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Thermosetting of Wheat Protein Based Bioplastics: Modeling of Mechanism and Material Properties

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Summary: Structural investigation of gluten-glycerol blends subjected to heat-treatment was carried out by size-exclusion high performance liquid chromatography (SEC) and stress-strain tests. SEC is a valuable tool to investigate the size distribution of gluten protein chains, while the molecular weight between network junctions (M_e) can be estimated from the elastic plateau modulus. Wheat gluten aggregation upon thermosetting seems to proceed through direct covalent cross-linking between glutenin oligomers and the gluten macropolymer. The time course of the reaction, which showed a slow-down of the reaction rate with time, was described by a simple mechanistic model. The deceleration of the reaction rate was presumably due to the development of a three-dimensional protein network, which decreased the accessibility of reactive groups. The network formation could be evidenced separately by the decrease of M_e during the heat-treatment.

Keywords: activation energy; kinetic model; network structure; SEC analysis; wheat gluten

Introduction

For many years, various raw materials from agricultural resources have been used to produce renewable, biodegradable, and edible packagings. Among agricultural products, proteins have been employed empirically as packaging materials (e.g. soybean lipoprotein films in Asia, collagen envelopes, etc.). The use of proteins for non-food applications may be a promising way to produce biodegradable materials with a large range of functional properties due to their unique structure. Wheat gluten has been widely investigated as a protein source, because it is a low-cost raw material, annually renewable and readily available.

Whatever the technique used, the processing of wheat gluten generally involves heat-treatment of the proteins (e.g. drying, curing, or extrusion). Temperature has an aggregating effect on wheat

gluten in that it induces the formation of covalent bonds between proteins. This aggregated structure will govern the properties of the final product. However, the mechanism of gluten aggregation due to thermosetting is not well understood yet.

Gluten, in contrast to homopolymers, has a very diverse structure. The amino acids which can be found in proteins, offer a large variety of possible chemical interactions and reactions. Vital wheat gluten is a mixture of different types of protein, i.e. glutenin, gliadin, albumin and globulin. Albumins and globulins constitute the metabolic proteins of wheat; gliadins, which can be further subdivided into different classes, are monomeric proteins; glutenins represent the polymeric protein fraction of wheat. Glutenin subunits are likely to be linked together end-to-end in order to form the gluten macropolymer the size and precise structure of which are still under discussion. It is however widely accepted that gluten size might extend several millions.[1],[2] Wheat storage proteins are characterized by their high content in glutamine and proline along with their low content in charged amino acids.[3] As a consequence, they are insoluble in water. Ionic detergents such as sodium dodecyl sulfate are needed to dissolve them.

The decrease of gluten extractability even in buffers containing denaturing agents is often taken as a measure of the protein aggregation. Various studies have been carried out describing the solubility loss of gluten heated at different moisture contents. Kinetic studies were performed to describe the influence of drying conditions of wheat grains or gluten on empirical parameters, such as the loaf volume of bread baked with heat-treated gluten. Those studies however, which were summarized by Weegels and Hamer[4], did not provide much insight in the aggregation mechanism and structure organization of gluten proteins during heat-treatment.

In order to use wheat gluten in technological processes to produce biodegradable materials, we need a clear understanding of gluten reactivity and its aggregating mechanisms on a molecular scale.

In that purpose we followed the gluten solubility loss by size-exclusion chromatography, which provided evidences in the change of the molecular size distribution of proteins, as well as quantification of the SDS-soluble protein fraction. A mathematical model of the gluten aggregation was formulated in order to provide an additional tool to investigate the protein aggregation

behavior. The mesoscopic structure of protein aggregates was finally approached thanks to rheological determinations.

Material and Methods

Vital wheat gluten was graciously provided by Amylum (Aalst, Belgium). Protein, starch, lipid, and ash contents amounted to respectively 76.5, 11.8, 5.0, and 0.8% on a dry mass basis. Moisture content was 7.2 % (wet matter basis).

A hand-mixed gluten-glycerol blend (35 % glycerol) was heat-treated in a water bath and under a heating press (Techmo PL 10T, France) for different times at various temperatures (70, 82, and 94°C).

Analysis of the protein size distribution was performed by size-exclusion high performance liquid chromatography (SEC) according to Redl et al.[5] Ground gluten samples were extracted in a sodium phosphate buffer (0.1 M, pH 6.9) containing 1% sodium dodecyl sulfate (SDS). Extracted protein (SDS-soluble protein fraction, F_s) was directly analyzed by SEC. The remaining SDS-insoluble protein fraction F_i was extracted in SDS-buffer by sonication and reduction of disulfide bonds with dithioerythriol. SEC analysis of F_i was carried out in order to check the recovery of the gluten protein mass. Calibration of the HPLC column (TosoHaas TSK-G 4000 SW XL) was done according to Dachkevitch and Autran.[6] The protein content of the ground samples was determined by Dumas method (NA 2000, Fisons Instruments). The differences in the chromatogram areas due to varying protein contents were normalized prior to data treatment.

Rheological analysis of samples was carried out with a dynamic mechanical thermal analyzer (DMTA MK III, Rheometric Scientific, USA). Samples were immersed in deionized water at room temperature (22.5°C). A tensile deformation (strain) was applied to the sample at a rate of 0.001 s^{-1}. Resulting stress and elongation ratio were recorded. Young's modulus (E) was calculated from the slope of the linear stress increase. The equilibrium Young Modulus can be related to the shear modulus (G) with the approximation $E(t) = 3 G(t)$.[7] According to the theory of rubberlike elasticity the density of cross-linked network strands may be estimated from the equilibrium modulus G_e[7]:

$$\frac{E_e}{3} \cong G_e = \frac{\rho}{M_e} RT \qquad\qquad (1)$$

where ρ is the density of the material ($\rho = 1.2 \cdot 10^6$ g m^{-3}), M_e is the average molecular weight between cross-links or entanglement coupling points (g mol^{-1}), R the universal gas constant (R = 8.314 J mol^{-1} K^{-1}) and T the temperature (K).

Results and Discussion

SEC results

The extraction of vital wheat gluten with SDS-phosphate buffer brought approximately 85% of the total protein into solution. Typical SEC profiles of a series of heat-treated samples are shown in Figure 1. The good resolution between protein polymers (glutenin, M_r >700,000 – 95,000) and monomers (gliadin, M_r 95,000 – 10,000) was achieved thanks to the strong disruptive power of SDS suppressing electrostatic and/or hydrophobic interactions between gluten proteins.[8]
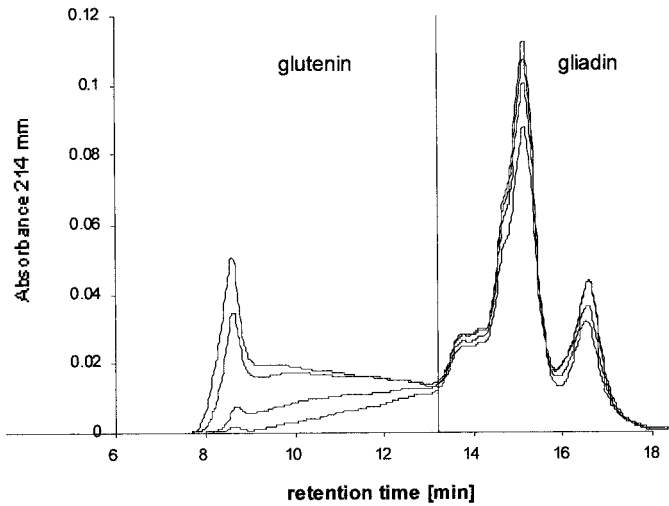

Figure 1. Changes of SEC elution profiles of the SDS-soluble protein fraction during heat-treatment at 82°C (top to bottom): control sample, 25 min, 420 min, 1140 min, and 1440 min.

The decrease of the chromatogram area evidences a progressive protein solubility loss during heat-treatment. Glutenin polymers seemed to be more susceptible to heat-treatment than monomeric gliadins. Large glutenin polymers reacted more quickly than smaller ones. The change of the elution profiles suggests that protein aggregation occur through direct cross-linking of SDS-soluble proteins with the SDS-insoluble gluten macropolymer (15% of total protein in vital gluten). A step-by-step coupling of monomers should typically result in the accumulation of middle size proteins during the treatment, which would give rise to a peak shifting towards the gel point of the proteins before its disappearance. Such a development, however, cannot be observed in Fig. 1. The successful extraction of the SDS-insoluble protein fraction (F_i) of heat-treated samples by sonication and reduction of disulfide bonds evidenced that protein solubility loss occurred predominantly due to the formation of disulfide cross-links.

Model development

The protein solubility loss in SDS-phosphate buffer is one of the most marked features of gluten aggregation. Schofield and coworkers[9] postulated that glutenin proteins unfolded upon thermosetting facilitating thereby sulfhydryl/ disulfide interchanges between exposed groups. The creation of new disulfide bonds may therefore lock the proteins into the denatured state.

Taking into consideration the proposition of Schofield and coworkers[9], the observation of gluten protein unfolding upon heating[10], and the evidence of direct gluten protein cross-linking derived from our analysis, we may formulate the following mechanistic model of the gluten aggregation: proteins unfolded exposing reactive cystine groups and aggregated then by direct cross-linking between the large gluten macropolymer (SDS-insoluble protein) and the SDS-soluble molecules.[11]

We describe the reversible change in the protein conformation by

$$P_s \underset{k_{-1,d}}{\overset{k_{1,d}}{\rightleftharpoons}} P_s^*$$

(2a)

where P_s and P_s^* represent a protein before and after this transformation, respectively, and $k_{1,d}$ and $k_{-1,d}$ are the corresponding rate constants. As an approximation, we neglect their possible

dependence on the molecular weight of the protein. In particular, this should be applicable to very large proteins, such as the SDS-insoluble macropolymer P_i:

$$P_i \underset{k_{-1,d}}{\overset{k_{1,d}}{\rightleftharpoons}} P_i^{*}$$

(2b)

We write the irreversible cross-linking reaction as

$$P_s^{*} + p_i^{*} \xrightarrow{k_{2,p}} p_a^{*}$$

(3)
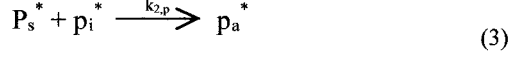
where p_i^{*} (in contrast to P_i^{*}) denotes merely a section (of yet unknown size) of the protein P_i^{*}. For the following we quantify its mass $m_{p_i^{*}}$ with the help of the relation $m_{p_s^{*}} = \alpha \cdot m_{p_i^{*}}$, where α is a freely adjustable parameter. In the same way p_a^{*} stands for a section of the aggregated protein and $k_{2,p}$ is the corresponding rate constant. The protein precipitation may progress additionally through a P_s^{*} becoming part of P_a^{*}, when reacting with it, i.e.:

$$P_s^{*} \xrightarrow[P_a^{*}]{k_{3,p}} p_a^{*}$$

(4)

P_a^{*} will finally contain all the insoluble protein. The reaction is driven by the rate constant $k_{3,p}$. Complete renaturation of P_a^{*} might be impossible due to the formation of covalent bonds, which lock proteins into the denatured state. Preliminary calculation of rate constants confirmed that the temperature dependency of the reaction followed Arrhenius law,

$$k = A \cdot \exp^{\left(\frac{E_a}{RT}\right)}$$

(5)

where k is the rate constant, A the pre-exponential factor, E_a the activation energy, R the ideal gas constant, and T the absolute temperature.

Computing the rate constants directly with Arrhenius law offers the advantage that the model constants can be optimized on the whole data set in the same run. The exact modeling procedure is described by Domenek et al. 2002.[11] The model parameters are given in table 1. The calculated activation energy of the precipitation reactions is consistent with previous studies, which were summarized by Weegels and Hamer.[4]

Table 1. Fitted model parameters of the heat-induced gluten protein precipitation.

Constant	Value	Constant	Value
$E_{a,D}$ [kJ mol^{-1}]	53.9	$E_{a,P}$ [kJ mol^{-1}]	172
$E_{a,R}$ [kJ mol^{-1}]	29.5	$A_{2,p}$ [min^{-1}]	11.7 x 10^{22}
$A_{1,d}$ [min^{-1}]	71.3 x 10^5	$A_{3,p}$ [min^{-1}]	0.0985 x 10^{22}
$A_{-1,d}$ [min^{-1}]	0.00998 x 10^5	α	1.041
	R^2	0.9910	

$E_{a,D}$ and $A_{1,d}$: activation energy and frequency factor of the unfolding reaction.
$E_{a,R}$ and $A_{-1,d}$: activation energy and frequency factor of the refolding reaction.
$E_{a,P}$: activation energy of both protein precipitation reactions.
$A_{2,p}$, and $A_{3,p}$: frequency factors of the precipitation reactions, α is a freely adjustable parameter.
R^2 correlation coefficient between the experimental data and the model calculation (n= 30).

Figure 2 shows plots of our model fitted with experimental data at three different temperatures. The proposed model described the protein aggregation behavior accurately ($R^2 = 0.9812$). In the beginning of the reaction protein solubility dropped sharply from 85 % to approximately 70 %. Then reaction proceeded with a significantly diminished rate. A delayed onset of the reaction due to protein unfolding was merely observed, even if looked at in more detail (Figure 3).
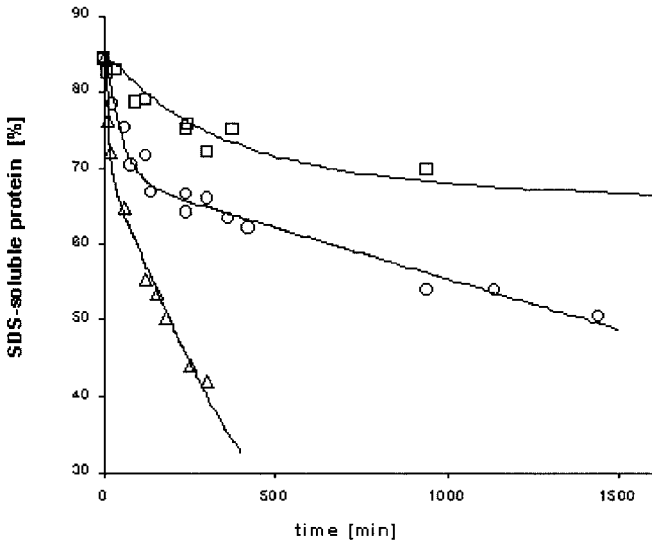

Figure 2. Model fit to the experimental data at different temperatures: $\square$ = 70°C, O = 82°C, Δ = 94°C. ($R^2 = 0.9812$).

Figure 3 shows, in more detail, the progression of the gluten aggregation and the time course of the model intermediates for the first 200 min at 82°C. The biphasic reaction behavior can be seen clearly. Unfolded protein species were formed quickly and depleted upon reaction with P_i^* and P_a^*. The reaction slow-down coincided with the almost disappearance of P_i^*.

Two different effects may contribute to the biphasic reaction behavior:

(i) Gliadin was shown to aggregate more slowly than glutenin (Figure 1). The sharp aggregation might therefore reflect the glutenin solubility loss, while the slow phase might be attributed to gliadin reaction. The rapid solubility loss, however, affected only 15 % of the total protein mass regardless of the treatment temperature, which is not entirely consistent with the percentage distribution of the different protein types in gluten (approximately 34 % glutenin, 51 % gliadin, and 15 % aggregated macropolymer in native gluten).

(ii) The Arrhenius time-temperature dependency of both precipitation reaction rates could be computed with a single activation energy, which indicated that both reactions followed the same pathway. The slow down of the reaction rate was expressed by the decrease of the pre-exponential factor (from $1.17 \cdot 10^{23}$ to $9.85 \cdot 10^{20}$), which might be interpreted as increasing hindrance of the reaction partners to meet. Due to the formation of a three-dimensional cross-linked structure, P_a^* would be less reactive than P_i^*, which is supposed to be an mostly linear polymer. The branching of native polymers to form a cross-linked structure would progressively diminish the accessibility of reactive cystine groups and so result in an overall reaction slow-down.
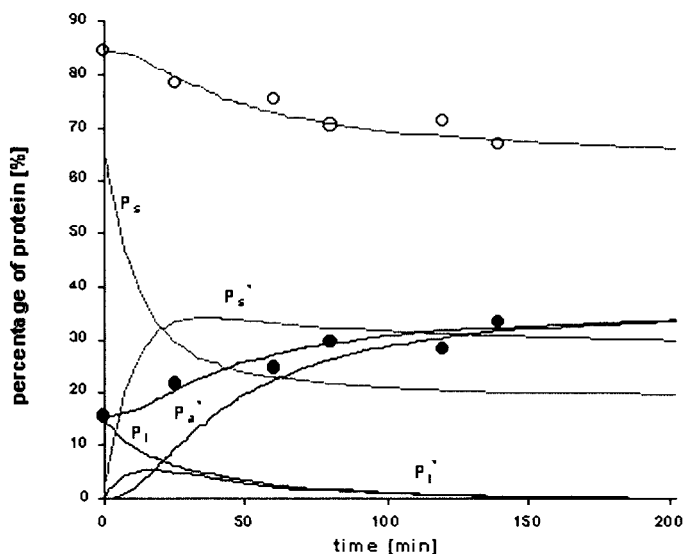
Figure 3. Time course of the intermediates calculated with the kinetic model at 82°C: O = F_s, ● = F_i. Gray lines correspond to the model calculation of the SDS-soluble proteins (native SDS-soluble protein P_s, unfolded SDS-soluble protein P_s^*), black lines to the calculation of SDS-insoluble proteins (native SDS-insoluble protein P_i, unfolded SDS-insoluble protein P_i^*, aggregated protein P_a^*).

Rheological measurements

Rheological methods are most valuable tools in order to investigate polymer network structures. In the conceptual scheme of cross-linking or entanglement coupling, an important parameter is the average molecular weight between of cross-links (M_e). It should be considered here as a measure of the spacing between topological restrains, which may not have a clear physical definition. Figure 4 shows that both system parameters, F_i and M_e, are related. The increase of F_i resulted in the decrease of M_e, which above 60 % F_i leveled slightly off. Above this value no dramatic change of material properties might be expected any more. The decrease of M_e reflects increasing network density in the gluten-glycerol blend, which can be assigned to the formation of a three-dimensional cross-linked structure, which replaced the linear native macropolymer during aggregation of wheat gluten.
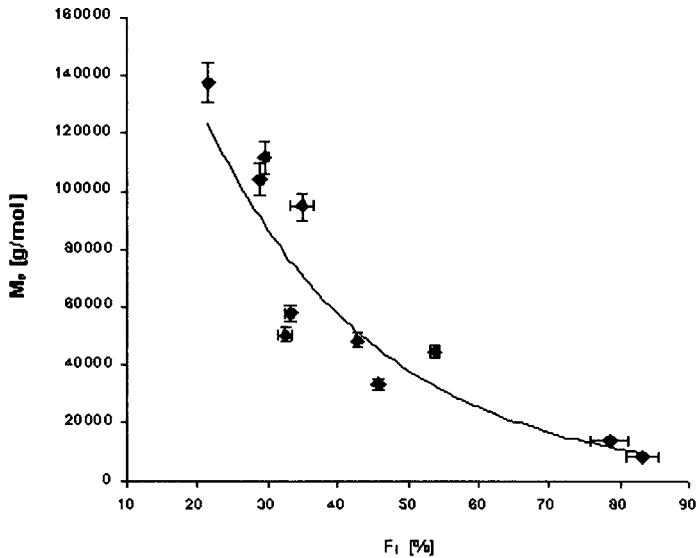
Figure 4. Relationship between the percentage of SDS-insoluble proteins (F_i) and the average molecular weight between network junctions (M_e).

Conclusion

Wheat gluten polymerization upon thermosetting seems to proceed through direct covalent cross-linking between middle-size glutenin oligomers and the gluten macropolymer. The biphasic behavior of this reaction, probably caused by the formation of a three-dimensional protein network, can be described by a simple mathematical model. Temperature has thus a strong structuring influence on the protein network, which will govern the material properties of gluten products. Understanding of the kinetics of wheat gluten cross-linking upon thermosetting may help to define parameters and limits of gluten texturizing processes from hot-molding over curing of biomaterials up to processes involving heat- and shear-treatments, such as mixing or extrusion.

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