The Influence of Thermal Crosslinking on Solubility of Films Prepared From Collagen Hydrolysate

by

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Abstract

The presented work describes a method of utilizing collagen hydrolysate obtained through enzymatic hydrolysis of chrome-tanned shavings for preparing biodegradable films. Films were prepared by casting from a solution of collagen hydrolysate with added plasticizer (glycerol) and cross-linking agent (starch dialdehyde). Films thus prepared were subjected to thermal annealing at 60, 70, 80 and 90 °C for 24 hours. Thermal annealing is a mode of physical cross-linking of biodegradable films. The work studied effect of thermal crosslinking on film solubility. Solubility curves are important for applying biodegradable films in industrial practice. Thermal annealing was found to affect the cross-linking degree of prepared film in significant manner, hence, also, its solubility. Results indicate that collagen hydrolysate, following certain modification by plasticizer and cross-linking agent is capable of producing films whose properties may be additionally modified.

Resumen

El trabajo presentado describe un método para utilizar una solución de un hidrolizado colagénico por hidrólisis enzimática de rebajaduras de cuero al cromo en la preparación de películas biodegradables. Películas fueron preparadas por vaciado desde una solución del hidrolizado colagénico con un plastificante añadido (glicerina) y un agente reticulante (almidón dialdehídico). Las películas así preparadas fueron sometidas a un fraguado térmico de 60, 70, y 90ºC durante 24 horas. Fraguado térmico es un método de reticulación de películas biodegradables. El trabajo estudió el efecto del fraguado térmico sobre la propiedad de solubilidad de la película. Curvas de solubilidad son importantes en las prácticas de aplicación industrial de películas biodegradables. El fraguado térmico, se encontró que afecta en forma significante el grado de reticulación de las películas así preparadas, luego entonces, su solubilidad. Resultados indican que el hidrolizado de colágeno, con consiguientes modificaciones por plastificante y agente reticulante, es capaz de producir películas cuyas propiedades pueden ser adicionalmente modificadas.

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INTRODUCTION

Economic development is gathering high tempo, meeting necessities of life and also bringing serious concern for global deterioration of the environment. Repeatedly employing waste as secondary raw material is important from viewpoint of ecology as well as economy of the whole process. Disposal of waste by mere landfill or incinerating is irrational, and at present also the least preferred. Potential waste recycling is winning ever more recognition. Wastes are getting regarded as valuable secondary raw materials and ways of utilizing them are under consideration. Waste processing often involves demanding and difficult solutions requiring sizeable investments in technological plant.

Dechromation of Tanned Wastes

Tanned wastes chiefly appear as chrome shavings, cuttings, grinding powder, and also as so-called clicking waste. They are quite "clean" waste containing, apart from chromic compounds, also a large quantity of collagen proteins and small quantities of inorganic salts. Chrome-tanned shavings, with a view to their volume, are an important waste of the tanning industry. Due to the collagen in question being chrome-tanned, there is an effort at such waste processing that obtains proteins in the form of hydrolysate, and remaining sludge that is based on chromium compounds. Cross-linking of collagen with basic chromic complexes makes its processing more difficult. In principle, the chromic cation may be reclaimed or removed from leather or wastes through the action of substances transforming insoluble Cr^{3+} complexes in leather into a soluble form [1]. According to employed agent, the hydrolysis in question is acid or alkaline.

Literature contains a whole number of concrete procedures focused on dechromation of shavings and leathers. An alkaline medium for hydrolysis may be produced, for example, by $Ca(OH)_2$, NaOH, KOH, K_2CO_3, MgO. Hydrolysis may run at high (up to 150 °C) or low temperatures, under atmospheric or high pressure (up to 15 MPa). Hydrolysis in an acid medium employs, for example, H_2SO_4, CH_3COOH, HCl. Dechromation may also be performed by peroxides in an alkaline environment [2]. One of the most prospective methods for dechroming tanned wastes is enzymatic hydrolysis giving a number of advantages such as, for instance, high efficiency, mild reaction conditions (temperature, pressure, and pH), reaction specificity or minimum side reactions [3].

When dechroming through the action of inorganic acids, dechromation efficiency decreases in a sequence of H_2SO_4 > HCl > HNO_3 and depends on concentration of acid. Its disadvantage lies in the fact that it leads to hydrolysates with chromic oxide of substantially higher content that can be subsequently reduced with difficulty. The Cr^{3+}–collagen bond is stable in a pH range of 2–7. At higher alkalinity, bonds get disrupted and soluble chromite is formed. At a concentration corresponding to pH 8, dechromation efficiency decreases in the following sequence: NaOH > NH_4HCO_3 > $NaHCO_3$ > Na_2CO_3 > $Na_2B_4O_7$. Protein hydrolysate isolated through the action of alkalis contains quantities of Cr in the order of tenths to units of ppm depending on conditions of hydrolysis, and also considerable quantities of sodium salts, potassium salts, calcium salts and magnesium salts. Repeated extraction by alkalis and acids effects easier disruption of the bond between chromium and collagen and the release of chromium into solution [4]. Because chromates, as also dichromates, do not exhibit any tanning effects, it is possible in principle to remove chromium from tanned materials after oxidation in an alkaline environment [5]. Compounds used as alkalis are sodium hydroxide, soda and ammonia. Presence of neutral salts also has a role; these suppress swelling and substantially affect the course of dechromation. A certain residual alkalinity of the solution is also necessary because oxidation stops at pH values smaller than 8 [6].

Enzymatic hydrolysis employing proteolytic enzymes runs in a mildly alkaline environment, at pH levels of 8.3 to 9.5 and temperatures up to 75 °C. Enzymes attack the alkali-denatured bond between collagen and chromium. An alkaline environment prevents dissolution of chromium and its transition into solution. Chromium is obtained in the form of $Cr(OH)_3$ in remaining undegraded sludge following separation of protein hydrolysate. When MgO together with other alkaline agents is used, higher protein solubility is achieved with a lower quantity of enzyme. The course of hydrolysis may be influenced by composition of mixtures and enzyme dosage and hydrolysis may be performed with steam under pressure [7]. Kolomaznik et al. developed and patented a two-stage technology for enzymatic hydrolysis of chrome-tanned shavings based on application of low-molecular amines (isopropylamine, diisopropylamine and cyclohexylamine) as bases, and proteolytic enzyme (Alcalase, NovoNordisk, Denmark). Resultant products following separation are low-molecular enzymatic hydrolysate (M_n < 20 kDa) and separated insoluble substances in the form of so-called filter cake. It was proved the application of volatile amines in hydrolysis brings a number of advantages, particularly increasing productiveness of protein in hydrolysate, increasing Cr_2O_3 content in filter cake and reducing content of ash in hydrolysate [8].

General Applications for Collagen Hydrolysate (Films)

Choice of conditions in hydrolysis of chrome-tanned wastes enables to obtain products of various properties and various chromium content, and even to conduct the process in such manner that hydrolysates containing chromium below standards for hygiene are obtained. Hydrolysates of chrome-tanned shavings may be employed in a number of industrial applications, for example, in manufacture of liquid and solid growth stimulants or as a feed for pigs, cattle and poultry [9].

Quite high water solubility of collagen hydrolysate and thus lower resistance of hydrolysate-based films, foils or gels to moisture has to be eliminated by increasing cross-link density in hydrolysate. For that reason we studied the influence of crosslinking of collagen hydrolysate hydrogels with polymeric dialdehyde starch and ageing time of prepared gels on gel rigidity and on gel melting temperature [10].

After modification with glutaraldehyde, collagen hydrolysate may be used as filler in leather production [11]. Its further use is in manufacture of biodegradable films or foils based on polyvinyl alcohol [12]. An interesting field of industrial application of collagen hydrolysate could be its use as a wall material for the production of (micro) capsules. For example, in agriculture (micro) encapsulation can be widely used for enclosing agro-technical chemicals, such as fertilizers, herbicides, pesticides, fungicides, insecticides. Further application fields of (micro) capsules are personal-care hygiene products, cleaning and disinfect products, industrial chemicals etc [13]. Collagen hydrolysate, obtained through microbial unaffected raw-material (e.g. collagen casings by-products), may be used in pharmaceutical and food industry or in cosmetics. In food packaging film/foil barrier properties for oxygen, carbon dioxide, water or aromatic substances can be controlled by crosslinking density [14].

Processing Technique for Proteins

Proteins may be processed in the presence of a large quantity of water (for example, into films, coatings) or low quantity of water (for example, extruded products). Casting and thermoplasticizing belong to the two principal processing procedures for proteins [15]. In general, preparation of films, coatings and other forms of products consists of three steps:

a) breakdown of native form: severing intermolecular bonds (non-covalent, or possibly covalent) stabilizing protein in its native form, by chemical or physical methods, thereby achieving greater mobility of polymeric chains
b) formation of shape: arranging of polymeric chains into desired shape
c) stabilization of shape: forming of new intermolecular bonds and interactions stabilizing the three-dimensional network

The principle of casting (also designated solvent method) consists in dissolving or dispersing protein in a solvent; the required shape of product arises by casting the solution, spraying it, or dipping the product to receive a coating into the solution, followed by evaporation of solvent. Dissolving of protein mostly proceeds in an aqueous environment (in some cases an acid, alkaline or alcoholic environment has to be used) at a low or high pH level, at elevated temperature which increases its solubility. Protein concentration usually does not exceed 20 percent (w/w). Ingredients frequently added are plasticizers, cross-linking agents, surfactant and others to modify final physico-chemical properties of film/foil. Casting the solution on a plate (glass, silicone, high-grade steel) produces a film after evaporation of solvent. Thermoplasticizing as opposed to the solvent method has an advantage in not requiring drying to evaporate solvent in order to stabilize resultant shape. Its principle consists in processing protein with added plasticizers by plastics technologies above the glass transition temperature (T_g), and then shaping rubbery mass so obtained into the desired product which acquires a stable shape on cooling [16]. However, extrusion of proteins is, in general, only possible in a limited window of operating conditions and the thermal properties of extrudates depend on the processing conditions in a quite complex way [17].

The plasticizing effect of hydrophilic plasticizers (glycerol and low-molecular polyethylene glycols) for collagen hydrolysate films assessed on the basis of T_g depression was studied by means of differential scanning calorimetry in our previous work [18]. As for thermal processing of collagen hydrolysate, not much has been published in this area. However, from the results of thermal processing of gelatin we can estimate the characteristics of collagen hydrolysate at thermoplasticizing. In this sense the study by Park et al. investigating the differences in film properties of gelatin films produced by a heat-pressing method (extrusion) and by a casting method could bring more details into the issue of thermal processing of collagen hydrolysate as well as mechanical and chemical properties of prepared films. From their results it is evident that gelatin film produced by an extrusion method showed lower tensile strength and modulus of elasticity and higher percentage elongation at break and water vapor permeability compared to gelatin films made by casting method [19].

The objective of this work was to find how thermal cross-linking of films prepared from collagen hydrolysate affects their water solubility.

EXPERIMENTAL

Films were produced by first preparing a 30 percent (w/w) aqueous solution of hydrolysate containing 10 percent (related to mass of hydrolysate) added cross-linking agent (starch dialdehyde) and 5 percent (related to mass of hydrolysate) added plasticizer (glycerol). Actual film was made by casting warm solution on a silicone plate and evaporating water under defined conditions.

Powdery hydrolysate obtained by enzymatic hydrolysis of chrome-tanned shavings designated E-Hycol was delivered by the Stospol Co., Valasske Mezirici (The Czech Republic). Hydrolysate was kept in a glass-stoppered bottle and its composition is shown in Tab. I.

TABLE I
Composition of Powdery Hydrolysate

Field 1	Field 2
Dry matter	90.3 %
Inorganic solids *	5.3 %
Total Kjeldahl nitrogen *	14.9 %
Chromium *	205 ppm
$-NH_2$ groups *	0.22 mmol g^{-1}

* in dry matter

Dry matter was determined by drying weighed sample in a glass weighing bottle at 103±2 °C for 12 hours and weighing on cooling. Ash was determined by carefully incinerating the sample in a ceramic crucible over a gas burner, followed by its annealing at 600 °C and weighing ash on cooling. Total Kjeldahl nitrogen was determined by mineralizing the sample for 30 min by boiling (at approx. 440 °C) in sulphuric acid with added catalyst. Nitrogenous substances were thus transformed into ammonium sulphate from which ammonia was released in an alkaline environment and steam-distilled, and subsequently determined by titration. Chromium was determined by flame atomic absorption using GBC 933 AA instrument (GBC Scientific Equipment Pty Ltd., Dandenong, Victoria, Australia) after mineralization of a sample with H_2SO_4 30 min at 440 °C. Primary amino groups were determined spectrophotometrically after reaction with ninhydrine [20].

Starch dialdehyde (CAS No 9047-50-1) was supplied by the Sigma Aldrich Co, U.S.A. (Product No P9265). Glycerol (CAS No 56-81-5) was supplied by the Sigma Aldrich Co, U.S.A. (Product No G9012). Apparatus and equipment: magnetic stirrer with temperature control Ika ETS-D4 fuzzy (Germany), drier WTB Binder E/B 28 (Germany), incubator WTC Binder B53 (Germany), electronic balance KERN 770/GS/GJ (Germany), pH-meter Picollo HI 1295 (Germany), Tescoma silicone plate of 18-cm diameter (The Czech Republic), filter paper Filpap KA-1 of 9-cm diameter (The Czech Republic).

Procedure for preparing films: Dry hydrolysate in a quantity of 30 g was weighed into a 150-ml beaker and 100 ml water (at a temperature of 22 °C) was added. Heating of the mixture started at a rate of 5 °C min^{-1} together with simultaneous stirring over magnetic stirrer. On reaching 60±0.5 °C, stirring continued until hydrolysate was completely dissolved, and went on for another 10 min. An addition of 4N NaOH adjusted solution pH to 11±0.2, and stirring still continued for 30 min. Level of pH was then checked and, if necessary, adapted to 11±0.2. Under constant stirring and with temperature held at 60±0.5 °C, 3.0 g starch dialdehyde (DAS) was added to the solution in small doses and stirring proceeded until it dissolved, after which it went on for another 30 min. Finally, 1.5 g glycerol (GLY) was added to the solution and stirring at 60±0.5 °C continued 15 more minutes. The solution was cast while warm onto the silicone plate. Film was formed in 75 hours in the drier (without air circulation) by solvent (water) evaporating at a temperature of 40±0.5 °C. After removal from the silicone plate, film was placed for 24 hours in a desiccator containing dried silica gel. Film thickness was subsequently measured in ten points and mean thickness calculated. Test samples measuring 2 by 2 cm were prepared from film by mechanical separation.

The procedure in thermal crosslinking of films was as follows: Film samples on Petri dishes were exposed to heat for 24 hours in a drier (without air circulation) at temperatures of 60, 70, 80 and 90 (±0.5) °C. They were then conditioned 48 hours in a desiccator over dried silica gel.

The dry matter *(D)* of 3 test samples from every thermal crosslinking was always determined. Samples were dried in a glass weighing vessel at 103±2 °C for 12 hours and after cooling in desiccator were weighed. Dry matter content served to calculate the coefficient of conversion to zero moisture content *(f)*. Conversion coefficient was used to convert the weighed-in quantity of sample for solubility (m_1) into dry matter content of weighed-in sample for solubility (m_2).

$$D = \frac{n_2}{n_1} 100 \qquad (1)$$

$$f = \frac{100}{D} \qquad (2)$$

$$m_2 = \frac{m_1}{f} \qquad (3)$$

Solubility tests of thermally crosslinked film samples were conducted at temperatures of 25 and 37 (±0.1) °C. A sample of film was placed in glass weighing vessel, weighed and covered with 40 ml distilled water preheated to 25 and 37 °C (see Fig. 1). The glass weighing vessel was then placed in the incubator. After a specified dissolving time, depending on the stage of dissolution the sample had reached, work proceeded in two alternative ways. Firstly, in case the film test sample after solubility test did not disintegrate into smaller fragments and stayed compact, it was carefully removed from dissolving medium (water) and weighed on a watch glass (m_3). The mass of swelled film sample after solubility test served to calculate the mass increment of film sample after solubility test *(I)*. The swelled film sample was subsequently placed on a Petri dish, left to dry for 12 hours

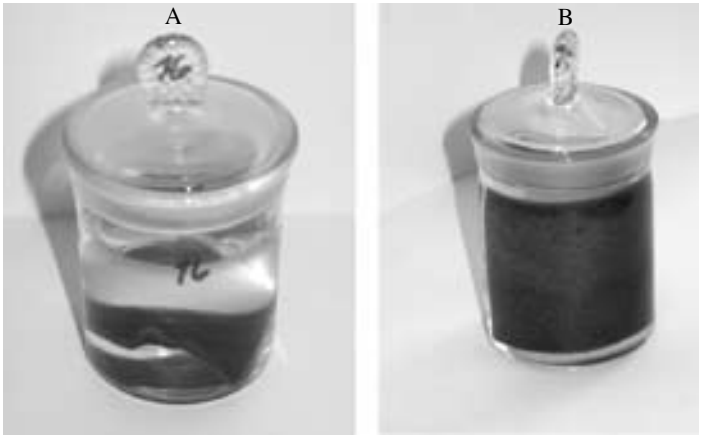

Figure 1: Film sample in a glass weighing vessel in solubility test: A– film at start of solubility test; B– swelled film at end of solubility test.

at 103±2 °C, and after cooling in desiccator was weighed. Thus, dry matter content of non-dissolved film sample after the solubility test *(m_4)* was found, and served to calculate the dissolved fraction of film sample after the solubility test *(X)*. Secondly, in case the film test sample after solubility test disintegrated into smaller fragments, these were separated by filtration through filter paper. Residues of film sample on filter paper were dried on a Petri dish for 12 hours at 103±2 °C, and after cooling in desiccator were weighed. Thereby, dry matter content of non-dissolved film sample after the solubility test *(m_4)* was found, and served to calculate the percentage of dissolved film sample after solubility test *(X)*. Each test of solubility was performed threefold and arithmetic mean calculated, standard deviation ranged within ±4.0%.

$$I = \frac{m_3}{m_1} \qquad (4)$$

$$X = \frac{m_2 - m_4}{m_2} 100 \qquad (5)$$

Results and Discussion

The prepared film felt tacky, it was odorless, russet, non-transparent, of average thickness 1.05 mm. After thermal crosslinking, samples of film exhibited dry matter content *(D)* presented below with the above-mentioned calculated coefficient of conversion to dry matter *(f)*. Film thermally crosslinked at 60 °C: D = 95.18 percent and f = 1.0506; film thermally crosslinked at 70 °C: D = 95.82 percent and f = 1.0436; film thermally crosslinked at 80 °C: D = 96.07 percent and f = 1.0409; film thermally crosslinked at 90 °C: D = 97.97 percent and f = 1.0207. Results of solubility tests of thermally crosslinked films are summarized in Tab. II (test of solubility at 25 °C) and in Tab. III (test of solubility at 37 °C).

The mass increments of swelled film samples after solubility tests are indicated in Fig. 2 (films thermally crosslinked at 60 and 70 °C) and in Fig. 3 (films thermally crosslinked at 80 and 90 °C). Mass increment of films is associated with their capacity to take in water during the solubility test. From graphic records it is obvious that capacity of films to take in water is markedly influenced by their previous thermal crosslinking. Fig. 2 shows an obvious difference between results of thermal crosslinking of films at 60 °C and 70 °C. Films thermally annealed at 60 °C swelled in short times during solubility tests. In the solubility test at 25 °C, it was 2 hours in which a sevenfold increment in film mass was recorded, and in the solubility test at 37 °C it was 1 hour in which a 3.88 multiple increment in film mass was recorded. During longer times, gradual disintegration of films occurred. On the contrary, films thermally annealed at 70 °C swelled considerably longer and also greater film mass increments were recorded. In the solubility test at 25 °C, film samples exhibited swelling even after 16 hours with mass increment also greatest – 10.42 times. Disintegration of film already took place during this time. In the solubility test at 37 °C, film samples displayed greatest swelling after 2 hours, when a 12.67 multiple increment of film mass was recorded, and after 8 hours it was still a 9.73 multiple increment of mass. Disintegration of film already occurred during this time. Fig. 3 indicates mass increments of film thermally crosslinked at higher temperatures and it is obvious from graphic records that the difference between thermal annealing at 80 °C and 90 °C is in no way essential. Despite that, a substantial difference is apparent on comparison with films thermally annealed at 60 and 70 °C. Films thermally annealed at 80 and 90 °C swelled for considerably longer times. In solubility tests at 25 °C, these films were still swelling after 190 hours, their mass increment being the greatest just after this time: 11.26 multiple (films thermally annealed at 80 °C) and 9.77 multiple (films thermally annealed at 90 °C). In the solubility test at 37 °C, films swelled even longer – 334 hours, with mass increment again being maximum – 12.54 multiple with films thermally annealed at 80 °C and 11.65 multiple with films thermally annealed at 90 °C. Fig. 4 compares surface dimensions of film sample before the solubility test and swelled sample after solubility test.

TABLE II
Results of Film Solubility Tests at Dissolution Temperature 25 °C

Thermal crosslinking of film samples

	60 °C		70 °C		80 °C		90 °C	
τ (hr)	I	X (%)	I	X (%)	I	X (%)	I	X (%)
1	5.02	32.2	6.48	31.7	8.96	25.6	7.02	24.8
2	7.00	36.2	7.65	34.9	9.38	27.9	7.15	25.3
4	–	69.4	9.35	37.3	9.45	30.2	8.46	28.6
8	–	74.4	9.42	43.8	9.46	32.4	9.01	31.2
16	–	81.1	10.42	50.1	9.49	35.1	9.20	33.9
30	–	82.3	–	66.8	9.88	37.9	9.28	36.3
66	–	84.4	–	78.9	10.89	41,2	9.28	39.4
120	–	88.8	–	81.3	11.04	45.4	9.37	43.6
136	–	92.6	–	82.6	11.12	47.3	9.59	45.4
190	–	95.3	–	85.3	11.26	50.5	9.77	48.1
334	–	98.4	–	88.4	–	61.9	–	59.1
400	–	98.9	–	89.6	–	66.7	–	63.2

τ … time of dissolution

I … mass increment of film sample after solubility test

X … dissolved fraction of film sample after solubility test

TABLE III
Results of Film Solubility Tests at Dissolution Temperature 37 °C

Thermal crosslinking of film samples

	60 °C		70 °C		80 °C		90 °C	
τ (hr)	I	X (%)	I	X (%)	I	X (%)	I	X (%)
1	3.88	39.2	11.35	38.1	9.01	28.9	7.75	26.3
2	–	45.7	12.67	40.8	9.12	31.8	7.96	28.0
4	–	71.3	11.25	45.5	9.32	33.1	8.52	30.7
8	–	77.8	9.73	47.8	9.48	35.6	9.07	33.9
16	–	84.2	–	52.2	9.59	39.8	9.42	37.8
30	–	91.4	–	71.0	9.69	44.5	9.81	40.2
66	–	96.1	–	85.6	10.05	48.2	10.02	44.8
120	–	99.7	–	87.5	11.75	57.9	10.51	52.0
136	–	100	–	88.2	11.79	59.3	10.89	53.9
190	–		–	89.7	11.81	60.8	11.05	56.8
334	–		–	90.5	12.54	64.0	11.65	60.4
400	–		–	92.3	–	68.3	–	63.6

τ … time of dissolution

I … mass increment of film sample after solubility test

X … dissolved fraction of film sample after solubility test

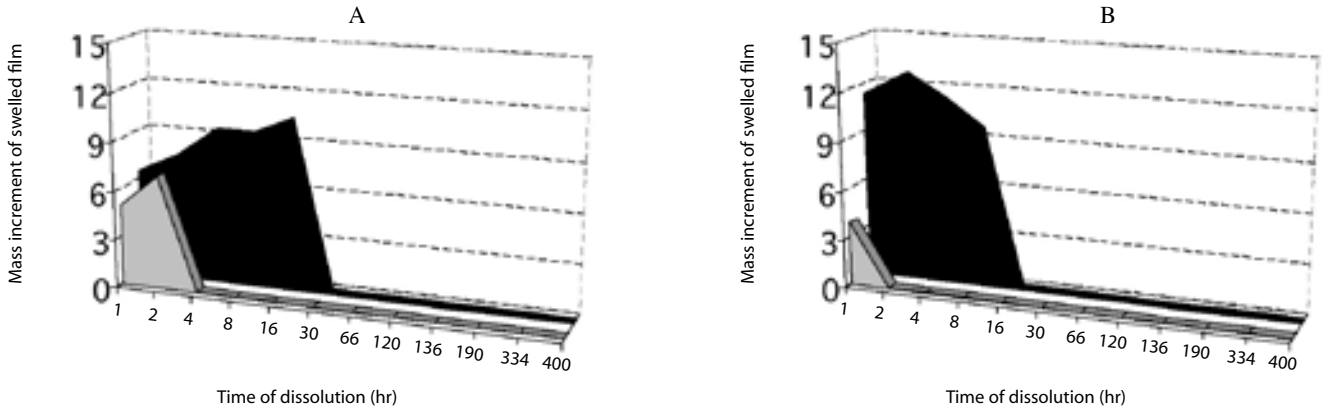

Figure 2: Mass increment of swelled film samples thermally crosslinked at 60 (grey color) and 70 °C (black color) after solubility test: A–solubility test at 25 °C; B–solubility test at 37 °C.

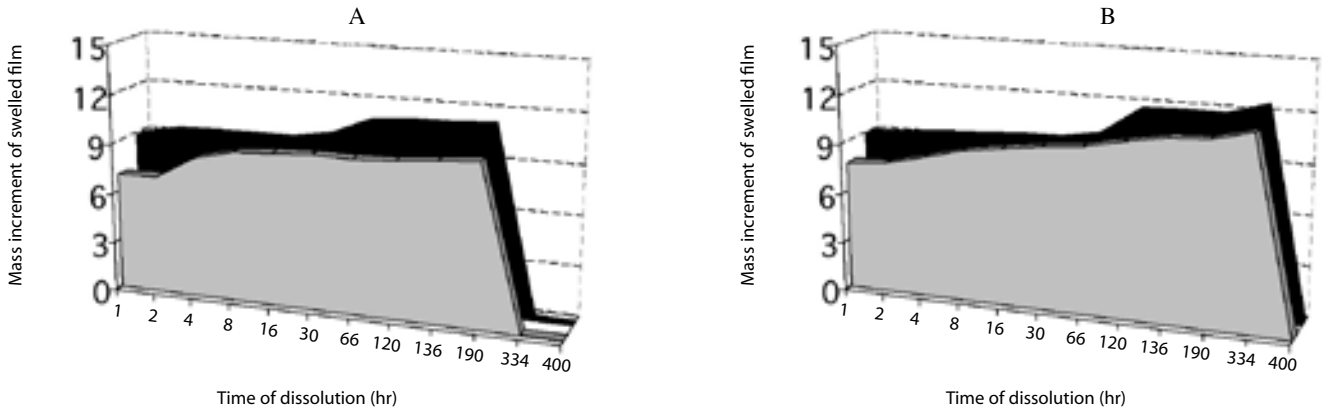

Figure 3: Mass increment of swelled film samples thermally crosslinked at 80 (black color) and 90 °C (grey color) after solubility test: A–solubility test at 25 °C; B–solubility test at 37 °C.

Figure 4: Swelled film sample after solubility test (left) and film sample before solubility test (right).

Curves of film solubility are indicated in Fig. 5 (solubility test at 25 °C) and Fig. 6 (solubility test at 37 °C). From the course of curves it is obvious that solubility of films is strongly influenced by their previous thermal crosslinking, with that allowing separating the film dissolution rates into two groups. The first comprises films thermally annealed at 60 and 70 °C. At the beginning of solubility tests there is no essential difference between films so treated. In the solubility test at 25 °C (see Fig. 5), 32.2 percent film (thermally annealed at 60 °C) and 31.7 percent film (thermally annealed at 70 °C) dissolved in 1 hour of dissolving. But a longer dissolution time was already marked by the influence of previous thermal annealing of films due to which film cross-linking occurred. After 16 hours, for example, 81.1 percent film thermally annealed at 60 °C dissolved, whereas only 50.1 percent film thermally annealed at 70 °C. After 400 hours, almost 99 percent film thermally annealed at 60 °C dissolved, and almost 90 percent film thermally annealed at 70 °C.

In the case of solubility tests at 37 °C (see Fig. 6), percentage of dissolved film was higher than with films dissolved at 25 °C. Films thermally annealed at 60 °C completely dissolved after 136 hours, while with films thermally annealed at 70 °C the quantity dissolved after that time was approx. 88 percent. Solubility curves of films thermally annealed at 80 and 90 °C exhibit merely slight differences under different dissolution conditions (temperatures 25 and 37 °C) (see Figs. 5 and 6). Films thermally annealed at 90 °C dissolved somewhat more slowly than films thermally annealed at 80 °C. However, a substantial difference in

dissolution course is obvious on their comparison with films thermally annealed at 60 and 70 °C. For example, the solubility test at 37 °C (see Fig. 6). After 4 hours of dissolving, dissolved quantities were approx. 71 percent film thermally annealed at 60 °C, 45.5 percent film thermally annealed at 70 °C, 33 percent film thermally annealed at 80 °C and 31 percent film thermally annealed at 90 °C. After 136 hours of dissolution, dissolved quantities were 100 percent film thermally annealed at 60 °C (dissolved completely), approx. 88 percent film thermally annealed at 70 °C, 59 percent film thermally annealed at 80 °C and almost 54 percent film thermally annealed at 90 °C. After 400 hours, the quantities were approx. 92 percent film thermally annealed at 70 °C, 68 percent film thermally annealed at 80 °C and almost 64 percent film thermally annealed at 90 °C.

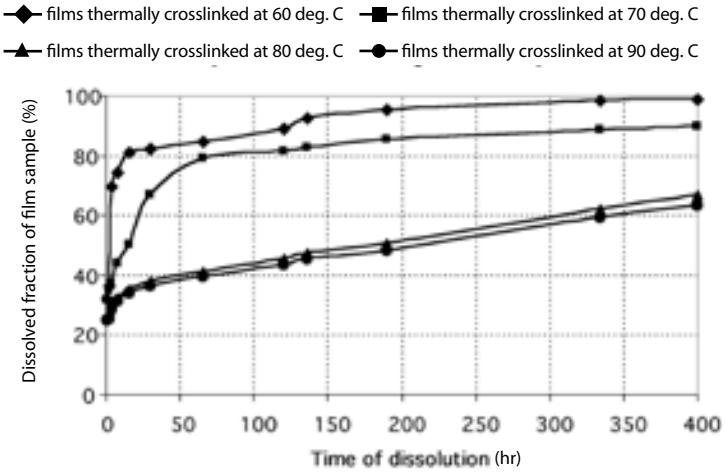

Figure 5: Solubility of films in solubility test at 25 °C.

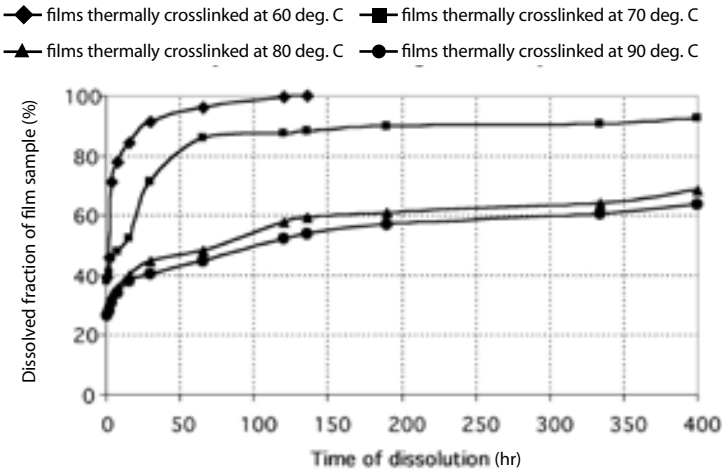

Figure 5: Solubility of films in solubility test at 37 °C.

CONCLUSION

This work studied the influence of heat history of films on their solubility. Films were prepared by casting from a 30 percent solution of collagen hydrolysate with 5 percent added glycerol and 10 percent added starch dialdehyde. Prepared films were subjected to the action of heat at 60, 70, 80 and 90 °C for 24 hours, producing further film cross-linking. The effect under study was influence of thermal crosslinking on film dissolution rate. Films were dissolved by immersion in water at temperatures of 25 and 37 °C. After dissolving, the sample of film was separated. In case the sample remained compact and swelled, the increment in film mass was determined (swelling degree, through changed surface dimensions). Film sample was then dried to constant mass at 103 °C and percentage of dissolved fraction was determined by means of gravimetric analysis. From test results it is obvious that additional thermal annealing significantly affects the swelling degree of prepared film and thus also its solubility. Greatest differences between solubility curves were recorded in thermal annealing at 60, 70 and 80 °C. It was also found that up to a certain dissolution time, increasing thermal annealing increases capacity of the sample to absorb water, which in several cases showed through an increment up to twelvefold in sample mass. After dissolution for 400 hours at 25 °C, almost 99 percent film thermally annealed at 60 °C dissolved, approx. 90 percent film thermally annealed at 70 °C, almost 67 percent film thermally annealed at 80 °C and merely 63 percent film thermally annealed at 90 °C dissolved.

Results show that collagen hydrolysate obtained by enzymatic hydrolysis of waste chrome-tanned shavings, following certain modification by plasticizer and cross-linking agent, is able to form films whose properties can be additionally modified by thermal annealing. A suitable cross-linking degree enables to control the dissolution rate of films, foils and (micro) capsules. Films meet with wide application chiefly in the field of packing materials. Solubility curves are important for applying biodegradable films in practice, especially for modeling rate of releasing the active component(s) from such films. It is important for maintaining the required concentration of active substance, for example, of farming chemicals (fertilizers, insecticides, pesticides, fungicides, herbicides and others) in soil, or of products for domestic and industrial detergents and disinfectants.

Preliminary results of laboratory experiments which have not been finished yet proved that collagen hydrolysate mixed with plasticizer and cross-linking agent to obtain dough-like material can be processed into biodegradable packaging also by procedures known in thermoplastic processing which combine the effect of temperature and pressure.

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LEGEND

D	dry matter content (%),
f	conversion coefficient to zero humidity,
I	mass increment of film sample after solubility test,
m_1	weighed sample of film for solubility (g),
m_2	dry matter of weighed sample of film for solubility (g),
m_3	mass of swelled film sample after solubility test (g),
m_4	dry matter of non-dissolved film sample after solubility test (g),
n_1	weighed sample before drying (g),
n_2	weighed sample after drying (g),
τ	time of dissolution (hr),
X	dissolved fraction of film sample after solubility test (%).