Study of the Temperature Effect on the Formation of Wheat Gluten Network: Influence on Mechanical Properties and Protein Solubility

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Modifications of mechanical properties of wheat dough during thermal treatments depend mainly on the capacity of wheat gluten proteins to establish intra- and intermolecular interactions when subjected to high-temperature processing. The present study investigates the effect of thermal treatments on the mechanical properties and protein solubility of wheat gluten-based network. The increase in treatment temperatures (from 80 to 135 °C) induces an increase in mechanical resistance of the gluten network (tensile strength increases from 0.26 to 2.04 MPa) and a decrease in deformability (elongation decreases from 468 to 236%). The increase in temperature (from 80 to 135 °C) also induces a very strong reduction of protein solubility in 2% SDS (from 68 to 0%) that could be correlated to the mechanical changes observed. It was concluded that the modifications of the wheat gluten network properties seem to depend mainly on the temperature level, as temperatures >108−116 °C allow activation of thermosetting reactions.

Keywords: *Wheat gluten; thermal treatment; mechanical properties; solubility*

INTRODUCTION

The manufacture of many cereal food products based on wheat (breads, crackers, cookies, snack bars, etc.) requires the preparation of a dough obtained through the formation of a wheat gluten network. Knowledge of the rheological properties of wheat gluten-based networks and of their evolution during processing is generally considered as critical for the quality of cereal foods. Modification of the rheological properties of wheat gluten-based doughs during kneading and baking strongly affects the structural characteristics of the final products.

Changes of the viscoelastic properties of wheat dough during thermal treatments (such as baking) depend mainly on the physicochemical characteristics of the wheat gluten and in particular on its capacity to establish intra- and intermolecular interactions when submitted to high temperatures (Hansen et al., 1975). The levels of temperature reached during thermal processing depend on the process: 100−190 °C in extrusion processing of flour-based foods, 30−150 °C in wheat flake processing, 100−140 °C in wheat biscuit or cracker processing, and 190−260 °C in commercial baking (Hansen et al., 1975; Donovan, 1977; Li and Lee, 1996). According to Hoseney et al. (1986) and Levine and Slade (1988), the properties of wheat gluten proteins can be described using the concept of glass transition. Heating amorphous proteins above their glass transition temperature produces soft and rubbery materials. Kokini et al. (1995) showed that glass transition in wheat gluten proteins is partially reversible because of the relative mobility of molecules and their high reactivity at high temperature, which could permit establishment of intermolecular covalent bonds (Levine and Slade, 1990) leading to the formation of three-dimensional aggregates. Thermal treatments between 100 and 150 °C of wheat flours favor the formation of protein aggregates stabilized by intermolecular covalent disulfide bonds (Hansen et al., 1975).

Kokini et al. (1994, 1995) have shown a large increase in the elastic modulus for gluten, gliadin, or glutenin dough during heating at >70−80 °C. For gliadins, the increase in the elastic modulus above 70 °C was attributed to cross-linking reactions resulting in the formation of a network (Levine and Slade, 1988, 1990; Kokini et al., 1994). The elastic modulus reaches a peak at 120 °C, indicating maximum structure buildup. At this temperature, the aggregation reaction appeared to be completed and a highly cross-linked network formed. When the temperature was increased to >130 °C, a softening of the cross-linked gliadins was observed (Kokini et al., 1994). For glutenins, the reaction zone starts at 90 °C, at the point where glutenins cross-link through disulfide bonds (Levine and Slade, 1988, 1990) and maximum structure buildup is reached at 135 °C, where the elastic modulus is maximum. As the temperature increases further, the elastic modulus drops dramatically at 150 °C, suggesting softening of the glutenin cross-linked network due to thermal degradation or apparent thermoplastic softening (Kokini et al., 1994). The transition temperatures (reaction zone) of glutenins are moisture independent at moisture contents >20%. In addition, the effects of temperature on wheat gluten were found to be synergistic with high-pressure treatments (200−800 MPa during 20−50 min) (Apichartsrangkoon et al., 1999).

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More recently, the formation and characterization of the wheat gluten-based networks were studied with edible films and coatings or biodegradable packaging applications (Cuq et al., 1998). Wheat gluten-based films are classically obtained by casting and drying of aqueous alcoholic protein solutions (Gontard et al., 1992; Gennadios et al., 1994; Herald et al., 1995; Ali et al., 1997). Wheat gluten-based films have also been produced by collecting the "skin" formed after the boiling of protein solutions (Watanabe and Okamoto, 1973) or by wheat gluten thermoplastic extrusion in the presence of disruptive agents (Mecham and Olcott, 1947; Guilbert and Gontard, 1995; Redl et al., 1999). These films based on wheat gluten are usually not water-soluble and have mechanical properties close to those usually observed for various protein-based films (Cuq et al., 1998). To improve the functional properties (water vapor barrier properties and mechanical properties) of films, the effects of thermal treatments of the raw materials (Herald et al., 1995) and of the films (Ali et al., 1997; Micard et al., 2000) were recently studied.

The present study investigates the effect of thermal treatments on the mechanical properties and protein solubility of wheat gluten-based network. A "thermoplastic" process, which combines simultaneously the formation of the proteinic network under low plasticizer content conditions and the thermal treatment of the film, was applied. Homogeneous blends of wheat gluten and glycerol (as plasticizing agent) were prepared and treated by thermal processing using a heating press.

EXPERIMENTAL PROCEDURES

Materials. The raw materials used for the preparation of wheat gluten-based films are as follows: commercial vital wheat gluten (Amylum Aquitaine, Bordeaux, France), glycerol (BDH Laboratory Supplies, Poole, U.K.), Sigma gluten (Sigma, G-5004), and Sigma gliadins (Sigma, G-3375). Total protein content of wheat gluten was determined in triplicate according to the Kjeldahl method using a conversion factor of 5.7 (Amylum gluten = 75 g of protein/100 g of gluten, Sigma gluten = 80 g of protein/100 g of gluten).

Sample Preparation. The fabrication of films based on wheat gluten requires the preliminary preparation of a homogeneous blend of wheat gluten and glycerol. One gram of wheat gluten (weighed at ±0.01 g with a Precisa balance, 3610 D-CFr, Balco, France) was mixed intensely with 0.4 g of glycerol (50 g of glycerol/100 g of proteins) using a mortar and pestle for 3 min until a homogeneous dough was obtained. The blend was then placed between two Teflon sheets into the heating press (Techmo, Nazelles-Negron, France). Films based on wheat gluten were obtained after pressing under 20 MPa for 10 min at a defined process temperature (80, 100, 110, 125, 135, or 150 °C). The films had surfaces of ~100 cm^2 and a dry matter content of ~14 mg of dry matter/cm^2.

Film Thickness. The thickness was measured with a hand-held micrometer (Braive Instruments, Checy, France) with 7.5 mm diameter faces, to the nearest 10^{-3} mm. Thickness values reported are means of nine measurements (confidence interval = ±5 μm) and ranged from 105 to 175 μm, depending on the treatment temperature.

Rheological Measurements. Tensile strength and percentage elongation at break were determined using a Rheo TAXT2 Rheometer (Champlan, France) operated according to ISO procedure (5A type, standard ISO 527-2, 1993) at 20 °C and 60−65% relative humidity. Wheat gluten protein-based films were first cut into dumbell shaped specimens with a punch of 75 mm length (width = 4 mm; length of the elongating part = 20 mm) and stored at 20 °C and 60−65% relative humidity for 3 days in an environmental chamber. The films were then fixed on the extensional grips before measurements. The grips moved at a speed of 0.3 mm s^{-1}. Tensile strength (megapascals) was calculated by dividing the peak load (newtons) at break by the cross-sectional area (square millimeters) of the film. Dividing the extension values by the initial length of the elongating part of the specimen (20 mm) yielded percentage elongation at break. The tensile strength−elongation curves were plotted until the break. A total of four to nine specimens was tested for each film type.

Protein Solubility. Pieces of films (7 cm^2) were weighed and placed into flasks containing 5 mL of 50 mM sodium phosphate buffer (pH 7.0) with or without 2% SDS or 2% SDS + 10 mM 2-mercaptoethanol. The flasks were agitated at 180 rpm and 30 °C for 18 h. The solutions were then centrifuged, and the protein concentration was determined using the bicinchoninic acid assay (Smith, 1985) according to the manufacturer's instructions. Standard curves using bovine serum albumin were made for each of the above solutions. For each temperature, at least two samples from two independent film fabrications were solubilized. The content in soluble proteins was calculated by dividing the weight of soluble protein in solution by the weight of total protein in the film. The total amount of protein in the film was calculated theoretically from the Kjeldahl value of the raw material considering that no losses occurred during the heat pressure treatment.

RESULTS AND DISCUSSION

The process allowed preparation of homogeneous films from wheat gluten. The films were elastic with relative transparency and a slight yellow color for all levels of treatment temperatures used (between 80 and 135 °C). The plasticizer content of blends was enough to make the glass transition temperature of wheat gluten proteins much lower than the temperatures used during the fabrication and thermal treatment of the films [T_g is estimated to be <20 °C according to Pouplin et al. (1999)]. The use of glycerol (a molecule much less volatile than water) as a plasticizing agent limited plasticizer loss by vaporization during the pressing at high temperature. Micard et al. (2000) demonstrated that the glycerol loss of heat-cured wheat gluten films (between 80 and 140 °C during 15 min) never exceeded 11% of the initial content.

Mechanical Properties. The mechanical properties of wheat gluten-based films are characterized by a relatively high variability among different sample measurements (average variation coefficients are 22% for strength values and 11% for elongation values). However, this relatively large variability in mechanical measurements is comparable with previous results on wheat gluten-based films reported in the literature (Micard et al., 2000) and could be explained by the presence of some imperfections in the films and by the difficulty of preparing "perfect" dumbell shaped samples. Despite this variability in measurements, we have taken into consideration the average values and the variation coefficients to study the effects of thermal processing on the mechanical properties of networks based on wheat gluten.

The effects of thermal treatments on the mechanical properties of wheat gluten films are illustrated by the changes in experimental strength−elongation curves as a function of the process temperature (Figure 1). An increase in treatment temperature induces a tensile strength increase at low elongations (<100%). These changes in mechanical properties at low elongations are similar to those previously observed by Lepizzera and Lambla (1997) in coalesced latex blends. Those changes were due to an increase in content of rigid components in latex and were explained by an increase in intramo-

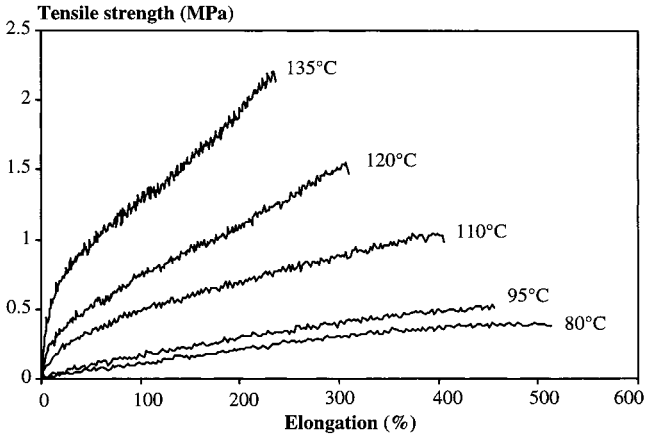

Figure 1. Influence of process temperature (between 80 and 135 °C) on mechanical properties illustrated by the strength−elongation curves (typical curves determined at 20 °C and 60−67% relative humidity) for the wheat gluten-based films (with 50 g of glycerol/100 g of protein).

Table 1. Influence of Process Temperature (between 80 and 135 °C) on the Tensile Strength and Elongation at Break (Determined at 20 °C and 60% Relative Humidity) for the Wheat Gluten-Based Films (with 50 g of Glycerol/100 g of Protein)[a]

process temp (°C)	tensile strength (MPa)	elongation (%)
80	0.26 a (± 0.09)	468 a (± 89)
95	0.42 a (± 0.09)	451 ab (± 33)
110	1.0 ab (± 0.13)	405 ab (± 39)
120	1.45 ab (± 0.25)	315 b (± 31)
135	2.04 b (± 0.45)	236 c (± 21)

[a] Values in parentheses are standard errors. Statistical analysis for the effect of treatment was performed separately for each temperature using Tukey's multiple comparison test. For each measured parameter, the same letters denote films that are not significantly different ($p \leq 0.05$).

lecular rigidity. For the wheat gluten proteins, thermal treatments could involve rigidification of some protein chains by formation of intramolecular covalent cross-links. This could explain the increase of mechanical strength at low elongations. The increase in treatment temperature induces also an increase of the slope of the strength−elongation curve at high elongation (between 100% and break point). These changes in mechanical properties at high elongations are similar to those observed in rubber (Yeoh and Fleming, 1997) with increasing cross-linking agent concentration. For the wheat gluten networks, thermal treatments could induce cross-linking of proteins by formation of intermolecular covalent bonds, which results in an increase of mechanical strength at high elongations. Both intra- and intermolecular covalent cross-links could thus be involved in the changes of mechanical properties of wheat gluten-based network treated at high temperature.

The effect of pressing temperature on the mechanical behavior at the break point appears to be significant (Table 1). The increase in pressing temperature (from 80 to 135 °C) induces an increase in mechanical resistance of gluten network (tensile strength increases from 0.26 to 2.04 MPa) and a decrease in deformability (elongation decreases from 468 to 236%). Some similar variations in mechanical properties of wheat gluten-based films have also been demonstrated by Ali et al. (1997) after a thermal treatment from 65 to 95 °C for 2 h. Micard et al. (2000) have also observed a decrease in

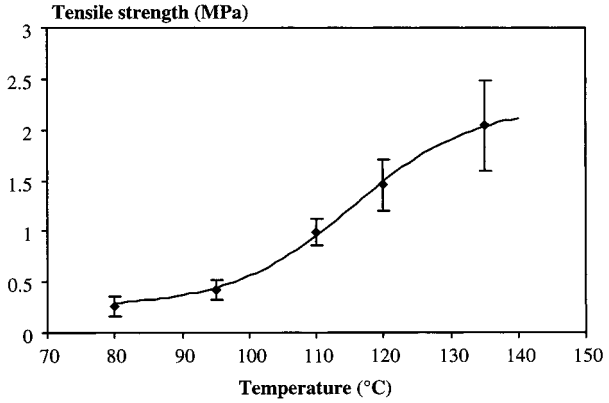

Figure 2. Influence of process temperature (between 80 and 135 °C) on tensile strength at break (determined at 20 °C and 60−67% relative humidity) for the wheat gluten-based films (with 50 g of glycerol/100 g of protein) (model parameters: $a = 9.11$, $Y_s = 2.336$, $T_c = 116$ °C, and $R^2 = 0.998$).

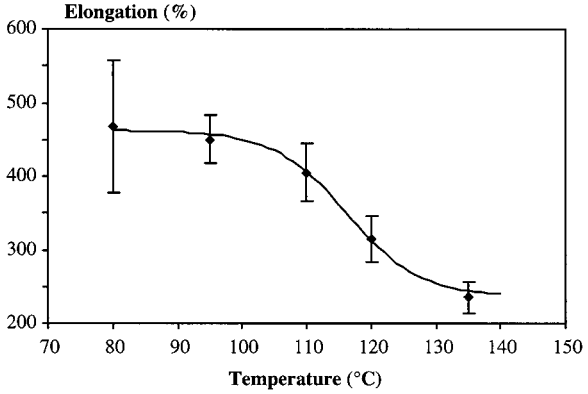

Figure 3. Influence of process temperature (between 80 and 135 °C) on elongation at break (determined at 20 °C and 60−67% relative humidity) for the wheat gluten-based films (with 50 g of glycerol/100 g of protein) (model parameters: $a = 5.66$, $Y_s = 462$, $T_c = 116$ °C, and $R^2 = 0.997$).

elongation properties (from 391 to 170%) and an increase in mechanical resistance (from 2.4 to 7.3 MPa) after thermal treatment of wheat gluten-based films at increasing temperature (from 80 to 140 °C for 15 min).

Changes in the mechanical properties of wheat gluten-based films as a function of temperature are observed over a temperature range of ∼40 °C and present a sigmoidal shape (Figures 2 and 3). The sigmoidal shape of curves was fit using a simple model (eq 1) that was proposed by Peleg (1994) to describe the variations of mechanical properties for wheat gluten proteins as a function of temperature

$$Y(T) = \frac{Y_s}{\left[1 + \exp\left(\frac{T - T_c}{a}\right)\right]} \quad (1)$$

where $Y(T)$ is the calculated parameter (tensile strength or elongation at break), Y_s is the maximum value of the parameter, T is the temperature, T_c is the temperature at the inflection point, and a is an empirical constant that characterizes the extent of the variation. The parameters Y_s, a, and T_c were calculated to fit experimental data from a nonlinear optimization procedure (Gauss Newton procedure) using Microsoft Excel 98. The calculated curves determined using the model and the corresponding experimental points are presented in

Table 2. Influence of Process Temperature (between 80 and 135 °C) on the Content of Soluble Proteins (Expressed in Grams of Soluble Protein/100 g of Protein in Film) for the Wheat Gluten-Based Films Prepared from the Amylum Gluten[a]

temp (°C)	buffer[b]	SDS[c]	SDS + 2-M[d]
80	5.1 a (0.1)	68.2 a (1.1)	64.1 a (3.4)
110	3.0 b (0.2)	28.0 b (1.4)	62.7 a (0.9)
135	2.0 c (0.1)	0 c (1.2)	59.2 a (4.6)

[a] Values in parentheses are standard errors. Statistical analysis for the effect of heat treatment was performed separately for each temperature using Tukey's multiple comparison test. For each measured parameter, the same letters (a, b, c) denote films that are not significantly different ($p \leq 0.05$). [b] Phosphate buffer 50 mM (pH 7). [c] Phosphate buffer 50 mM (pH 7) + 2% SDS. [d] Phosphate buffer 50 mM (pH 7) + 2% SDS + 10 mM 2-mercaptoethanol.

Figures 2 and 3. Experimental values fitted well with the model equation ($R^2 = 0.997-0.998$). The values of the parameter a (5.7−9.1) represent a rather important spreading out of the temperature zone for the mechanical property changes.

The mechanical properties of wheat gluten-based films are affected by the pressing temperature above 90−95 °C, and the changes are very significant between 95 and 125 °C (the inflection point is estimated by the model at 116 °C). From 130 to 140 °C, it seems that the mechanical properties of wheat gluten-based films are less affected by the thermal processing. Increasing pressing temperature from 95 to 125 °C involves a very significant increase of film cohesion (increase in tensile strength and decrease in elongation). According to the rheological characterization of polymeric materials (Ferry, 1980), these changes could be explained by a cross-linking effect of thermal treatment on the polymeric network (Levine and Slade, 1988, 1990). Various studies have suggested that the effects of heating on tensile strength and elongation for protein-based films were partially attributed to the development of heat-induced cross-linkages within the film structure (McHugh et al., 1994; Ali et al., 1997; Micard et al., 2000).

Solubility of the Proteins. The solubility properties of wheat gluten proteins as a function of thermal treatment of films are presented in Table 2. At 80 °C, the proteins from the wheat gluten-based films were not very soluble in pH 7 buffer (nearly 5% solubility). These results of solubility in buffer are in conformity with the characteristics of wheat gluten proteins, which are as insoluble in water and buffers. Proteins from wheat gluten-based films are mainly solubilized in 2% SDS (nearly 68% solubility for the films pressed at 80 °C). This value is comparable to the extractability in SDS (~70%) reported for "native" wheat gluten (Weegels et al., 1994a). The solubility of wheat gluten proteins in 2% SDS and 10 mM 2-mercaptoethanol is not very different from that observed in 2% SDS. These results suggest a great participation of the hydrophobic interactions in the stabilization of the gluten networks obtained at low temperature (80 °C). At this temperature, disulfide bonds do not seem to play an important role in the stabilization of wheat gluten networks. However, 35% of proteins remain insoluble in 2% SDS + 10 mM 2-mercaptoethanol. These insoluble proteins could probably be stabilized by interactions and bonds that are not accessible to the disruptive agents during solubility tests.

Increasing the temperature of film formation from 80 to 135 °C induces significant modifications of the solubility properties of wheat gluten-based networks (Table 2). A slight reduction in protein solubility in buffer (from 5 to 2%) and a very strong reduction in solubility in 2% SDS 2% (from 68 to 0%) were observed from 80 to 135 °C. This important decrease in solubility was not observed when 10 mM 2-mercaptoethanol was added to the solubilization buffer, but insoluble protein content slightly increased (from 35 to 40%) with the temperature. Increasing the temperature during the treatment of wheat gluten-based films modifies significantly the type of interactions stabilizing the protein network. Variations of solubility with temperature increases could be explained by an increasing participation of disulfide bonds for stabilization of the protein network. This is in accordance with the data (Table 2) which show that in order to recover a similar percentage of protein solubilization in SDS buffer for films made at 80 °C, it is necessary to add a reducing agent in the buffer for films made at 135 °C. To characterize further the effect of temperature on modifications of protein solubility, we plotted the changes in protein solubility in 2% SDS of wheat gluten-based films as a function of temperature (Figure 4). The sigmoid model (eq 1) describes well the experimental data of protein solubility ($R^2 = 0.993$). The value of the parameter a (8.1) is of the same order of magnitude as those calculated for the mechanical properties and represents the important temperature range for solubility changes. The changes in protein solubility in 2% SDS seem to be significant above 80−90 °C and become very important between 95 and 122 °C (the inflection point is calculated at 108 °C). From 125 to 130 °C, thermal processing does not seem to affect significantly solubility in 2% SDS. The insolubilization of wheat gluten proteins mainly occurs between 95 and 125 °C. Changes in solubility as a function of temperature and the position of the inflection temperature (108 versus 116 °C) are similar to those previously described for the mechanical properties (Figures 2 and 3).

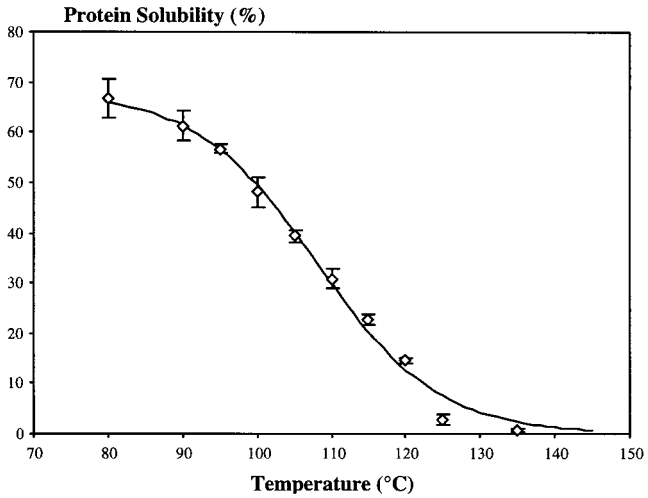

Figure 4. Influence of process temperature (between 80 and 135 °C) on the 2% SDS soluble proteins fraction (expressed in grams of soluble protein/100 g of protein in film) for wheat gluten-based films prepared from the Amylum gluten (model parameters: $a = 8.15$, $Y_s = 68.0$, $T_c = 108$ °C, and $R^2 = 0.993$).

These results were compared with the data obtained with other types of wheat proteins (wheat gluten and gliadins purchased from Sigma) (Figure 5). The decrease of solubility in 2% SDS shows a sigmoid shape and is well described by the model ($R^2 = 0.942-0.999$). The variations in solubility are almost similar to those

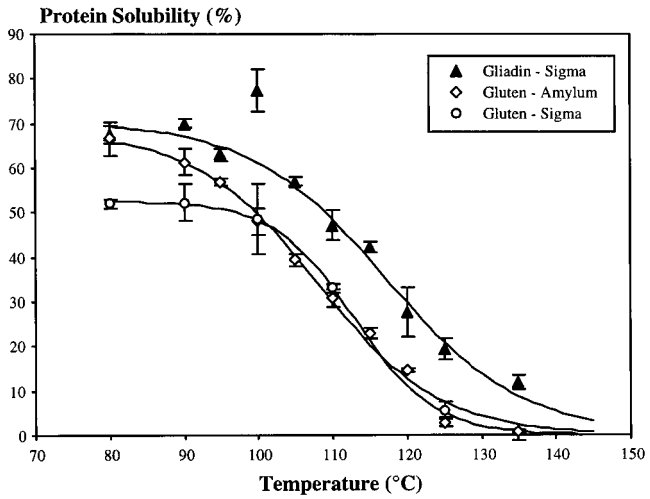

Figure 5. Influence of process temperature (between 80 and 135 °C) on the 2% SDS soluble proteins fraction (expressed in grams of solubilized protein/100 g of protein in film) for the wheat protein-based films prepared from Amylum gluten (model parameters: $a = 8.15$, $Y_s = 68.0$, $T_c = 108$ °C, and $R^2 = 0.993$), from Sigma gluten (model parameters: $a = 5.36$, $Y_s = 52.6$, $T_c = 113$ °C, and $R^2 = 0.942$), or from Sigma gliadins (model parameters: $a = 9.11$, $Y_s = 70.4$, $T_c = 117$ °C, and $R^2 = 0.999$).

previously described in Figure 4, with significant changes between 95 and 125 °C (the inflection points were calculated at 113 °C for Sigma gluten and at 118 °C for Sigma gliadins). It seems that except for a slight 5 °C shift, gliadin solubility is affected by the temperature in the same way as the wheat gluten from which they have been extracted.

Numerous publications describe the network formation by protein−protein aggregation and the decreased extractability of proteins when gluten dough was heated at temperatures >80 °C (Pence, 1953; Jeanjean et al., 1980; Schofield et al., 1984). A reduced solubility in SDS after heating was also described by these authors and could be explained by hypothesizing that hydrophobic groups are largely exposed to such an extent that the protein aggregates; the aggregated state is fixed by disulfide bonding (Weegels et al., 1994b). The decrease in hydrophobicity was found to be due to decreased extractability, mainly of the glutenin fraction (Weegels et al., 1994a). It appears that there is a rearrangement of disulfide bonds presumably through sulfhydryl−disulfide interchange reactions, which are facilitated through thermally induced unfolding of the protein conformational structure (Schofield et al., 1984).

The changes in solubility and aggregation of glutenins and gliadins at high temperature are usually considered to be responsible for modifications of wheat gluten properties (Jeanjean et al., 1980; Weegels et al., 1994a; Li and Lee, 1996). These changes in solubility can be used to explain the variations of mechanical properties for wheat gluten-based films previously described (Figures 2 and 3). The increase in cohesion of the wheat gluten-based films (i.e., increase in tensile strength and decrease in elongation) could be due to partial cross-linking of the gluten network, resulting in an increase in the number of intermolecular covalent bonds, and these changes could thus be considered as similar to traditional thermosetting (Levine and Slade, 1988, 1990).

From our experiments, it can be hypothesized that the modifications of protein solubility for the wheat gluten proteins subjected to thermal treatment at high temperatures either depend on the gliadin fraction or do not depend on the precise nature of proteins (gliadins, glutenins, or gluten) but rather on the level of temperature. The temperature level used allows the activation of thermosetting reactions that induce the insolubilization of the protein network (formation of intra- and intermolecular covalent bonds). However, it is necessary to confirm this hypothesis by complementary experiments that will compare the behavior of both components of wheat gluten (glutenin and gliadin). These results would help to define the main factors or components that are responsible for the behavior of wheat gluten networks subjected to thermal treatment.

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