini, J. Thermal Anal. 47 (1996) 1339.
[25] G.P. Johari, G. Sartor, J. Chem. Soc., Faraday Trans. 92 (1996) 4521.