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Thermal properties of raw and processed wheat gluten in relation with protein aggregation

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Abstract

The glass transition temperature (T_g) and change in heat capacity (ΔC_p at T_g) of processed wheat gluten samples, including wet (casting) and dry processes (thermal treatment, mixing, thermomoulding and mixing followed by thermomoulding) were determined by modulated differential scanning calorimetry (MDSC) as a function of water content. An increase of T_g and a drastic decrease of ΔC_p were observed when gluten was dry processed. Casting process gave a film with calorimetric properties close to those of native gluten. The molecular size distribution of proteins in native and processed glutens was measured by size exclusion chromatography. The thermomoulding and the use of chemical cross-linker during casting resulted in a drastic drop of SDS-soluble proteins. In contrast, mixing of gluten, even using high specific mechanical energies, gave only a slight polymerisation of the proteins. Therefore, except for treatments where a high temperature was applied to gluten, the modification of calorimetric parameters accounting for reticulation phenomena was not related to the SDS-insoluble protein content. T_g, ΔC_p and protein SDS extractability could account for different kinds of protein networks.

Keywords: Wheat gluten; Processing; MDSC

1. Introduction

Wheat gluten is a renewable and biodegradable material, which possesses, like other proteins, thermoplastic properties relevant to technical (non-food) applications [1–3]. Two technological processes can be used to make materials based on proteins: a wet process leading to a film formation ('casting') and dry processes using the proteins' ability to change in a reversible way from a rigid to a soft state through the glass transition phenomena. The polymer properties are drastically modified when the temperature rises above the glass transition temperature (T_g). For example, a change in the heat capacity occurs, which enables T_g to be determined calorimetrically. The glass transition is promoted by a temperature increase and/or plasticiser addition [4]. The change in T_g due to the plasticising effect of water may be described by theoretical approaches as Gordon–Taylor, Couchman–Karasz or Kwei equations [5–7]. These three models have already been used to predict the dependence of the glass transition upon the composition in protein and plasticiser blends [8–14].

Apart from the plasticiser-depressing effect, the T_g value of a polymer is generally governed primarily by chemical composition and secondarily, by structural features, such as the chain molecular weight, side branches, steric hindrance, covalent and non-covalent bonding (disulphide and hydrogen bonding, hydrophobic interactions) within the molecular structure, ratio of amorphous and crystalline regions, and other physical conditions such as pressure [15,16]. As far as proteins are concerned, it has been shown that T_g could be estimated using the Matveev's method [17] if its amino acid composition is known [18]. The ΔC_p occurring during the glass transition phenomena is related to the weight fraction affected by the glass transition [19]. It is generally admitted that T_g increases and ΔC_p decreases when strong cross-linkages or intermolecular hydrogen bonds or weaker van der Waals forces increase, restraining the chain mobility and increasing the stiffness of the polymer chains [15,20–22]. The increase of T_g after cross-links has been largely studied for synthetic polymer networks and to a lesser extent for enzymatic or chemical cross-linked natural polymers, as arabinoxylans cross-linked via the diferulic bridge formation [23] or gelatin microspheres cross-linked with glutaraldehyde [24].

A thermal treatment of cereal proteins classically

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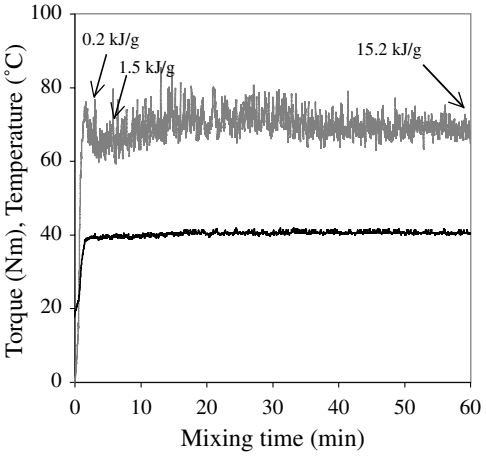

Fig. 1. Torque (—) and temperature (▬) evolution during the mixing process of gluten.

undergoes aggregation/cross-linking phenomena [13]. This thermally irreversible thermosetting reaction would be analogous to the curing of epoxy resin or the vulcanisation of rubber [25]. The thermal treatment, by modifying the interactions between proteins, leads to variations of T_g and ΔC_p determined by differential scanning calorimetry. An increase in T_g and a decrease in ΔC_p values have, therefore, been observed for commercially hot-air dried corn gluten meal by di Gioia et al. [18]. Accorded to Sartor and Johari [26], the degradation and heat polymerisation of wheat gluten, respectively, decreases and increases its T_g value. Furthermore, a decrease of ΔC_p reflects a decrease in configurational degrees of freedom [26]. Mixing of flour dough has generally been reported as inducing a protein depolymerisation phenomena. The combined effect of temperature and shear rate, encountered in a process such as high-temperature short-time extrusion, on the interactions or cross-links in gluten, has also recently been studied [27–30]. Resulting rearrangements depend on characteristics of shear rate vs thermal treatments. For instance, the polymerisation is dominant if a severe thermal treatment is applied. The key role of sulfhydryl–disulphide interchange in protein interactions during the extrusion process has been emphasised. According to Li and Lee [27,28], the hydrophobic interactions play a key role in disulphide cross-linking of wheat proteins during extrusion. Strecker et al. [30] hypothesised that disulphide bonds were superimposed upon the network already formed by cross-linking resulting from non-disulphide bonds. The effect of the thermomoulding treatment on proteins has never been studied. However, a high-pressure (800 MPa) treatment of β-lactoglobulin and bovine serum albumin showed aggregation phenomena with the intervention of disulphide bonds and perhaps exposed hydrophobic regions [31].

While some studies demonstrated that the network creation, operating when temperature or cross-linker is applied to polymer, modifies its T_g and ΔC_p, no study was attempted to follow the changes in glass transition parameters as a function of the treatment applied to proteins. In the present study, thermal properties (T_g, ΔC_p) of native and processed glutens have been investigated using modulated differential scanning calorimetry (MDSC). MDSC, allowing the determination of thermal properties in a single scan, was chosen in preference to the conventional DSC, in order to avoid a possible thermal modification of the sample during the DSC run [32]. Various treatments were applied to the native gluten: 'casting' (i.e. protein solubilisation followed by solvent evaporation), thermal treatment (simulated by an appropriate heating rate during DSC run), mixing (combined with the thermomoulding or not) and thermomoulding alone. An attempt to relate the change in thermal properties with the biochemical changes occurring in the gluten networks (polymerisation and depolymerisation, determined by protein solubility in SDS) is also presented in this study.

2. Material and methods

2.1. Material

Vital wheat gluten was provided by Amylum (Aalst, Belgium). Protein, starch, lipid and ash represented 76.5, 11.8, 5.0 and 0.8% of its dry matter, respectively. Its moisture content was determined as 7.2% in dry mass (dm).

2.2. Experimental

2.2.1. Preparation of processed glutens

Casted gluten films were prepared from a film-forming solution of vital wheat gluten ($N \times 5.6 = 76.5\%$) in an absolute ethanol/acetic acid/water solvent. The gluten concentration (12.8 g (dm)/100 ml solution), ethanol concentration (32 ml/100 ml solution), and pH of the solution (4, adjusted with acetic acid) were chosen according to Gontard et al. [33], who described casting procedure more precisely and observed that such a formulation gave a resistant, homogeneous and transparent film without insoluble particles or excessive increase of water vapour permeability. In our experiment, the gluten was first mixed with a reducing agent (sodium sulphite, 30 mg) and water (~20 g) for 15 min before adding ethanol. A cross-linking agent (formaldehyde 37% v/v, 0.8 ml) was added to the ethanolic medium. The film-forming solution was then heated at 50°C under magnetic stirring. After bubble and foam removal, it was immediately poured and spread onto a crystal PVC plate ($30 \times 40 \times 0.8$ cm) placed on a level surface with the help of a thin layer chromatography spreader bar (0.8 mm) (Braive instruments, Checy, France). The film-forming solution was dried for 2.5 h at room temperature (17°C, 37% RH) before peeling from the PVC support.

Mixed gluten was obtained using a two-blade counter-rotating batch mixer turning at a 3:2 differential speed (Plasticorder W 50, Brabender, Duisburg, Germany) connected to a computer interface and a controller unit

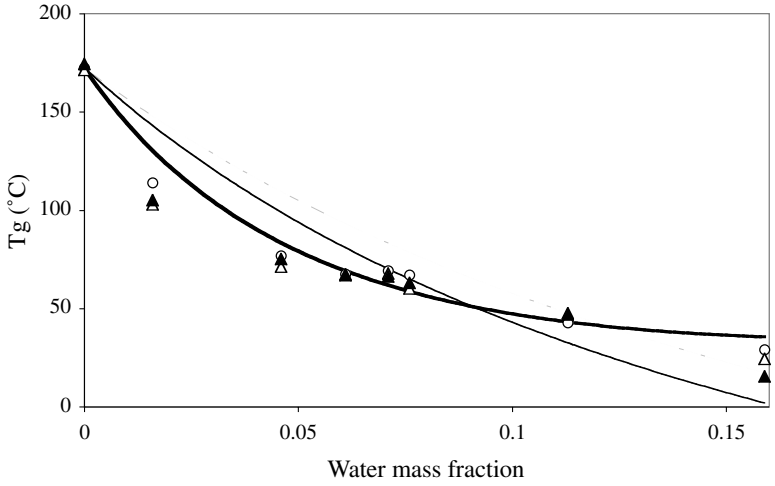

Fig. 2. T_g of gluten determined at first heating, cooling and second heating with a 5°C/min heating rate as a function of water content. (○): experimental values at first heating; (△): experimental values at cooling; (▲): experimental values at second heating; (—): Couchman–Karasz; (▬): Gordon–Taylor; (▬): Kwei fits at first heating.

(PL2000, Brabender O.H.G, Duisburg, Germany). The torque and the product temperature were continuously recorded during the mixing process. The mixing chamber was thermostated at 20°C. The sample temperature observed through out the process has been shown never to exceed 40°C irrespective of the mixing time (Fig. 1). The mixing chamber (volume: 50 cm^3) was filled with a 50 g total mass (gluten and water), which corresponded approximately to filling ratios of 80%. The mixing speed was 30 rpm. The specific mechanical energy (SME), expressed in kJ/g, provided to the product is computed from the torque curve, following the equation:

$$\mathrm{SME} = \frac{\omega}{m} \int_0^{t_{\max}} C(t)\, \mathrm{d}t$$

where ω is the rotor speed (in rad/s), m the sample mass (in g), $C(t)$ the torque at time t (in N m) and $t_{\max}$ the mixing time (in s).

In order to obtain a homogeneous material made from gluten for DSC experiments, a water content of ~25 g/100 g of wet material was chosen. The mixing was realised at different specific energies (0.2, 1.5 and 15.2 kJ/g corresponding to 1.5 and 7 min and 1 h of mixing, respectively) (Fig. 1).

Thermally treated gluten was obtained by running MDSC (see Section 2.2.2) at 2°C/min heating rate with amplitude and period of modulation of 0.5°C and 60 s, respectively. Gluten was subjected to three scans: heated at observed T_g + 60°C, cooled to observed T_g − 60°C, and heated again.

Thermomoulded gluten was obtained by moulding 10 g of gluten, preliminarily equilibrated at 85% relative humidity (using KCl solution at 20°C), for 2.5 min at 100 bars and 130°C with a thermomoulder TECHMO (PL 10T; 0–250 bars).

Mixed and thermomoulded gluten resulted from moulding of the gluten sample mixed for 1 h in the Plasticorder (in the same mixing conditions as those described above).

2.2.2. DSC measurements

Measurements were performed on a TA instrument 2920 CE modulated DSC coupled with a refrigerating cooling system (RCS). Nitrogen was used as a purge gas at a flow rate of 50 ml/min and as a cooling gas line at a flow rate of 150 ml/min. The temperature and the heat flow were calibrated by the measurement of the melting of indium (156.61°C) and water (0°C). The heat capacity was calibrated with sapphire (aluminium oxide; TA instruments, New Castle, USA). In all calibrations, the experimental conditions (e.g. pan type, purge gas, heating rate, modulation amplitude and period) have been identical to those used in subsequent sample evaluations. The glass transition temperature was recorded from the inflection point of the change in heat capacity on the reversing heat flow signal. Before analysis, all the fresh samples (except mixed and thermomoulded gluten which was freeze-dried between the two processes) were equilibrated at the desired relative humidity (>5 days). After equilibration, the moisture of the samples was determined by weighing after heating at 104°C for 24 h. For each gluten material, four pans (with approximately 10 mg of material) were analysed for each water content. Pans were sealed with inverted lids to achieve an optimum thermal conductivity. For the measurement of T_g and ΔC_p of dry samples, the pans were equilibrated for 3 weeks in P_2O_5 before analysis.

To characterise the thermal properties (T_g and ΔC_p) of native and processed glutens, the heating rate was optimised in order to avoid an excessive thermal treatment of gluten. Since MDSC typically involves heating rates of 1–5°C/min [34], 5°C/min has been tested as the heating rate reducing the temperature exposure time of gluten during the run. The period and the amplitude of modulation were 60 s and

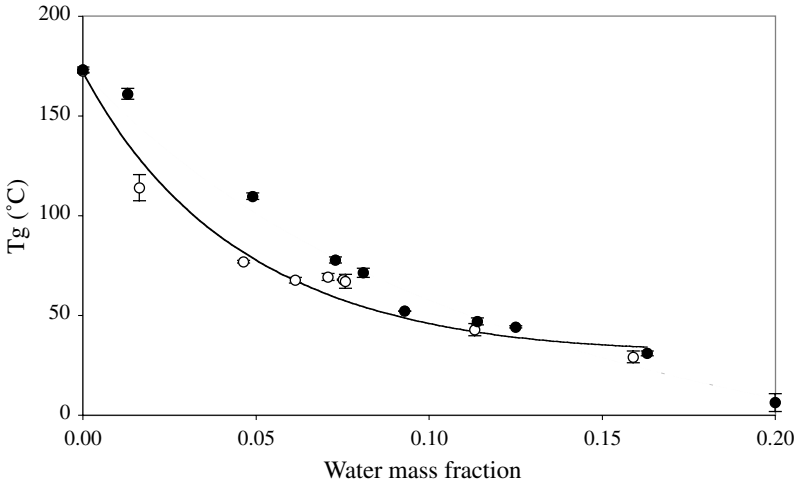

Fig. 3. T_g determined at first heating as a function of water mass fraction and heating rate. (●): experimental values at 2°C/min; (○): experimental values at 5°C/min; (—): 2°C/min and (▬): 5°C/min Kwei fits.

0.796°C. Three scans were performed for each sample: heating up to observed T_g + 60°C, cooling up to observed T_g − 60°C, and heating again. The T_g of gluten observed during the three scans as a function of water content is presented in Fig. 2. The T_g drops, at first rapidly then more slowly, from approximately 175°C to 20°C when the water content increased from 0 to 16%. This general feature has been previously observed for gluten [12,35,36] and its fractions, gliadin and glutenin [9,37,38] by DSC or DMTA experiments. The glass transition temperature determination during first scan was difficult to visualise when water content increased above 16%. However, no or slight differences in T_g was observed during the three scans irrespective of the water content tested, showing that a 5°C/min heating rate preserves gluten from an excessive heat treatment. This heating rate has been chosen for T_g and ΔC_p determination of processed glutens in this study.

Thermodynamical models (Couchman–Karasz [5] Eq. (1); Gordon–Taylor [6] Eq. (2)) or empirical model (Kwei [7] Eq. (3)) were applied for the prediction of the water plasticisation of gluten (Fig. 2). Applications of the Gordon–Taylor and Kwei equations, required the optimisation of one (k) and two (k and q) constants, respectively. The Couchman–Karasz equation was applied without fitting, with $\Delta C_p = 1.94\ \mathrm{J\ g^{-1}\ °C^{-1}}$ for water [12] and $\Delta C_p = 0.4\ \mathrm{J\ g^{-1}°C^{-1}}$ for gluten (measured by MDSC for gluten).

$$T_g = (W_1 T_{g1} \Delta C_{p1} + W_2 T_{g2} \Delta C_{p2})/(W_1 \Delta C_{p1} + W_2 \Delta C_{p2}) \quad (1)$$

$$T_g = (W_1 T_{g1} + k W_2 T_{g2})/(W_1 + k W_2) \quad (2)$$

$$T_g = (W_1 T_{g1} + k W_2 T_{g2})/(W_1 + k W_2) + q W_1 W_2 \quad (3)$$

where W_1 and W_2 are the weight fractions, T_{g1}, T_{g2} glass transition temperatures, ΔC_{p1}, ΔC_{p2} change in heat capacity of the components (1 = gluten, 2 = water).

The Kwei equation, giving the best fit (Fig. 2), has been chosen for the further modelling of the DSC results.

2.2.3. Size distribution of gluten proteins

The size distribution of proteins in native and processed glutens was studied by size-exclusion high-performance liquid chromatography (SE-HPLC) as previously described by Redl et al. [29,39]. Gluten samples were extracted twice with a sodium phosphate buffer that included sodium dodecyl sulfate (SDS, 1%). The second extraction comprised a sonication treatment in order to extract SDS-insoluble proteins [39,40]. Prior to the extraction, the mixed gluten samples (~0.8 g) were frozen in liquid nitrogen and grounded with ~4 g of starch in a ball mill (Prolabo, France) for approximately 2 min. The solid-to-solvent ratios used for the first extraction (SDS-soluble proteins) were 3.75 mg/ml for the "mixed gluten–starch" blend and 0.75 mg/ml for all other samples. For the second extraction, ratios four times higher were used. Once corrected for their different solid-to-solvent ratios during extraction, areas of SDS-soluble and SDS-insoluble proteins (in arbitrary units) were added and the sum (i.e. total extractable proteins (TEP)) was expressed as percents of the corresponding area calculated for native gluten (on equivalent dry protein basis). For all samples, extractions were performed in duplicate.

SE-HPLC profile of SDS-soluble proteins (first extract) was arbitrary divided into five peaks (F1–F5). The calibration of the column, according to Redl et al. [29], showed that F1 and F2 correspond to proteins ranging from $M_r = 7 \times 10^6$ (the claimed molecular weight exclusion limit of the column used) to $M_r = 150 \times 10^3$.The range corresponded to the known glutenin polymer molecular weight range [41]. Peaks F3 and F4 corresponded to proteins ranging from 150 to 20×10^3 and, therefore, could be assimilated

Table 1
Constants issue of the application of Gordon–Taylor and Kwei equations

MDSC heating rate	2°C/min[a]	5°C/min
k (Gordon–Taylor)	5.1	6.4
k (Kwei)	6.5	12.2
q (Kwei)	184	601
(Experimental T_g − Theoretical T_g)^2		
Gordon–Taylor	617	2376
Kwei	368	553

[a] Value from Micard and Guilbert [32].

to gliadins [40]. The second extract, obtained after solubilisation by sonication, allowed characterisation of insoluble proteins ($F_{(sonicated)}$) whose molecular weight exceeded 7×10^6 before sonication.

2.2.4. Statistics

The ΔC_p values obtained for native gluten and wet and dry processed glutens were subjected to variance analysis (Statgraphics version 5.1). The source of variation between (6 degrees of freedom) and within (35 degrees of freedom) groups and, a multiple range analysis, were realised on native and processed gluten samples.

3. Results and discussion

3.1. Thermal properties of gluten severely heated during MDSC run

In order to study the influence of a severe heating of gluten on its thermal properties (T_g and ΔC_p), MDSC runs were performed with a 2°C/min heating rate as 5°C/min has been shown to preserve gluten from an excessive heat treatment (see Section 2). Results obtained for gluten during the first scan, with water content from 0 to 20%, are presented in Fig. 3. For comparison, experimental results obtained during the first scan with a 5°C/min heating rate are also presented.

For both heating rates, T_g drops from approximately 175 to 20°C, when water content increased from 0 to 16%. As for a 5°C/min heating rate (Fig. 2), the Gordon–Taylor, Couchman–Karasz and Kwei equations were applied to experimental values obtained with a 2°C/min heating rate. The sum of squared difference between the theoretical and the experimental T_g calculated for the Gordon–Taylor and Kwei models showed that Kwei gave best fit irrespective of the heating rate 2 or 5°C/min (Table 1). However, the good compliance of the Kwei equation to the experimental values was especially demonstrated at 5°C/min heating rate. The experimental values obtained at 2°C/min and 5°C/min heating rates fitted with the Kwei model are presented in Fig. 3. The Kwei model, which was originally developed for blends of synthetic polymers, has been used for blends of biopolymers and water [10,32,42,43], in order to take into account the plateau currently observed at a high water content. The fitted values of *k* and *q* are given in Table 1. The values obtained at 5°C/min ($q = 601$; $k = 12.2$) were close to those obtained by DSC or DMTA for numerous biopolymers [10,43]. The *q* value provides a relative measure of the secondary interactions (like hydrogen bonds), a larger value suggesting a more facile contact between the two components of the blend [7,42,43]. So the higher *k* and *q* values obtained by the Kwei fitting for gluten heated at 5°C/min (Table 1) could indicate that the protein, less reticulated (as also indicated by the lower T_g, see below), is able to create more interactions with water.

T_g values for gluten heated at 2 and 5°C/min (first scan) were very similar for water content higher than 7% (Fig. 3). But the 2°C/min heating rate gave values higher than the 5°C/min heating rate for water contents from 0 to 7%. For

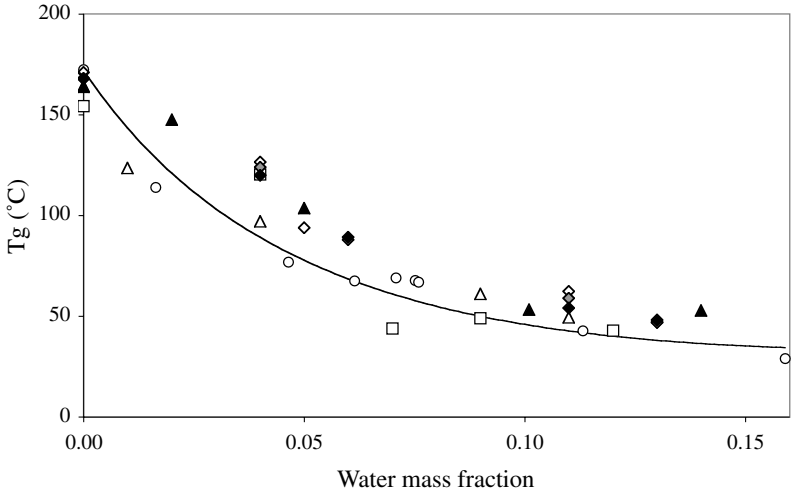

Fig. 4. T_g of native gluten and materials made from gluten by wet and dry processes determined at first heating (5°C/min) as a function of water content. (○): native gluten; (□): film (△): thermomoulded gluten; (◇): fluten mixed at 0.2 kJ/g; (◆): gluten mixed at 1.5 kJ/g; (◆): gluten mixed at 15.2 kJ/g; (▲): gluten mixed at 15.2 kJ/g and thermomoulded; (—): Kwei fit at first heating for native gluten.

Table 2
ΔC_p of native gluten and processed glutens measured by MDSC at 5°C/min heating rate

Samples	ΔC_p (J g^{-1} °C^{-1})[a]	Standard deviation
Native gluten	0.365[a]	0.024
Film (with formaldehyde)	0.468[a]	0.030
Mixed gluten		
0.2 kJ/g	0.238[b]	0.055
1.5 kJ/g	0.248[b]	0.066
15.2 kJ/g	0.241[b]	0.020
Thermomoulded gluten	0.211[b]	0.077
Mixed and thermomoulded gluten	0.199[b]	0.025

[a] Any two means followed by same superscript letter are not significantly different ($P > 0.05$).

conventional polymers (e.g. polystyrene and polyethylene), T_g value determined by MDSC exhibited no apparent heating rate dependence [44,45]. For thermally unstable polymers, long scanning times (i.e. slow heating rates) may result in a thermal alteration of the material [34]. As the T_g of gluten with 0–7% water is relatively high, an increase of the exposure time to temperature could induce reticulations in the protein material as showed by the increase of T_g. When gluten with water contents from 0 to 10% was subjected to three successive scans (heating, cooling, heating) at 2°C/min, a strong increase in T_g was observed between the first and the second scan (results not shown) contrary to the results obtained at 5°C/min (see Section 2, Fig. 2).

These results suggest that studies of T_g determinations performed at low heating rate and during the second scan (for avoiding thermal history of the sample) have to be interpreted with caution in the case of heat-sensitive biopolymers. Similarly, the dependence of the fitting of experimental data with models on experimental conditions of DSC measurements (heating rate) has to be taken into account.

Table 3
Total SDS extracted protein (TEP) of native gluten and dry and wet processed glutens (expressed as a function of the initial gluten protein content)

Samples	TEP[a] (%)
Native gluten	97.8 (2.0)
Film (without formaldehyde)[b]	93.7 (4.0)
Film (with formaldehyde)	0.2 (0.3)
Mixed gluten	
0.2 kJ/g	93.3 (1.4)
1.5 kJ/g	91.4 (0.9)
15.2 kJ/g	92.5 (2.5)
Thermomoulded gluten	1.2 (0.3)
Mixed and thermomoulded gluten	2.6 (2.6)

[a] Values in parenthesis are standard deviation.
[b] Results from Micard et al. [46].

3.2. Thermal properties of processed glutens

The glass temperature as a function of the gluten water mass fraction for the various processed gluten samples and for the native gluten is presented in Fig. 4. All the DSC measurements were performed with a 5°C/min heating rate (during first scan) to reduce the incidence of the thermal treatment during the T_g and ΔC_p determinations. Dry processed gluten samples (mixing, thermomoulding and mixing followed by thermomoulding) tended to show higher T_g values than the native gluten and the casted gluten samples, especially when the water content was not exceeding 10%. However, the establishment of a relation between the process applied on gluten and the increase of T_g has not been possible.

The ΔC_p values of native and processed glutens are presented in Table 2. The analysis of variance with a confidence level of 95% showed that the ΔC_p values were significantly different between the samples (F-ratio = 6.872; $P = 0.0001$). The multiple range analysis distinguished two homogeneous groups constituted by (1) the native gluten and the film of gluten and (2) all the dry processed gluten samples. The difference of ΔC_p between the wet and the dry processes, also observed for T_g (see above), could be related to a contrasted structure of the protein network of samples obtained from these two kinds of processes. The casting of gluten probably leads to the formation of a loose and open network structure, since the weight fraction of proteins undergoing the glass transition phenomena remained similar to those observed with the native gluten. Casting is a minimal process, where unfolding of proteins is optimised during the solvation step and the chain association is progressive during the solvent evaporation. In contrast, dry processed samples exhibited lower ΔC_p than native gluten, a feature characteristic of a more structured and cross-linked network. Since, mixing, thermomoulding or both in combination gave samples with similar ΔC_p, they might induce the formation of similar types of networks. It is also possible that T_g and ΔC_p variations are not sensible enough to distinguish slight differences in the network structure (types and number of protein interactions, etc).

3.3. Biochemical changes in processed gluten samples

The size distribution of proteins, from native gluten and processed samples, was examined using SE-HPLC. Total extractable proteins from the different samples are given in Table 3.

Proteins from the wheat gluten film, when a cross-linking agent (formaldehyde) was included in the film-forming solution, were no longer extractable with SDS and by sonication, as shown in a previous study on a formaldehyde post-treated wheat gluten film [46]. The thermomoulded

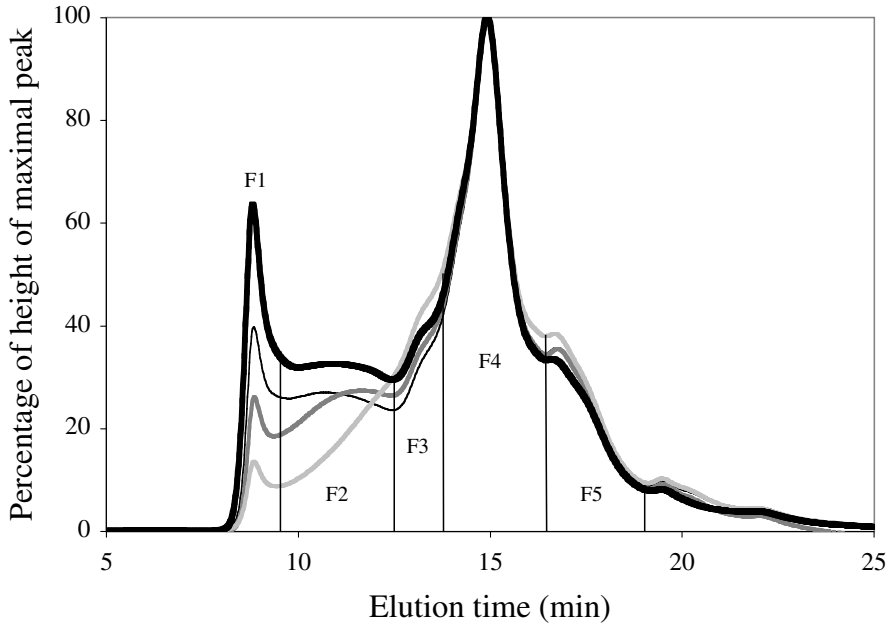

Fig. 5. Elution profiles obtained by size-exclusion HPLC of native and mixed glutens (0.2, 1.5 and 15.2 kJ/g). (—): native gluten; (▬): 0.2 kJ/g mixed gluten; (▬): 1.5 kJ/g mixed gluten; (▬): 15.2 kJ/g mixed gluten.

gluten gave similar results. The extraction of protein in SDS buffer is driven by the disruptive effects of SDS on the electrostatic, hydrophobic and hydrophilic interactions. Gluten proteins are brought into solution by the sonication, through a reduction of the huge size of glutenin polymers. It has been postulated by Singh et al. [40] that sonication causes the shear degradation of disulphide bonds that connect the glutenin subunits together. The sonication would not affect other type of covalent linkages (peptide bonds). In this respect, formaldehyde treatment and thermomoulding of gluten would result in the formation of additional covalent bonds (isopeptide bonds, methylene bridge etc), rendering proteins unextractable.

The glutenin polymerisation, resulting in a dramatic decrease in protein solubility of the thermomoulded sample, can be attributed to the thermal treatment (130°C), even if a weak shear stress also occurred. Indeed, a severe thermal treatment (140°C) applied to a gluten film gave similar

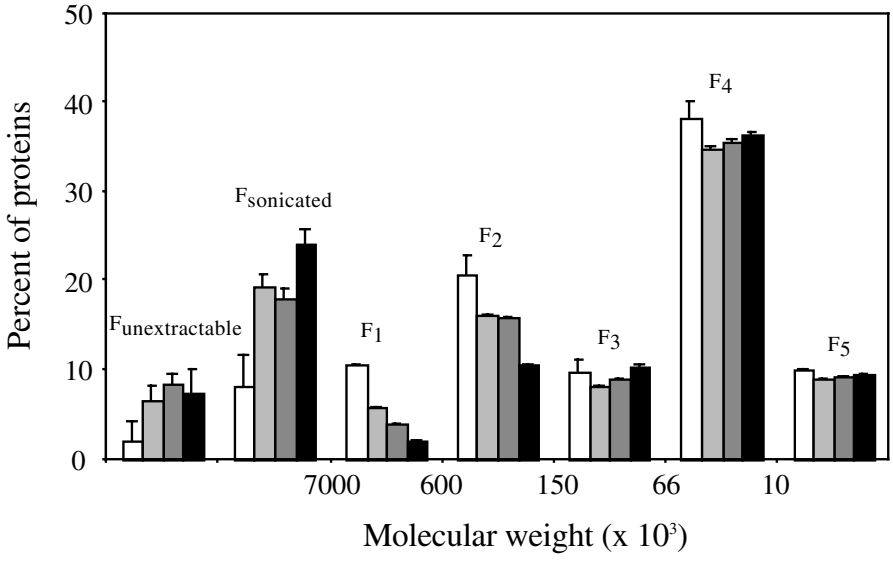

Fig. 6. Peak surfaces of elution profiles shown in Fig. 5. Error bars represent standard deviations of duplicate experiments: (□) native gluten; (▬) gluten mixed at 0.2 kJ/kg; (▬) gluten mixed at 1.5 kJ/kg; (■) gluten mixed at 15.2 kJ/kg.

result [46]. Furthermore, during an extrusion process, where a shear stress is combined with a thermal treatment, it is generally assumed that the protein polymerisation depends on the time–temperature history, whereas the depolymerisation reaction is due to the mechanical shear stress [29,30,47,48]. The severity of the thermomoulding process was further confirmed by the brown dark colour of the samples.

On the contrary, the mixing of gluten at a low temperature (40°C) with water (25 g/100 g of total weight), even at intense energy input (15.2 kJ/g), induced a minor change in the protein extractability (Table 3). Typical elution profiles of the SDS-soluble proteins of native and mixed glutens are shown in Fig. 5. The corresponding areas (F1–F5) and total areas obtained for SDS-insoluble proteins ($F_{(sonicated)}$) and that calculated for unextractable proteins are shown in Fig. 6. The increase of the specific mechanical energy input from 0.2 to 15.2 kJ/g, decreased the percentage of larger (F1) and smallest (F2) SDS-soluble glutenin polymers from 6 to 2% and 16 to 10%, respectively. At the same time, the percentage of proteins extracted by sonication ($F_{(sonicated)}$) and the unextractable proteins increased from 19 to 24% and from 6.7 to 7.5%, respectively. The percentage of gliadins (F3 and F4) was not significantly modified. These changes indicated that SDS-insoluble glutenin polymers and even unextractable protein complexes were produced at the specific expense of the SDS-soluble glutenin polymers. In our case, gluten proteins were not, as for flour dough mixing, diluted with starch (filler) or by the high water content. The reactivity of gluten would have been different explaining why we observed a polymerisation of proteins instead of a depolymerisation mechanism commonly reported for mixing of dough [49,50].

The mixing of wheat gluten at a low plasticiser content has been recently studied by Redl et al. [39], with experimental conditions similar to those used in this study, except the nature of the plasticiser (glycerol instead of water in our case). In our study, the changes in size distribution of proteins between 0.2 and 15.2 kJ/g, was less pronounced than that observed between native gluten and gluten mixed at 0.2 kJ/g. The main biochemical changes would have so occurred in a short time interval (less than 1.5 min) before maximal torque registration (Fig. 1). According to Redl et al. [39], the decrease in SDS-solubility of proteins occurred above a temperature threshold of 50–60°C. At this temperature, proteins undergo a denaturation, which is supposed to involve at first, hydrophobic interactions of proteins followed by thiol–disulphide exchange [51]. In our conditions, where the temperature never exceeded 40°C (Fig. 1), the formation of SDS-insoluble proteins at 0.2 kJ/g, was similar or even greater than that was observed by Redl et al. [39] for samples mixed for 1–2 kJ/g. Furthermore, unextractable proteins were even produced, indicating the formation of iso-peptide linkages. The observed changes could involve the formation of local overheating zones at the beginning of mixing inducing a thermal denaturation. Indeed, during the first minute of mixing where the main biochemical changes occurred, the temperature sharply rose from 20 to 40°C, despite the thermostatic control of the mixer.

4. Conclusions

The structure of gluten network is differently approached depending whether calorimetric parameters (T_g and ΔC_p) or protein solubility are taken into account. Indeed, casting of gluten films seems to build a loose and open network regarding the ΔC_p value, although the formaldehyde creates a high polymeric network. In the same way, mixed glutens give lower ΔC_p values, leading to assume that mixing causes a network reticulation, almost equivalent to that obtained after thermomoulding. According to the biochemical analyses, the protein network induced by mixing, even at intense energy input is not recognised as being so highly cross-linked than that induced by thermomoulding. According to these results, we would suggest that the restricted segmental motion of protein chains, would result more from a strengthening of the gluten network through hydrophobic inter-chains bonds, than from covalent bonds arising from a high thermal denaturation or from formaldehyde cross-link.

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