



HAL
open science

Film extrusion of sunflower protein isolate

Antoine Rouilly, Alexandra Mériaux, Céline Geneau, Françoise Silvestre, Luc Rigal

► **To cite this version:**

Antoine Rouilly, Alexandra Mériaux, Céline Geneau, Françoise Silvestre, Luc Rigal. Film extrusion of sunflower protein isolate. *Polymer Engineering and Science*, 2006, 46 (11), pp.1635-1640. 10.1002/pen.20634 . hal-03594499

HAL Id: hal-03594499

<https://hal.science/hal-03594499>

Submitted on 2 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Film Extrusion of Sunflower Protein Isolate

Antoine Rouilly, Alexandra Mériaux, Céline Geneau, Françoise Silvestre, Luc Rigal

Laboratoire de Chimie Agro-Industrielle UMR 1010 INRA/INP-ENSIACET, 118, Route de Narbonne, F-31077 Toulouse Cedex 04, France

Film extrusion of sunflower protein isolate (SFPI) was studied. The influence of die temperature (85–160°C), water and glycerol contents were investigated through appearance, mechanical and thermomechanical properties, and swelling behavior in water of films. It was demonstrated that highest temperature, well above SFPI denaturation temperature in the compound, highest glycerol content (70 parts for 100 parts of SFPI), and medium water content (20 parts for 100 parts of SFPI) gave the most regular and smoothest film (as seen on SEM micrographs). Its ultimate tensile strength, Young's modulus, and strain at break were, respectively, 3.2 MPa, 17.7 MPa, and 73%. Soaked in water, its swelling was about 186% w/w but the film was quiet insoluble. Effect of temperature and plasticizer content were discussed in relation to the kinetic of SFPI denaturation. These first results are very promising for the development of biodegradable protein-based films. POLYM. ENG. SCI., 46:1635–1640, 2006. © 2006 Society of Plastics Engineers

INTRODUCTION

Nowadays, there has been a rise in the market of biodegradable polymers. The major reasons of this development are the environmental consequences of the intensive use of synthetic plastics, the oil price increase, and the new and emerging applications for biodegradable materials. In this domain, new synthetic biodegradable polymers appear, PLA being actually the leader. Their price should rapidly decrease and so they should be able to compete with conventional plastic in a near future, especially in packaging. However, they have a major drawback; they degrade in compost conditions, so their elimination will necessitate an efficient sorting and the development of more composting units.

The other way is the use of the plastic properties of natural biopolymers. For example, thermoplastic starch [1] is studied for a long time in material applications and is now essentially used in multiphase systems [2]. Biopolymer processing has many advantages: use of renewable carbon, low energetic cost, biodegradability, and some drawbacks re-

lated to moisture: special processing conditions and water sensitivity of formed materials.

Among the wide range of biopolymers, proteins appear to be particularly promising. The combination of the 20 natural amino acids gives complex structures and interactions as well as interesting mechanical properties. Almost all available proteins have been studied to make films by casting [3]. But to make protein-based plastics it is likely for proteins to be denaturated [4]. The unfolding of polypeptide chains followed by their rearrangement and the possible formation of new covalent bonds give better materials. Thermomolding is the most widespread technology and has been used on soy protein [5], corn zein [6], wheat gluten [7], or fish myofibrillar proteins [8], for example. With an appropriate combination of plasticizers, the formed materials show some interesting properties: high tensile strain, good barrier properties, relatively high water resistance, but the process is non-continuous.

The processing of proteins by extrusion is more difficult because of the complex association/dissociation phenomena under shear and heat treatment and has essentially been studied for food applications [9]. To make biodegradable materials, according to our knowledge, the only existing studies concern the extrusion of foamed sheets [10] and the injection-molding of biomaterials [11] from soy protein, and the low temperature extrusion of zein-oleic acid resin [12].

Sunflower protein isolate (SFPI) is obtained by alkaline extraction of the sunflower oilcake. It contains two major groups of protein, 11S globulin and 2S albumin [13], as well as a significant amount of phenolic compounds. The sunflower oilcake being a low value residue, the nonfood use of its proteic fraction could add value to this crop, especially in a complete nonfood cultivation of sunflower, the oil being used for biofuel synthesis. Plastic properties of SFPI have already been demonstrated and SFPI-based materials formed by casting [14], thermomolding [15], and injection-molding [16] tested. The aim of this new study was to demonstrate the possible forming of SFPI films by extrusion, monitoring the influences of operating temperature and plasticizers content.

Correspondence to: A. Rouilly; e-mail: Antoine.Rouilly@ensiacet.fr

EXPERIMENTAL SECTION

Materials and Methods

The SFPI was obtained by alkaline extraction from sunflower oilcake on a semi-industrial scale. SFPI composition has already been reported [16]. After centrifugation, the soluble proteins are precipitated at their isoelectric point by adding concentrated sulphuric acid. A second centrifugation allows their separation from the aqueous phase. The third stage consists in spray drying at 50°C, followed by a conditioning in 15-kg bags.

The glycerol used as a plasticizer was “reagent” grade and was purchased from Aldrich (St Quentin Fallavier, France). Glycerol was used without prior treatment.

SFPI, glycerol, and water were mixed using a Perrier 32.00 mixer (Montrouge, France). The mixture generally contained 100 parts (by weight) of SFPI, 10 to 70 parts of glycerol, and 10 to 50 parts of water. Mixtures were then conditioned in airtight containers for 12 hr at 25°C.

Thermal Study

The thermal study of mixtures and produced films denaturation was performed on a Pyris 1 power modulation Differential Scanning Calorimeter (Perkin Elmer), equipped with an Intracooler. The measurement cells were purged with dry nitrogen. The temperature was calibrated by use of indium ($T_f = 156.6^\circ\text{C}$) and distilled water ($T_f = 0^\circ\text{C}$). The capsules used for this study were airtight, steel capsules fitted with a rubbery O-ring. They are designed to resist high pressure (40 bars) and were therefore well adapted to study low moisturized protein at high temperatures. The samples weighed approximately 10 mg. Each sample was tested in triplicate. The measurements were taken during the first scan, between 25 and 200°C. The heating rate was 10°C/min for compound mixture and films and varied between 3 and 40°C/min for the kinetic study. For film analysis, we were only looking for the existence of a denaturation peak.

Film Extrusion

A Rheomex single screw extruder (Thermo Polylab System, Karlsruhe, Germany) equipped with a fan-tail die (width: 10 cm and thickness between 0.05 and 1 mm) was used for film production. The temperature of the die (T_D) varied between 85°C and 160°C. Film thickness was set according to mixture flowing properties to get the thinner film as possible. The temperature of each unit of the barrel was 10°C lower than that of the previous unit or die. The temperature of the supply tank was fixed at 50°C for all measurements. A temperature probe was fitted in the last barrel section, just before the die, giving the temperature of the mixture inside the extruder. The screw speed was set between 20 and 200 rpm. The characteristics of the extruder screw were: diameter 19 mm, L/D = 25, and compression rate 1.8.

Mechanical Properties

A 5-kN H5KT (JFC, Villemur sur Tarn, France) universal testing machine fitted with 100 N load cell was used to assess the tensile properties of the test specimens. The test samples, obtained with a cutting die, were 65 mm long and 5 mm wide. Their thickness was measured at five points with a digital micrometer (model IDC-112B, Mitutoya, Tokyo, Japan) and the mean value was recorded. A speed of 1 mm/s was used, with an initial grip separation of 55 mm. Film test specimens were cut, dried overnight at 50°C, and equilibrated at 25°C and 60%RH for at least 2 weeks before being tested.

Thermomechanical Analysis

DMA experiments were carried out on a Tritec 2000 DMA (Triton Technology, Keyworth, UK). Film samples (4 mm width and 20 mm length) were cut and equilibrated at 25°C and 60%RH for at least 2 weeks before being tested with a 5 Hz frequency, 20 μm displacement, and a 3°C/min heating rate on a -80 to 100°C range. DMA spectra of SFPI films show two distinct $\tan\delta$ peaks attributed to α - and β -transitions [10]. The transition that occurred in low temperature range was reported as T_β and used to make the comparative study of the different films thermal properties.

Measurement of Solubility in Water and Water Uptake

Samples (2 cm diameter) were cut out of the film, weighed, and immersed in demineralized water for 24 hr at 25°C. The surfaces of the samples were then wiped and the samples were weighed in a Petri dish to minimize water exchange with the atmosphere. The samples were then dried for 24 hr in an oven at 105°C and weighed again. The difference between the sample initial mass and the mass after immersion was used to calculate the water uptake or swelling. The difference between the initial mass of the dried sample (dry matter) and the mass after immersion and drying gave the amount of soluble matter in the film. Measurements were made in triplicates.

Microscopy

Micrographs were obtained with a binocular microscope (Nikon SMZ1500) equipped with a digital camera (Nikon DMX 1200). Scanning electron microscopy observations were carried out on a LEO 435VP. All samples were dried at 60°C during 48 hr in a vacuum desiccator before being metallized.

RESULTS

Influence of Extrusion Temperature

Temperature appears to be the critical parameter of protein processing. As for many polymers, the mobility

TABLE 1. Film properties according to extruder die temperature.

Property/die temperature (°C)	$T_D = 85^\circ\text{C}$	$T_D = 105^\circ\text{C}$	$T_D = 125^\circ\text{C}$	$T_D = 140^\circ\text{C}$	$T_D = 160^\circ\text{C}$
Probe temperature (°C)	73	82	99	109	135
Film denaturation peak	Y	Y	N	N	N
T_β (°C)	-35.8	-42.6	—	-41.9	-49.4
Ey (MPa)	14.0 ± 2.7	17.6 ± 3.5	—	27.0 ± 9.7	17.9 ± 4.2
UTS (MPa)	0.6 ± 0.2	1.0 ± 0.3	—	1.9 ± 0.8	1.5 ± 0.5
Strain at break (%)	9.3 ± 2.4	15.0 ± 5.6	—	25 ± 11	36 ± 23
Thickness (mm)	2.4 ± 0.1	2.5 ± 0.2	—	1.4 ± 0.2	1.5 ± 0.2
Water soluble content (%) w/w	33.1 ± 0.1	30.9 ± 1.5	32.5 ± 0.5	32.9 ± 0.1	31.3 ± 1.0
Swelling (% w/w)	50.8 ± 0.8	84.3 ± 11.5	82.8 ± 8.4	97.4 ± 6.2	137.9 ± 7.3
Corresponding micrograph	1a	1b	1c	1d	1e

Film composition: 100 parts of SFPI, 50 parts of glycerol, and 20 parts of water. Screw speed 20 rpm.

of polypeptides is increased with the increase of temperature but their movement is restricted because of structure ramification, of hydrophobic interactions becoming stronger and of coagulation which follows protein denaturation [17]. So thermomechanical processing of proteins was preferably performed under their denaturation temperature [12, 18] or by using a very high water content [10]. However, it is now well known that the best globular protein-based materials are obtained by hot pressing at high temperature [6, 15, 19]. Our first idea was so to investigate the extrusion of SFPI on a large range of temperature: from 85 to 160°C using a constant screw speed of 20 rpm. The mixture used for this study contained 100 parts of SFPI, 50 parts of glycerol, and 20 parts of water. This SFPI/glycerol ratio corresponding to the best thermomolding conditions [20]. The SFPI denaturation temperature in such a mixture was 123.7°C.

Results of this study are shown in Table 1. At low die temperature, lower than 105°C, only thick sheets were obtained ($e \sim 2.4$ mm). They exhibited a rough surface and at 85°C a mat grey color becoming nonuniformly bright at 105°C (Fig. 1a and 1b). While proteins were not denatured during forming, the increase of temperature from 85 to 105°C enhanced film quality. Their tensile strength and modulus increased respectively from 0.6 to 1.0 MPa and from 14.0 to 17.6 MPa. Surprisingly, their elongation and swelling were largely increased as well. This could be due to a small extent of cross-linking occurring at a temperature well below denaturation temperature as recorded probe temperature was only 82°C. This phenomenon, already reported by Micard et al. [19], is linked to the effect of shear on

proteins structure. Consequently, when die temperature reached the SFPI denaturation temperature (around 125°C) they were denatured while probe temperature just before the die was only 99°C. But shear induced denaturation was responsible for an heterogeneous flow of the mixture and the resulting film was not uniform, pierced everywhere, as if some unfused particles were hindering the forming of the film (Fig. 1c).

When die temperature reached 145°C, films appearance was completely changed. They were thinner ($e \sim 1.5$ mm), brighter and smoother (Fig. 1d and 1e). Their mechanical properties were drastically enhanced as well. UTS and tensile modulus reached, respectively, 1.9 and 27.0 MPa for the film produced at a die temperature of 145°C (Table 1). But the real change was obtained with a die temperature of 160°C. Film UTS and tensile modulus decreased compared with those of the 145°C film but the film was more homogeneous and more plastic-like. Its elastic elongation increased and its total elongation reached 36%. Its swelling behavior was as well completely modified; the total mass of water absorbed in the film was 138% of its initial mass while it was only 51% at 85°C and 97% at 145°C. This indicated a higher extent of cross-linking at high temperature; this behavior has already been reported in studies of protein-based materials [7, 19]. Temperature increase had another consequence: a decrease of films transition temperature T_β . The DMA $\tan\delta$ peak was at -35.8°C when film was extruded at 85°C, around -42°C at 105 and 145°C, and reached -49.4°C at 160°C.



FIG. 1. Film morphology according to extruder die temperature (a: 85°C, b: 105°C, c: 125°C, d:145°C, and e: 160°C). Scale arrow length: 5 mm.

TABLE 2. Mixture denaturation temperature, extruder screw speed, and film properties according to water content.

Property/water content (parts)	0	10	20	30
T_D (°C)	128.0	124.9	123.7	118.5
N (rpm)	150	20	20	10
Probe temperature (°C)	149	134	135	108
T_β (°C)	-44.7	-46.8	-52.0	—
Film thickness (mm)	2.1 ± 0.2	1.7 ± 0.1	1.4 ± 0.2	—
Ey (MPa)	25.5 ± 2.6	36.6 ± 4.9	17.9 ± 4.2	—
UTS (MPa)	2.2 ± 0.6	2.6 ± 0.4	1.5 ± 0.5	—
Strain at break (%)	43 ± 23	24.7 ± 5.4	36 ± 23	—
Water soluble content (% w/w)	34.2 ± 0.1	56.7 ± 0.6	31.3 ± 1.0	53.1 ± 2.5
Swelling in water (% w/w)	116.5 ± 2.9	97.1 ± 2.1	137.9 ± 7.3	106.4 ± 9.6
Corresponding micrographs	2a	2b	2c	2d

Glycerol content: 50 parts and die temperature: 160°C.

Influence of Water Content

Mixtures contained two different plasticizers: water and glycerol. Water having the greatest affinity to biopolymers is used essentially as rheology modifier. But as moistened biopolymers tend to degrade over time and as SFPI extrusion was obtained with a low mechanical energy, we wanted to investigate a possible processing with an amount of added water as low as possible. Extruded with a die temperature of 160°C, mixtures containing 0, 10, 20, and 30 parts of water and 50 parts of glycerol have been tested. Special operating conditions and film properties are gathered in Table 2.

The increase of mixture water content decreased its denaturation temperature T_D from 128°C without any added water to 118.5°C with 30 parts of water. Influence of water was finally not so important as glycerol content is high (50 parts). Film made from the 30 parts mixture was too irregular to make it possible to take some test specimens even at low screw speed (Fig. 2d). Feeding the screw with the mixture containing only glycerol and no added water was particularly difficult so we had to increase screw speed to 150 rpm. And this had a direct consequence: a severe self-heating inside the extruder, up to 149°C. Concerning the evolution of films mechanical properties, the trend was not obvious. With 10 parts of water the film was stronger, had the highest tensile strength and modulus, respectively, 2.6 MPa and 36.6 MPa, but the highest strain was obtained without water while the corresponding film was the thickest. Film transition temperatures decreased with the water content of the mixture before extrusion, from -44°C to

-52°C. The swelling was maximum for 20 parts and for 10 and 30 parts the soluble matter content was especially high reaching 56.7 and 53.1%, indicating that not only glycerol was dissolved.

Qualitatively, best film was obtained with 20 parts of water; it was smoother and more regular (Fig. 2c), with a lower water content film surface was irregular as if the flow of the “melted” mixture was not uniform (Fig. 2a and 2b). And so studies on the influence of the glycerol content have been performed with this amount of water.

Influence of Glycerol Content

As a first consequence, the increase of glycerol content caused a decrease of the mixture denaturation temperature (Table 3): a 12.5°C drop between 30 and 50 parts and only a 1.2°C drop between 50 and 70 parts of glycerol. All extrusions being made with a 160°C die temperature, denaturation occurred in all cases.

With 30 parts of glycerol, extruded film had some very interesting mechanical properties (Table 3): very high stress at break (6.7 MPa) with a high modulus (95 MPa) and a high strain (46%). Its structure was well maintained: its soluble content corresponded to the glycerol amount in the film (i.e., 23%) and the film had the highest swelling (209%). Unfortunately, its flowing behavior was not as good as for higher glycerol content. The film was irregular and test specimens had to be taken in chosen areas (Fig. 3a). The increase of glycerol content to 50 parts gave a smoother

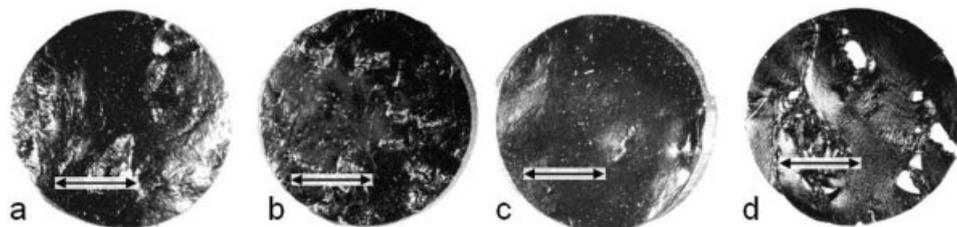


FIG. 2. Film morphology according to mixture water content (a: 0 parts, b: 10 parts, c: 20 parts, and d: 30 parts). Scale arrow length: 5 mm.

TABLE 3. Mixture denaturation temperature and film properties according to glycerol content.

Property/glycerol content (parts)	30	50	70
T_D (°C)	136.2	123.7	122.5
Thickness (mm)	0.8 ± 0.1	1.4 ± 0.2	0.43 ± 0.04
T_B (°C)	-30.2	-52.0	-59.9
E_y (MPa)	95 ± 22	17.9 ± 4.2	17.7 ± 1.8
UTS (MPa)	6.7 ± 1.6	1.5 ± 0.5	3.2 ± 0.6
Strain at break (%)	46 ± 18	36 ± 23	73 ± 24
Water soluble content (% w/w)	23.9 ± 0.8	31.3 ± 1.0	21.4 ± 0.3
Swelling (w/w) (%)	209 ± 22	138 ± 7	186 ± 34
Corresponding micrograph	3a	3b	3c

Water content: 20 parts, die temperature: 160°C and screw speed: 20 rpm.

film (Fig. 3b) but with a decrease of all film properties (Table 3). Another addition of 20 parts of glycerol gave the best results. Extruded film was the thinnest obtained (i.e., 0.43 mm); it was completely homogeneous (Fig. 3c) and had some good mechanical properties (Table 3). Its strain at break was the highest obtained (73%) with a good stress at break of 3.2 MPa. Obviously, as glycerol acts an external plasticizer, it had the lowest T_B (-59.9°C), but the T_B difference between films containing 70 and 50 parts of glycerol was less marked than between 30 and 50 parts, respectively, -30.2°C and -52.0°C.

Film obtained with 70 parts of glycerol had another interesting characteristic. Its water soluble content was lower than its glycerol content: 21.4% against 41%, indicating a loss of glycerol or an insolubilization of a part of the glycerol, eventually by grafting. Such behavior had never been observed in past experiments [20].

DISCUSSION

Forming of SFPI by extrusion is driven by an excessively complex phenomenon: denaturation. Denaturation is an endothermic phenomenon involving the loss of quaternary, tertiary, and secondary structure of proteins [21]. It is most of the time irreversible and causes a drastic change of their physicochemical properties (i.e., solubility, surface hydrophobicity). Buried groups of polypeptides are liberated and they could be more hydrophobic than the surface groups. When denatured, under thermal constraint, chains become more mobile but their movement is restricted because of protein branched structure,

to the increase of hydrophobic interactions intensity and to coagulation that follows denaturation [9]. To make proteins flow, use of plasticizers is then necessary.

In our case, we tried first using 20 parts of water and 50 parts of glycerol for 100 parts of SFPI to find the most appropriate temperature to film extrusion. It appears that using a die temperature (~160°C) well above denaturation temperature of SFPI in the mixture (~125°C) gives the best results (Table 1). Above 160°C, we got too much evaporation and films were disrupted. But things get more complicated when looking to kinetics. Indeed all presented results were obtained with a 20 rpm screw speed. Increasing or decreasing this value led to a complete modification of films, especially at high temperature. Experimentally, DSC study of SFPI denaturation temperature and enthalpy according to heating rate gave some interesting results (see Fig. 4). This particular behavior should be addressed to both fundamental effect of heat transfer and instrumental resolution of thermograms. But increasing the heating rate from 3 to 40°C/min caused a 16°C increase of T_D and a decrease of the denaturation enthalpy. Considering this kinetic effect and the very low residency time in a single screw extruder, thermal denaturation of SFPI may only occurred completely with a 160°C die temperature. Below this temperature, shear would then be responsible for SFPI denaturation as

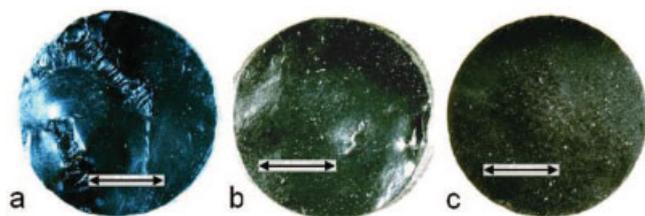


FIG. 3. Film morphology according to mixture glycerol content (a: 30 parts, b: 50 parts, and c: 70 parts). Scale arrow length: 5 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

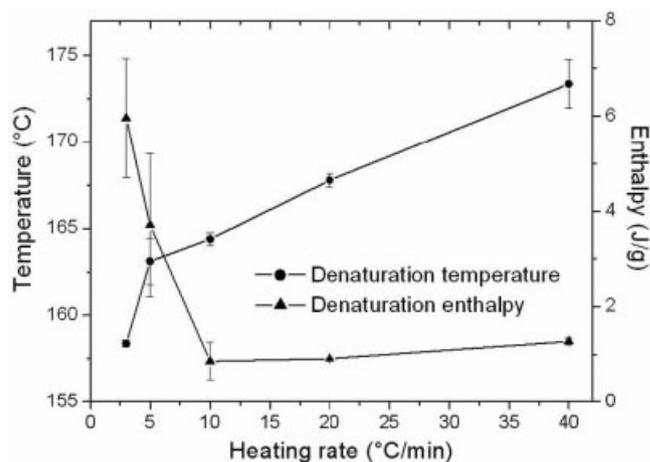


FIG. 4. SFPI denaturation temperature and enthalpy according to heating rate (equilibrated at 60% RH).

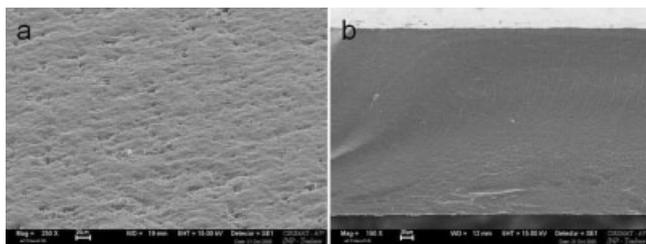


FIG. 5. SEM micrographs of film freeze fracture (a) and film surface (b). Composition 100 SFPI/20 water/70 glycerol.

no denaturation peak has been observed on thermograms of films extruded at 125 and 145°C (Table 1). And increasing thermomechanical energy provided to protein, cross-linking reactions are favored. This increase of cross-linking is responsible for the increase of swelling (Table 1). Protein structural modifications under mechanical shear have already been reported [22, 23] but never in relation with denaturation.

Thermal denaturation could be responsible for the T_{β} decrease as well. Die temperature and water content increase involved a decrease of film T_{β} (Tables 1 and 2). This decrease of T_{β} is not caused by polypeptides shortening under shear as water acts as a lubricant, but could then be due to a better disentanglement of polypeptide chains at high temperature, above denaturation temperature, liberating side chains from their native organization. Effect of water content is then only a plasticizer effect, increase of plasticizer content involves a decrease of SFPI denaturation temperature [24]. Glycerol content effect is different as glycerol stays in the film after conditioning, so it was expected that T_{β} decreases with the increase of glycerol content (Table 3).

Plasticization of SFPI is then more efficient at high temperature and with a high amount of external plasticizer. Best composition was indeed 20 parts of water and 70 parts of glycerol for 100 parts of SFPI. With its high boiling point (~270°C), glycerol acts as a plasticizer (decrease of SFPI denaturation temperature in the mixture and of polypeptides T_g) without evaporating at the die exit. It is possible as well that glycerol avoids the coagulation of SFPI facilitating the flow of the mixture, but more experiments have to be done for this to be proved. In these conditions, the film is homogeneous (Fig. 5a) and shows a smooth surface (Fig. 5b), indicating the complete plasticization of SFPI. As film mechanical properties are quite good (Table 3), this composition could be very promising for the development of biodegradable protein-based films.

CONCLUSION

Best conditions to get a smooth and homogeneous film from SFPI by extrusion were found to be: an addition of 20 parts of water and 70 parts of glycerol to 100 parts of SFPI, a die temperature of 160°C, and a screw speed of 20 rpm. Temperature had a positive effect on film properties related to polypeptides disentanglement. Addition of glycerol gave a regular flow off the die. Water was necessary to obtain a satisfactory feeding of the extru-

sion screw and to decrease SFPI denaturation temperature. In these conditions, film properties were: a 3.2 MPa tensile strength, a 17.7 MPa Young's modulus, and a 73% strain at break. Side chains relaxation temperature measured in DMA was -59.9°C. SFPI films were sensitive to water and tended to swell (~180% after a 24 hr soaking in water) but protein-based structure was quite insoluble.

REFERENCES

1. I. Tomka, "Thermoplastic Starch," in *Water Relationships in Food*, H. Levine and L. Slade, editors, Plenum Press, New York, 627 (1991).
2. L. Averous, *J. Macromol. Sci. Polym. Rev.*, **44**, 231 (2004).
3. A. Rouilly and L. Rigal, *J. Macromol. Sci. Polym. Rev.*, **42**, 441 (2002).
4. L.A. De Graaf, *J. Biotechnol.*, **79**, 299 (2000).
5. H.-J. Sue, S. Wang, and J.-L. Jane, *Polymer*, **38**, 5035 (1997).
6. F.X. Budi Santosa and G.W. Padua, *J. Agric. Food. Chem.*, **47**, 2070 (1999).
7. C. Mangavel, N. Rossignol, A. Perronnet, J. Barbot, Y. Popineau, and J. Gueguen, *Biomacromolecules*, **5**, 1596 (2004).
8. B. Cuq, N. Gontard, and S. Guilbert, *Polymer*, **38**, 2399 (1997).
9. J.A.G. Arêas, *Crit. Rev. Food Sci. Nutr.*, **32**, 365 (1992).
10. J. Zhang, P. Mungara, and J. Jane, *Polymer*, **42**, 2569 (2001).
11. C.M. Vaz, J.F. Mano, M. Fossen, R.F. Van Tuil, L.A. De Graaf, R.L. Reis, and A.M. Cunha, *J. Macromol. Sci. Phys.*, **41**, 33 (2002).
12. Y. Wang, A.M. Rakotonirainy, and G.W. Padua, *Starch/Staerke*, **55**, 25 (2003).
13. S. Gonzalez-Perez, J.M. Vereijken, K.B. Merck, G.A. van Koningsveld, H. Gruppen, and A.G.J. Voragen, *J. Agric. Food Chem.*, **52**, 6770 (2004).
14. F. Ayhllon-Meixueiro, C. Vaca-Garcia, and F. Silvestre, *J. Agric. Food Chem.*, **48**, 3032 (2000).
15. O. Orliac, A. Rouilly, F. Silvestre, and L. Rigal, *Polymer*, **43**, 5417 (2002).
16. O. Orliac, F. Silvestre, A. Rouilly, and L. Rigal, *Ind. Eng. Chem. Res.*, **42**, 1674 (2003).
17. J.I. Boye, C.-Y. Ma, and V.R. Harwalkar, "Thermal Denaturation and Coagulation of Proteins," in *Food Proteins And Their Applications*, S. Damodaran and A. Paraf, editors, Marcel Dekker, New York, 25 (1996).
18. S.H. Wang and C.-H. Chen, U.S. Patent 6,379,725 (2002).
19. V. Micard, M.H. Morel, J. Bonicel, and S. Guilbert, *Polymer*, **42**, 477 (2000).
20. O. Orliac, A. Rouilly, F. Silvestre, and L. Rigal, *Ind. Crops Prod.*, **18**, 91 (2003).
21. S. Damodaran, "Food Proteins: An Overview," in *Food Proteins And Their Applications*, S. Damodaran and A. Paraf, editors, Marcel Dekker, New York, 1 (1996).
22. T. Fisher, *Eur. Food Res. Technol.*, **218**, 128 (2004).
23. M. Pommet, A. Redl, M.-H. Morel, S. Domenek, and S. Guilbert, *Macromol. Symp.*, **197**, 207 (2003).
24. A. Rouilly, O. Orliac, F. Silvestre, and L. Rigal, *Thermochim. Acta*, **398**, 195 (2003).