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Thermoplastic Processing of Protein-Based Bioplastics: Chemical Engineering Aspects of Mixing, Extrusion and Hot Molding

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Summary: Proteins, as heteropolymers, offer a large range of possible interactions and chemical reactions. The thermoplastic behavior of proteins has been studied in order to produce bioplastics by thermal or thermomechanical processes such as mixing, extrusion or hot molding. The extrusion trials were performed by using a co-rotating twin-screw extruder, recording torque, temperature and die pressure. Batch mixing was done in a two blade counter-rotating mixer, with continuous recording of torque and product temperature. Proteins were alternatively extruded, mixed or hot molded under a large range of processing conditions. Protein aggregation during each process was estimated from the accumulation of SDS-insoluble protein fraction.

Protein aggregation evidences a cross-linking reaction the activation energy of which was dependent on the thermoplastic process used. The increase in network density appears to be induced by the severity of the treatment: temperature and shear strongly affect the structural characteristics of the protein-based bioplastics.

Keywords: activation energy; biomaterials; network structure; proteins; thermoplastic processing

Introduction

Proteins are natural polymers that for a long time have been used empirically to produce edible packaging and materials (soybean lipoprotein sheets in Asia, collagen envelopes, etc.). At the outset of the 20th century, these agricultural, renewable and biodegradable resources were considered as interesting raw materials for making plastics to eventually replace cellulose-based materials. Numerous vegetable proteins (corn zein, wheat gluten, soy proteins...) and animal proteins (milk proteins, collagen, gelatin, keratin and myofibrillar proteins...) can be used to form bioplastics [1]. We studied more particularly wheat gluten, because of its specific viscoelastic properties.

Proteins, contrary to homopolymers or copolymers in which one or two monomers are repeated, are heteropolymers comprising more than 20 different amino acids, each with specific sequences and structures. This molecular diversity means that proteins have a considerable potential for the formation of linkages that differ with respect to their position, nature and/or energy [2]. Moreover, proteins are specifically characterized by their potential reactivity to cross-link [3]. Protein-based materials could then be defined as a stable three-dimensional macromolecular network stabilized by low-energy interactions and strengthened by covalent bonds such as disulfide bonds between cysteine residues. A large range of properties can thus be expected from those protein-based bioplastics compared to processed homopolymers.

The thermoplastic behavior of proteins has been studied and used to make bioplastics by thermal or thermo-mechanical processes under low moisture conditions. The glass transition characterizes the change from a glassy state to a rubbery state and involves variations in the physical properties of the proteins such as increases in free volume and mobility of the macromolecules [4-5]. It is in part affected by the presence and content of plasticizers [6]: gluten transition occurs at lower temperature as the plasticizer content increases. Heating plasticized proteins above the glass transition produces soft and rubbery material that can be easily shaped. Polymerization could also occur, depending on the processing conditions and the plasticizer nature. Then cooling to room temperature can reconvert rubbery products to glassy materials with the desired structure. Control of the different processing operations will allow to design specific structures for requested properties.

Materials and Methods

Commercial vital wheat gluten was graciously obtained from Amylum Aquitaine (Bordeaux, France). Its protein content was 79.8% in dry mass (dm), and its humidity 6.2% (dm). Anhydrous glycerol was purchased from Fluka Chemie (Buchs, Switzerland). Chemicals for biochemical analysis of the samples were obtained from Sigma or Merck in p.a. quality.

Thermoplastic processing conditions

The extrusion trials were performed using a co-rotating twin-screw extruder as described by Redl *et al.* [7]. The influence of feed rate (1.9, 4.9 and 8.1 kg/hr) and screw speed (50, 100 and 200 rpm) were investigated at a constant barrel temperature (80°C). Torque, temperature and die pressure were continuously recorded during the extrusion process.

Hot molding was carried out in a heated press (PL10T, Techmo). Gluten/glycerol blends were pressed between two Teflon sheets, at different temperatures (70, 80, 100 and 120°C), for different times.

Mixing experiments were performed in a two blade counter-rotating batch mixer turning at a 3:2 differential speed (Plasti-corder W 50, Brabender, Duisburg, Germany). Mixing speed was 100 rpm, and the mixing chamber was regulated at 40, 60 and 80°C using a cryostat (Lauda RC 20) and water circulation in the double chamber of the mixer (75 g/s). Torque and product temperature were continuously recorded during the mixing process.

Biochemical analysis

SDS-insoluble proteins were characterized using size exclusion chromatography (SEC) as described by Redl *et al.* [8]. Briefly, processed materials were ground in liquid nitrogen by using a laboratory ball mill (Prolabo, France) and then blended with soluble starch (1/5 g/g). Samples were stirred for 80 min at 60°C in 20 ml of 0.1 M sodium phosphate buffer (pH 6.9) containing 1% sodium dodecyl sulfate (SDS). SDS is known to be an efficient dissociating detergent able to disrupt hydrophobic, hydrogen and ionic bonds. Phosphate-SDS buffer is thus likely to disrupt all non-covalent interactions. Extraction was followed by centrifugation (30 min, 37000 g, 20°C) in a Beckman centrifuge. 20 µl of the supernatants were directly submitted to SEC fractionation.

The insolubility in SDS buffer of the pellet proteins is mainly due to covalent disulfide bonds that stabilized the protein polymeric structure. The pellets were then suspended in phosphate-SDS buffer containing 20 mM dithioerythritol (DTE) and sonicated. Thanks to DTE reduction and sonication, gluten polymer undergoes chain scissions that decrease the polymer size. It is thus possible to bring the SDS-insoluble protein fraction into solution. After centrifugation, 20 µl of

supernatants was submitted to SEC fractionation. As the proportion of this SDS-insoluble protein fraction (Fi) is related to the density of covalent bonding between protein chains, it is a good parameter to study the extent of protein cross-linking during thermoplastic processing.

Results

Extrusion process

Extrusion is a highly efficient method for the continuous shaping of thermoplastic materials. Using this well-known technology with a renewable agricultural raw product such as wheat gluten represents a real challenge for the production of bioplastics.

The different applied operating conditions greatly affected the aspect of extrudates: increasing screw speed (from 50 to 200 rpm) and decreasing flow rate (from 8.1 to 1.9 kg/hr) both resulted in disrupted extrudates. Examples of a continuous (A) and a disrupted extrudate (B) are represented on figure 1 with their processing conditions.

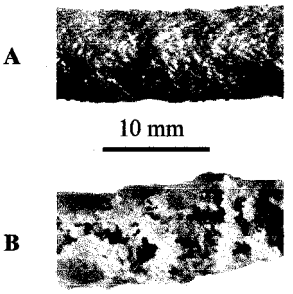

Figure 1. Extrudates obtained under different extrusion conditions [7]. A: screw speed 100 rpm, flow rate 4.9 kg/hr, die temperature 108°C, specific mechanical energy 735 kJ/kg B: screw speed 200 rpm, flow rate 4.9 kg/hr, die temperature 139°C, specific mechanical energy 1476 kJ/kg.

The appearance of surface irregularities and extrudate breakup seems to be induced by the severity of the thermomechanical treatment. In fact, both specific mechanical energy and maximum measured temperature were particularly high for extrusion conditions that resulted in disrupted extrudate. Apparently, high screw speed led to important viscous heat dissipation that resulted in excessive product heating. High temperature, together with significant mechanical

energy input, might have resulted in a more cross-linked structure in which the critical strain was decreased substantially, explaining the observed extrudate rupture. This was confirmed by size exclusion chromatography analyses.

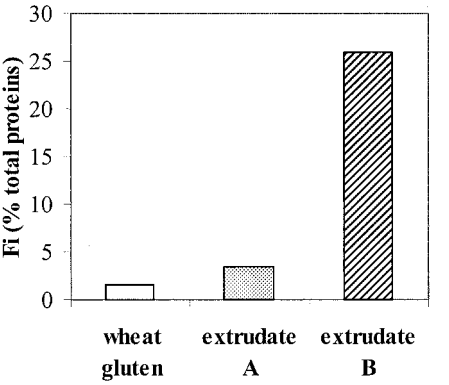

Figure 2. SDS insoluble protein fraction of native wheat gluten and of continuous and disrupted extrudates.

Cross-linking of gluten proteins was found to be favored by extrusion since this process resulted in an increase in the insoluble fraction Fi. And this increase was much more important for disrupted extrudates (figure 2).

The insoluble fraction (Fi) could therefore be related to the severity of the blend treatment all along the screws. The insoluble fraction increased effectively with energy and temperature (figure 3). However, as these parameters are partially codependent, it was difficult to isolate the influence of each of them.

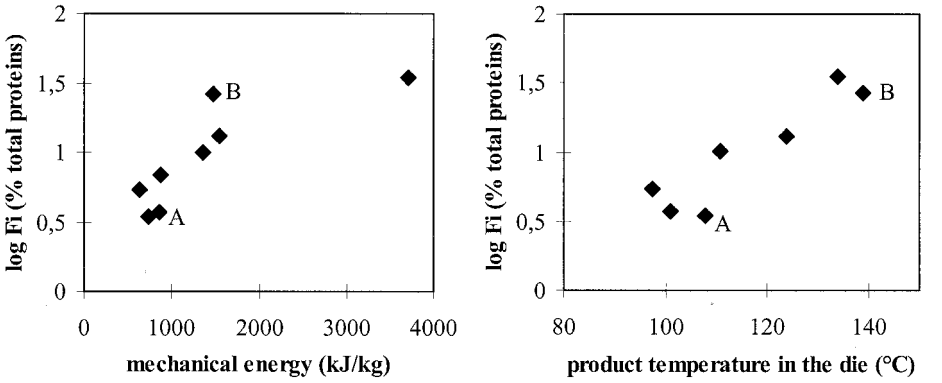

Figure 3. Variations in the SDS insoluble fraction (Fi) of different processed extrudates as a function of specific mechanical energy (left graph) and maximum temperature (right graph) [7].

The extrusion process involves temperature, pressure and mechanical energy. To distinguish the influence of each processing parameter on the protein cross-linking kinetic, two other thermoplastic processes were studied: hot molding, which involves temperature and pressure, and mixing, which involves temperature and mechanical energy.

Hot molding

Wheat gluten/glycerol blends were hot molded during different time, at different temperatures. Protein molecular size distribution of resulted films was analyzed by using SEC.

Data show that the insoluble protein fraction (Fi) increases as a function of time, according to a biphasic reaction mechanism, which might reveal a diffusion limited reaction in this static system. Moreover, biochemical changes enhance with temperature, suggesting the occurrence of heat activated reactions. The experimental data were fitted according to the following first-order kinetic expression:

$$Fi\ (\%) = 100(1 - \alpha - \beta \exp(-k_1 \cdot t) - \gamma \exp(-k_2 \cdot t)) \qquad \text{(eq. 1)}$$

with $(\alpha + \beta + \gamma = 1)$ as constraint (α is the percentage of non-reacting protein and $\beta + \gamma$ the percentage of reacting protein) and where Fi is the percentage of insoluble protein, t is the

processing time and k_1 and k_2 two rate constants, supposed to be temperature dependent according to Arrhenius law *i.e.*:

$$k = A \exp(-Ea/RT) \qquad \text{(eq. 2)}$$

where A is the frequency factor for the reaction leading to the insolubilization of gluten protein, Ea the activation energy of this reaction, R the universal gas constant (8.314 J/mol/K) and T the absolute processing temperatures.

From equations (1) and (2), it comes:

$$Fi\ (\%) = 100(1 - \alpha - \beta \exp(-A_1 \cdot t \cdot \exp(-Ea/RT)) - \gamma \exp(-A_2 \cdot t \cdot \exp(-Ea/RT))) \qquad \text{(eq. 3)}$$

Optimization of the Arrhenius parameters resulted in an activation energy of 170 kJ/mol. Reduction of the time scale with this value enabled to bring all experimental data on a single master curve, irrespective of the treatment temperature (figure 4b).

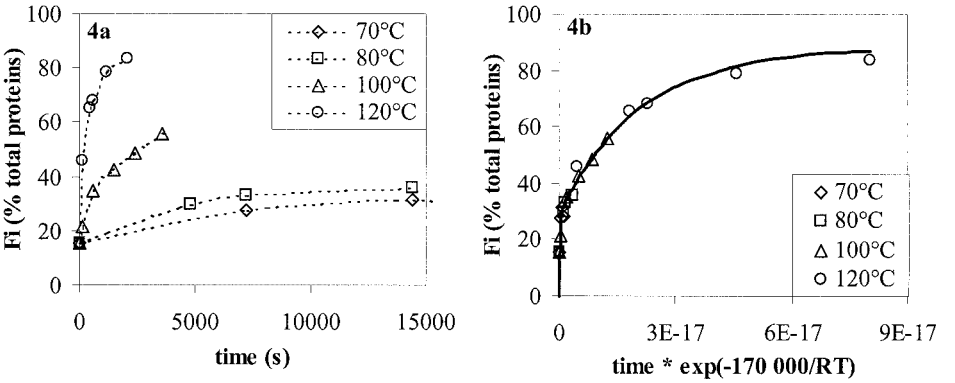

Figure 4. Change in the SDS insoluble gluten fraction as a function of time and temperature of the hot molding process. 4a: raw data. 4b: data as a function of reduced time.

Consequently, the gluten cross-linking reaction induced by hot molding seemed to have an activation energy of 170 kJ/mol.

Mixing process

Typical torque and temperature curves recorded during the mixing process are represented in figure 5.

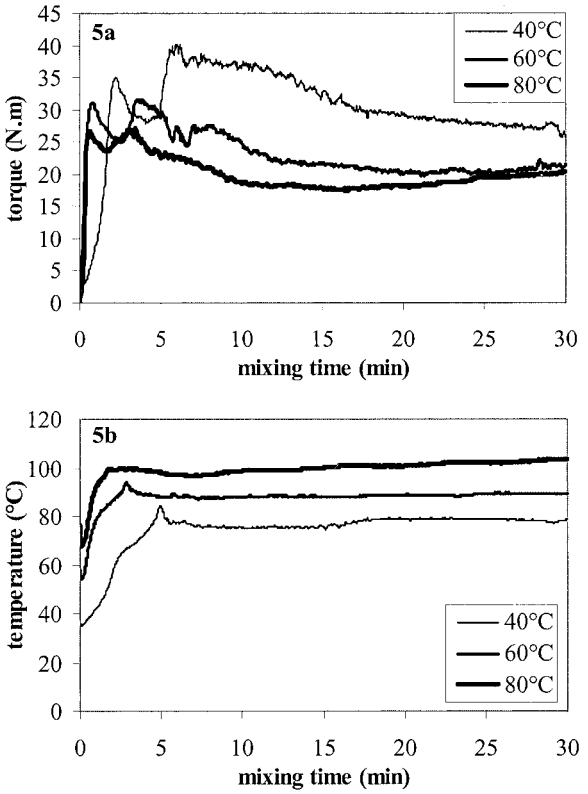

Figure 5. Torque (5a) and temperature (5b) evolution during the mixing process at different thermostat temperatures (rotation speed 100 rpm) [9].

The torque curves (figure 5a) showed 2 torque maxima. Lower thermostat temperatures resulted in higher torque values, and consequently in higher mechanical energy input. Three sets of samples differing in their thermo-mechanical history were thus generated.

The temperature curves (figure 5b) are characterized by a sigmoidal shaped increase, followed by an almost stable phase. Heat dissipation led to stabilization temperatures from 20 to 40°C higher than the thermostat temperatures (stabilization temperatures at 77, 88 and 100°C for programmed

temperatures of 40, 60 and 80°C respectively). These stabilization temperatures were the real temperatures at which the materials were processed.

Size exclusion chromatography analyses of the resulting materials were performed in a similar way as hot molding. The percentage of SDS-insolubilized protein (Fi) increased with mixing time (figure 6a). The rate of Fi-increase increased with the temperature, suggesting an Arrhenius dependency for the formation of gluten network.

In this case the diffusion limited reaction was no more observed in contrast to hot molding process possibly because mixing promoted molecular contacts between proteins. Accordingly, the data could be fitted by a simple first-order kinetic expression:

$$Fi\ (\%) = 100(1 - \alpha - \beta \exp(-k \cdot t)) \qquad \text{(eq. 4)}$$

with ($\alpha + \beta = 100$) as constraint (α is the percentage of non-reacting protein and β the percentage of reacting protein) and where Fi is the percentage of insoluble protein fraction, t the mixing time, and k the rate constant of the cross-linking reaction, supposed to be temperature dependent according to an Arrhenius law as expressed in equation (2).

From equation (2) and (4) it comes:

$$Fi\ (\%) = 100(1 - \alpha - \beta \exp(-A \cdot t \cdot \exp(-Ea/RT))) \qquad \text{(eq. 5)}$$

Finally, an optimized activation energy of 33.7 kJ/mol was calculated and enabled to collapse all experiments data into one master curve (figure 6b).

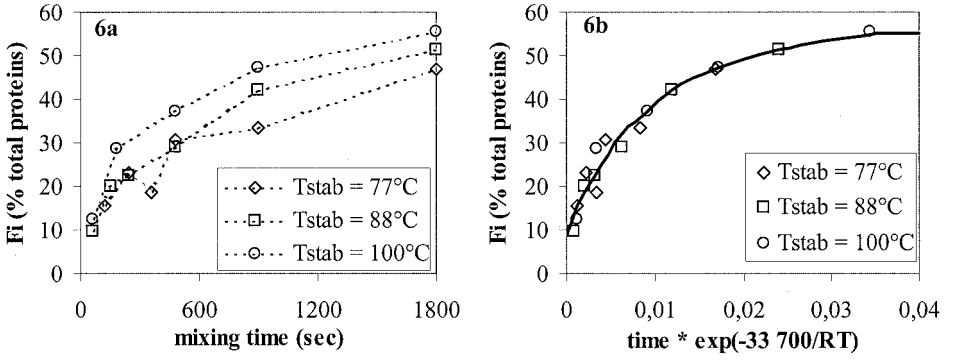

Figure 6. Change in the SDS insoluble gluten fraction as a function of mixing time and stabilization temperature [9]. 6a: raw data. 6b: data as a function of reduced time.

Although different kinetic models were applied for hot molding and mixing processes, the obtained activation energies can be compared, since they are independent of the chosen models, contrary to rate constants.

The activation energy obtained for mixing process (33.7 kJ/mol) is far below the previously calculated activation energy for wheat gluten cross-linking in static conditions (170 kJ/mol, determined by hot molding). Mechanical energy provided by mixing might favor protein interaction and increase their reactivity, thereby lowering the energy of activation of protein crosslinking.

Discussion

We observed that aggregation of gluten proteins displayed a time-temperature dependency, irrespective of the thermoplastic process. Thermal energy seems therefore to have a strong impact on the wheat gluten cross-linking reaction.

Furthermore, as the activation energy of cross-linking was significantly lowered by mechanical energy input upon mixing, we may hypothesize that mechanical energy plays a key role in protein solubility loss. This mechanical energy could be decomposed into two components that will have different effects: work input, and shear.

Work input, for its part would account for the thermal history of the sample, through the viscous dissipation of mechanical energy and thus participate to the thermodependency of the cross-linking reaction.

However, radical compounds might be created due to shear during mixing and extrusion [10]. It is proposed that radical compounds are catalysts of the chemical reaction leading to the increasing connectivity of the gluten network [11]. By this way, shear might decrease the activation energy for protein solubility loss from 170 kJ/mol in a static system to 33.7 kJ/mol in a shear stressed system.

Conclusion

Thermoplastic processing of wheat gluten plasticized with glycerol induced marked changes in the connectivity of the gluten network. Cross-linking of wheat gluten is a temperature controlled phenomenon, but high shear conditions can significantly decrease the activation energy of this reaction (from 170 to 33.7 kJ/mol). Consequently, extrusion of proteins is only possible in a limited window of operating conditions, avoiding early formations of a strongly cross-linked structure that reduces the mobility of the polymeric chains and inhibits the elastic recovery without rupture after processing. On the other hand, materials properties can be tuned for a wide range of applications by changing the thermoplastic processing conditions (temperature and/or shear) to obtain the desired structure.

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