Modelling the Solubility of Films Prepared from Collagen Hydrolysate

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Abstract

The work deals with modeling the solubility of biodegradable films prepared from collagen hydrolysate. To this purpose, we studied the effect of added plasticizer, cross-linking agent or additional thermal annealing on the dissolution rate of films; experiments were planned as factorial tests of the 2^3 type. The basis for preparing films was a 15% (w/v) solution of hydrolysate with various additions of plasticizer - glycerol (GLY) in quantities of 0, 5 and 10% (related to hydrolysate dry matter, w/w), cross-linking agent - dialdehyde starch (DAS) in quantities of 0, 4 and 8% (related to hydrolysate dry matter, w/w).

Films were prepared by casting solution on silicone plates with subsequent evaporation of solvent (water) in 72 hours at 35°C. A part of the films was subjected to additional thermal annealing at 62.5 and 90°C. Solubility tests on films were performed in water at 25°C. The percentage of dissolved film was determined through gravimetry.

The results of film solubility were assessed statistically and graphic models were produced. It was found that added dialdehyde starch and additional thermal annealing very prominently affect the course of film dissolution. Qualities further studied with prepared films were thermal properties by DSC and TGA techniques.

INTRODUCTION

Industrial utilization of collagen is very wide. The greatest proportion of collagen proteins obtained from raw hides has so far, been processed by the tanning industry into leathers. Great attention has been given to using atelocollagen proteins in humane medicine for producing collagen fibres (absorbable surgical stitches), films, foils, membranes, tapes, scaffolds (covering wounds, haemodialysis, substitute tendons), sponges, fleece (medical tampons, laparotomy waddings, medicine dosage reservoirs), tubes (prosthetic tubes, surgery of hollow organs), powders (haemostatic agent), soluble collagen (injectable collagen, drug carrier), gels (vitreous material substitute, moisturizing agents), and finally for producing collagen joint preparations.[1-6]

In the food industry, edible sausage casings are made from loosened-fibre collagen mass; collagen is further employed in the form of powders or capsules as a food complement, for regulating the viscosity of drinks, fibrous collagen is used to impart a desired shape and structure to meat products and powdered collagen to modify the structure of bakery products.[7-11]

A considerable proportion of collagen is consumed in the manufacture of food gelatins that have a number of functional properties (as gel and mousse, thickening agent, emulsifier, stabilizer, protective colloid). Gelatin is also used to produce (transparent and flexible) foodstuff films and foils (made by casting) and coats (made by spraying, dipping or pouring). In the pharmaceutical industry, gelatin is utilized to manufacture hard (HGC) or soft (SGC) capsules, tablets or microcapsules. Gelatin is also applied in the photographic industry (carrying emulsions of silver halides) and in technical applications (adhesives, coatings and paintings, protective colloids, perchings, finishes, anticorrosive coatings, match production.[12-16]

Collagen hydrolysate is denatured and partly hydrolysed protein. Collagen hydrolysates may be prepared through acid hydrolysis (mostly dilute H_2SO_4, HCl or H_3PO_4), alkaline hydrolysis (for example NaOH, KOH or $Ba(OH)_2$), enzymatic hydrolysis (using alcalases) or microbial breakdown. The procedure particularly favored lately is enzymatic hydrolysis in which few side products are created, and is the least energy demanding technique for producing collagen hydrolysates.[17-25] Collagen hydrolysates display properties that depend on the raw material source and processing method. Suitably selecting the method of hydrolysis and technological conditions particularly, enables us to regulate ash contents and molecular weight of hydrolysates. Alkaline or enzymatic hydrolysis allows preparation of collagen hydrolysate possessing molecular weights of 10-50kDa. Acid hydrolysis mostly leads to hydrolysates of low molecular weight (up to 1kDa) which is required, for example, in production of detergents.[26-29]

When collagen hydrolysates are made from microbiologically acceptable raw material sources of native collagen (hides, bones, cartilages, tendons, intestines, fish and others), the hydrolysates find

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particular use in the food industry (food complements, flavoring mixes, viscosity regulators and drink stabilizers) and in cosmetics (humectants, shampoos and hair conditioners, nail varnishes).

Tanning and leather industries produce a sizable part of collagen wastes in different stages of processing - from untanned wastes (trimmings of hides and white [limed] hides) through tanned wastes (chrome-tanned shavings) to tanned and treated wastes (coloured, finished) such as clicking (offcuts) waste. Collagen hydrolysates prepared from waste raw materials find use in technical applications (liquid and solid growth stimulants, mulching foils, sowing tapes, adhesives, fillers for plastics, rubber compound components, microbiological cultures, ingredients of cleansing agents, manufacture of wood-chip boards.[30-32]

Mechanical and chemical properties of collagen hydrolysates (depending above all on their molecular weight and amino acid composition) are quite poor, which limits their application. Reactive groups of amino acids enable us to modify hydrolysate properties. The methods employed most when chemically modifying hydrolysate properties are cross-linking (particularly employing aldehydes, hexamethylene diisocyanate, epoxides, epichlorhydrin, enzymes), increasing hydrophilicity (incorporating polar groups) or hydrophobicity (incorporating nonpolar groups) of hydrolysate. The most reactive protein groups are those with the amino acids serine, (primary -OH), hydroxyproline (secondary -OH), threonine (secondary -OH), tyrosine (phenolic -OH), aspartic and glutamic acid (-COOH), lysine (-NH_2) and arginine (-C(NH)NH_2).

Concerning physical modification methods, we may mention, for example, adding plasticizers, fillers, effect of temperature, UV radiation, gamma radiation, influence of pressure.[33-43] Hydrolysate properties to be modified in the first place are: solubility, adhesion to various substrates, mechanical properties, barrier properties, sensitivity to water, and also processing properties.[44,45]

The objective of this work was to prepare biodegradable films from collagen hydrolysate and model film dissolution by altering the addition of plasticizer and cross-linking agent and by additional thermal annealing of films. Experiments were planned by factorial tests and the data obtained were evaluated statistically.

EXPERIMENTAL PROCEDURE

Powder collagen hydrolysate obtained by enzymatic hydrolysis of chrome-tanned shavings was delivered by the Stospol Co., Valasske Mezirici (The Czech Republic). The hydrolysate was kept in a glass-stoppered bottle and its composition is: dry matter = 88.9%, number average relative molecular mass (M_n) = 16.9kDa; on dry matter: inorganic solids = 4.2%, total Kjeldahl nitrogen = 15.3%, chromium = 139ppm and -NH_2 groups = 0.34mmol g^{-1}. Chemicals: dialdehyde starch (CAS No 9047-50-1) supplied by Monomer-Polymer and Dajac Labs Inc. (Feasterville, USA); glycerol (CAS No 56-81-5) supplied by Sigma Aldrich Co., (St. Louis, USA); sodium hydroxide of analytical grade (CAS No 95077-05-7) supplied by Petr Lukes Co. (The Czech Republic).

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), analytical balance KERN 440, electronic balance KERN 770 (Germany), pH-meter Picollo HI 1280 (Germany), thickness meter TGL 7682-1 (Germany), silicone plate 270 x 210mm (Tescoma, The Czech republic), filter paper Filpap KA-1 (The Czech Republic), differential scanning calorimeter DSC 2010 (TA Instruments, New Castle, USA), thermogravimetric analyzer TGA Q500 (TA Instruments, New Castle, USA).

Planning and evaluating experiments

In order to assess the influence of selected parameters acting during preparation of biodegradable films on film solubility, experiments were planned utilizing factorial tests of the 2^3 variety (3 examined factors on two levels – minimal and maximal) with one repetition in the middle of experiment.

Factors studied were: Factor A = glycerol (GLY) added to the mixture used for preparing films (related to hydrolysate dry matter, w/w): bottom limit 0%, upper limit 10%. Factor B = dialdehyde starch (DAS) (related to hydrolysate dry matter, w/w): bottom limit 0%, upper limit 8%. Factor C = thermal annealing of films produced: bottom limit 35 ± 0.2°C, upper limit 90 ± 0.2°C. The lower limit of thermal annealing corresponds to the temperature at which films were prepared (see *'Procedure for preparing biodegradable films*'); in another part of this contribution such films are designated 'thermally unexposed'. Organization of factorial tests is surveyed and summarized in Table I. Modelling of film dissolution was carried out using the statistical program Statgraphics.[46]

TABLE I
Conditions of film preparation–organization of factorial tests

Film No.	Monitored factors		
	Factor A added glycerol (%, w/w)*	Factor B added dialdehyde starch (%, w/w)*	Factor C thermal annealing (°C)**
1	0	0	35
2	0	0	90
3	0	8	35
4	0	8	90
5	5	4	62.5
6	10	0	35
7	10	0	90
8	10	8	35
9	10	8	90

*Note: related to dry matter of hydrolysate
**Note: symbol 35 indicates thermally unexposed films

Procedure for preparing biodegradable films

Powder hydrolysate was weighed into a 500mL beaker in an amount that corresponds to 40g dry matter. Further additions were 267mL water and

plasticizer – in the case of 5% (w/w) addition it was 2g GLY and in the case of 10% (w/w) addition it was 4g GLY. The beaker containing the mixture was placed over a magnetic stirrer with temperature control. The mixture was stirred at 60 ± 0.5°C until the hydrolysate had completely dissolved and then for another 10 minutes. Solution pH was adjusted to 11 ± 0.2 by adding 11mL 5N NaOH and the mix was further stirred for 15 minutes. Then (with temperature held at 60 ± 0.5°C) the cross-linking agent was added – in the case of 4% (w/w) addition it was 1.6g DAS and in the case of 8% (w/w) addition it was 3.2g DAS. The cross-linking agent was added slowly in small doses over 5min and mixture was then stirred for 1 hour. After this time the mix was cooled with constant stirring (5 min) to 25 ± 2°C. The solution was subsequently cast onto a silicone plate (measuring 270 x 210mm) and the film was produced on evaporation of solvent (water) in a drier – without air circulation – at 35 ± 0.2°C over 72 hours. Test samples were prepared from these films, measuring 2 x 2cm and subsequently conditioned for 24 hours in a desiccator over dried silica gel at room temperature (22 ± 2°C).

Thermal annealing of biodegradable films

Samples of film were placed on Petri dishes and thermally annealed for 24 hours in a drier (without air circulation) at 62.5 ± 0.2°C and 90 ± 0.2°C. After this treatment, film samples were put for 24 hours in a desiccator containing dried silica gel at room temperature. Dry matter of samples was always determined with 3 thermally unexposed and thermally annealed films by drying at 103 ± 1°C for 24 hours; arithmetical mean was calculated, standard deviation ranged within ± 0.2%. Thickness of film samples was also measured.

Tests of film solubility

Samples of film were tested for solubility in water at 25 ± 0.1°C. This test was executed in tanning dishes where samples were immersed in 50mL water. When the dissolution test was finished, samples of film were filtered under slight vacuum on a Büchner funnel through low-density filter paper. The undissolved fraction of film sample on filter paper (in tanning dish) was dried at 103 ± 1°C to constant weight. Each test of solubility was performed threefold and the arithmetic mean calculated, standard deviation ranged within ± 3.5%.

Note: *Tanning dishes are glass dishes 66mm diameter and 29mm high with ground glass lids.*

Thermal study of films

Temperature co-ordinates of characteristic peaks and mass loss were determined by differential scanning calorimetry in open aluminum crucibles and by thermogravimetric analysis in open platinum crucibles. In both cases a quantity of approx 6mg of film was weighed into the crucible and measurements were conducted under a nitrogen atmosphere at a flow rate of 60mL min^{-1} over a temperature interval 20-400°C, dT/dt = 10°C min^{-1}. Each test was performed threefold and arithmetic mean calculated, standard deviation ranged within ± 2.0%.

RESULTS AND DISCUSSION

Films without added plasticizer are fragile and of low flexibility; added plasticizer makes the films more flexible. Prepared films display a characteristic brown-orange color which is produced by added dialdehyde starch. Depending on the added glycerol and dialdehyde starch, the thickness of films ranged from 0.95 to 1.0 mm; dry matter of thermally untreated films was 91.05-93.55%, for films thermally exposed at 62.5°C dry matter was 92.86-95.96%, and with films exposed at 90°C thickness 96.90-99.84%.

When studying thermal properties of films through DSC and TGA technique we focused on 4 characteristic peaks and transitions (see model DSC and TG curve in Fig. 1). The first significant endothermal peak visible on the DSC record (designated T1) is related to evaporation of remnant sorbed water from the sample of film (T_{SW}). Another important transition on the DSC record is the S-shaped transition (T2) attributed to the glass transition temperature (T_g). The second endothermal peak, clearly distinct on the DSC record (T3), is associated with melting of the sample (T_m). The last characteristic peak (T4) is then the start of thermal degradation (T_c).

Results of DSC and TGA measurements of films are arranged in Table II. The minimum of first endothermal peak (T_{SW}) was recorded at temperatures 49-68°C (depending on type of film). At these temperatures the TGA record confirmed a 1.8 to 3-5% loss in film weight (-Δm, see Table II) related to evaporation of remaining moisture.

Glass transition temperatures (T_g) were recorded (depending on type of film) in the 160-185°C range. At temperature T_g a drop of 7.1-9.8% in film weight was recorded (-Δm, see Table II). The level of T_g is particularly affected by added glycerol (GLY) and dialdehyde starch (DAS); thermal annealing of films affects T_g to a lower degree. Increased addition of GLY lowers T_g – see differences in T_g between films Nos. 1 and 2 (T_g = 175 and 176°C) as against films Nos. 6 and 7 (T_g = 160 and 162°C), or films Nos. 3 and 4 (T_g = 184 and 185°C) against films Nos. 8 and 9 (T_g = 165 and 167°C). On the other hand, increased DAS addition is accompanied by a higher T_g. For example, with films Nos. 1 and 2 (0% GLY and 0% DAS) T_g is 175 and 176°C, whereas with films Nos. 3 and 4 (0% GLY and 8% DAS) T_g increased to 184 and 185°C. Similar differences are obvious between films Nos. 6 and 7 as against films Nos. 8 and 9.

Melting temperature of films (T_m) is in the 200-233°C range and the drop in weight at this temperature is 10.0-17.5% (depending on type of film, see Table II). The influence of GLY addition on T_m is insignificant, but with added DAS the T_m level increases by 20-25°C.

That is obvious, for example, in the difference between films Nos. 1 and 2 (0% GLY and 0% DAS), in which T_m is 213 and 209°C, against films Nos. 3 and 4 (0% GLY and 8% DAS) in which T_m increased to 233 and 231°C.

Glass transition temperature and melting temperature are important thermal parameters when processing films through thermoplastic technology (extrusion, compression molding) where the mixture has to be processed at temperatures between T_g and T_m. Depending on film type, the difference between T_g and T_m is 33-66°C, which is sufficient for this processing mode. The start of thermal degradation of films (T_c) was recorded at temperatures ranging from 275 to 298°C, and at these levels a drop of 21.5 to 38.7% in film weight takes place (see Table II).

Overall results of tests on solubility of films prepared under examined conditions are presented in Table III. The statistical significance of studied factors (A – added glycerol, B – added dialdehyde starch and C – thermal annealing of films) on the assessed quantity (% film dissolved) was monitored by the standard Fisher test.[47] Assessed results are given in Table IV, from these results it is obvious that factor A (addition of glycerol) is a statistically insignificant quantity for % dissolved film. Factor B (addition of dialdehyde starch) very significantly affects the % dissolved film at dissolution times up to 240 minutes. Factor C (thermal annealing of produced films) is also a statistically significant factor at dissolution times of up to 240 minutes. Combinations of factors AB and AC appeared in all cases as statistically quite insignificant. Combination of factors BC was significant after dissolution times of 240 minutes, after dissolution lasting 120 minutes it was on the limit of significance. At dissolution times longer than 1920 minutes the values of the F-test for all factors under study (A, B, C) and their interactions (AB, AC, BC) are smaller than 6.35 and, therefore, statistically insignificant for the amount of dissolved film (see Table IV).

Models indicating the amount of dissolved film at selected dissolution times (5, 15, 30, 60, 120, 240) were made by evaluating measured data in the statistical program Statgraphics – see contour graphs in Figures 2-7. Contour graphs indicate influences of statistically significant factors (axis x = factor B – added dialdehyde starch, axis y = factor C – thermal annealing of produced films) on amount of dissolved film, and that always with minimal and maximal addition of plasticizer – glycerol (factor A).

Figure 2 indicates the amount of dissolved film when prepared without added glycerol (Fig. 2a) and with 10% added glycerol (Fig. 2b) after 5 minutes dissolution. It is obvious from graphs that suitably combined additions

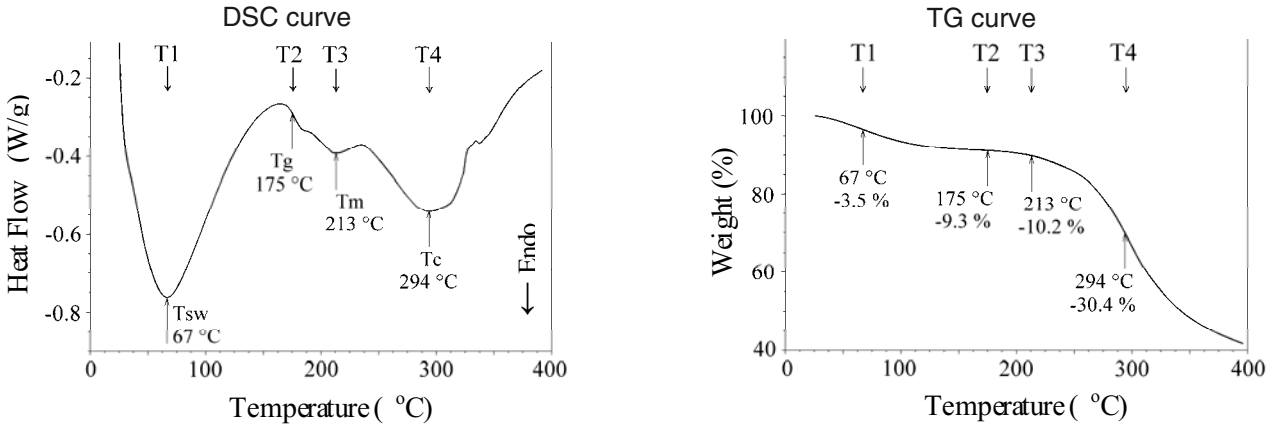

Figure 1. Model DSC and TG curve of film from collagen hydrolysate

TABLE II

Results of thermal analysis of films from collagen hydrolysate. Temperature co-ordinates of detected endothermal DSC peaks and transitions (T, °C) and related average cumulated mass loss on TG curves (–Δm, mass %)

Film No.	T1		T2		T3		T4	
	T_{SW} (°C)	–Δm (mass %)	T_g (°C)	–Δm (mass %)	T_m (°C)	–Δm (mass %)	T_c (°C)	–Δm (mass %)
1	67	3.5	175	9.3	213	10.2	294	30.4
2	68	2.8	176	7.6	209	10.0	293	29.0
3	50	3.4	184	9.8	233	13.6	280	22.9
4	56	2.3	185	8.4	231	11.2	278	21.5
5	52	2.4	172	8.5	223	13.5	275	23.5
6	49	2.3	160	8.5	206	14.7	298	38.7
7	51	2.0	162	7.3	200	12.5	297	37.4
8	50	2.2	165	9.0	231	17.5	276	26.0
9	54	1.8	167	7.1	224	13.4	275	22.0

T1, T2, T3, T4 – characteristic peaks and transitions of film on DSC record
T_{SW} – temperature related to evaporation of remaining sorbed water from film
T_g – glass transition temperature of film
T_m – melting temperature of film
T_c – start of thermal degradation of film

TABLE III
Percentage of film dissolved in water at 25°C*

Film No.	Time of dissolution (min) 5	15	30	60	120	240	480	960	1920	3840	7680	15360	30720
1	78.9	97.7	—	—	—	—	—	100	—	—	—	—	—
2	69.0	91.5	97.6	98.6	—	—	—	100	—	—	—	—	—
3	54.1	79.7	91.5	95.0	97.5	98.0	98.4	98.8	99.5	99.7	—	100	—
4	23.3	30.5	35.9	47.2	58.1	65.4	76.7	83.1	95.0	98.0	98.7	99.0	100
5	61.7	86.7	92.5	95.6	98.0	98.6	98.8	99.5	—	—	—	100	—
6	74.6	92.7	98.8	—	—	—	—	100	—	—	—	—	—
7	64.7	88.5	94.1	96.8	99.0	—	—	100	—	—	—	—	—
8	44.8	72.4	80.6	90.8	96.0	97.7	98.2	98.3	98.8	99.6	—	100	—
9	15.3	20.2	33.2	45.8	55.0	60.9	65.3	77.5	84.1	90.9	96.2	96.6	97.1

*Note: related to weighed-in dry matter of film sample for the solubility test

TABLE IV
Results of analysis of the Fisher test for statistical significance of studied factors and their interactions for percentage of dissolved film

Monitored factors	Time of dissolution (min) 5	15	30	60	120	240	480	960	1920-30720
A	2.54	0.80	0.47	0.12	0.09	0.09	0.64	0.39	
B	84.70*	34.35*	31.54*	29.14*	24.99*	23.70*	17.80	18.93*	values less
C	24.25*	15.28	17.15	20.33*	19.39*	18.76*	14.08	14.10	than 6.35
AB	0.29	0.11	0.11	0.03	0.04	0.09	0.64	0.39	
AC	0.01	0.00	0.05	0.00	0.02	0.07	0.59	0.28	
BC	6.20	10.13	13.01	16.67	18.45	18.76*	14.08	14.10	

$F_{crit}^{95\%}{}_{(1;2)} = 18.51$
*Note: statistically significant factor

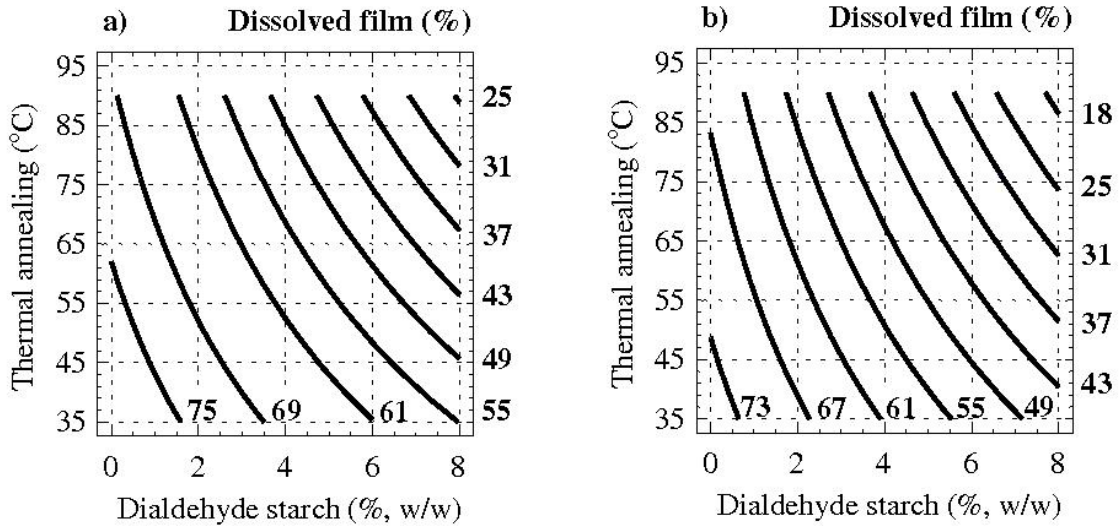

Figure 2. Contour graph of dissolved film (%) after 5 minutes dissolution: a) film without glycerol; b) film with 10% glycerol (w/w).

of dialdehyde starch (0-8%, w/w) together with thermal annealing (35-95°C) allow us to model dissolution of films in such manner that their solubility falls within very wide limits: 25-75% dissolved film (film without added GLY, Fig. 2a) or 18-73% dissolved film (film with 10% added GLY, Fig. 2b). With maximal addition of DAS and maximal thermal treatment, the percentage of dissolved film is 3-4 times smaller (depending on GLY addition) than when mentioned factors were at their minimum.

A very similar dependency of the amount of dissolved film on DAS addition and thermal annealing may be traced after 15min dissolution (Fig. 3).

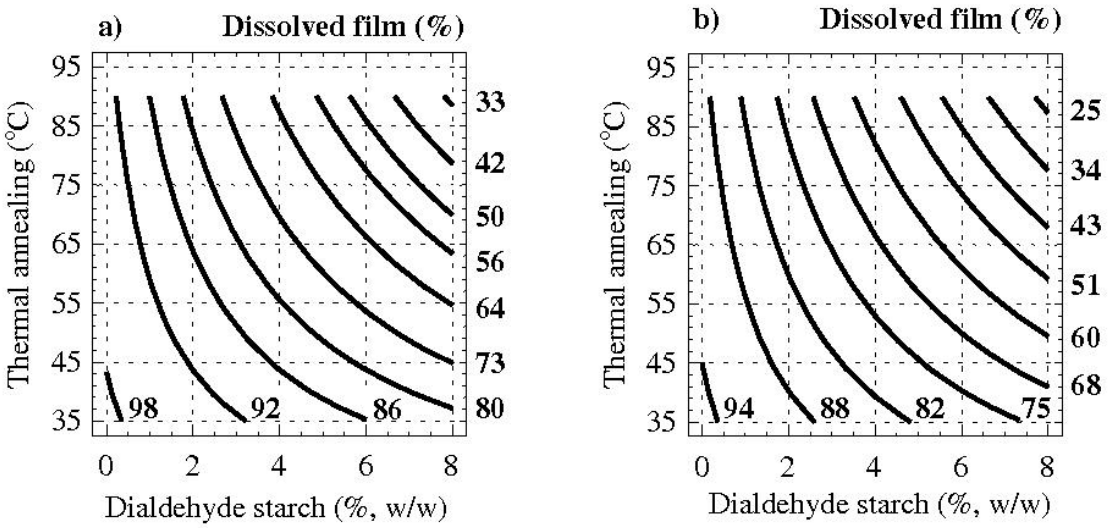

Figure 3. Contour graph of dissolved film (%) after 15 minutes dissolution:
(a) Film without glycerol, (b) film with 10% glycerol (w/w).

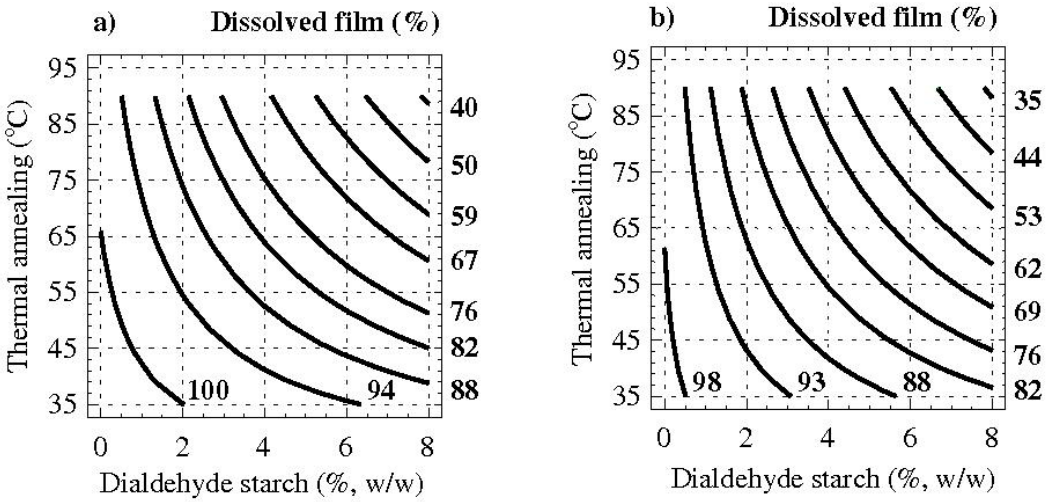

Figure 4. Contour graph of dissolved film (%) after 30 minutes dissolution:
a) film without glycerol; b) film with 10% glycerol (w/w).

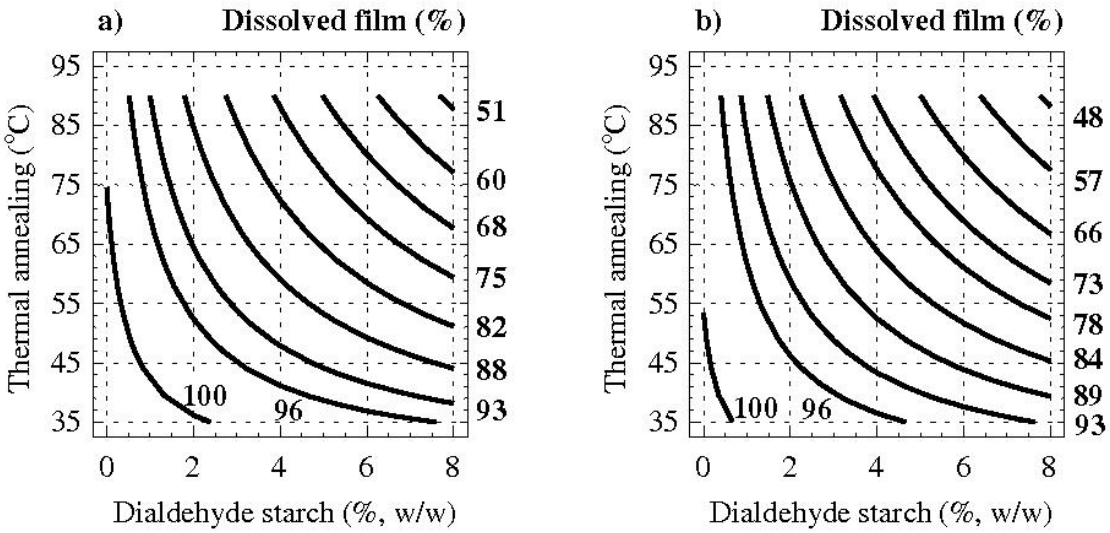

Figure 5. Contour graph of dissolved film (%) after 60 minutes dissolution:
a) film without glycerol; b) film with 10% glycerol (w/w).

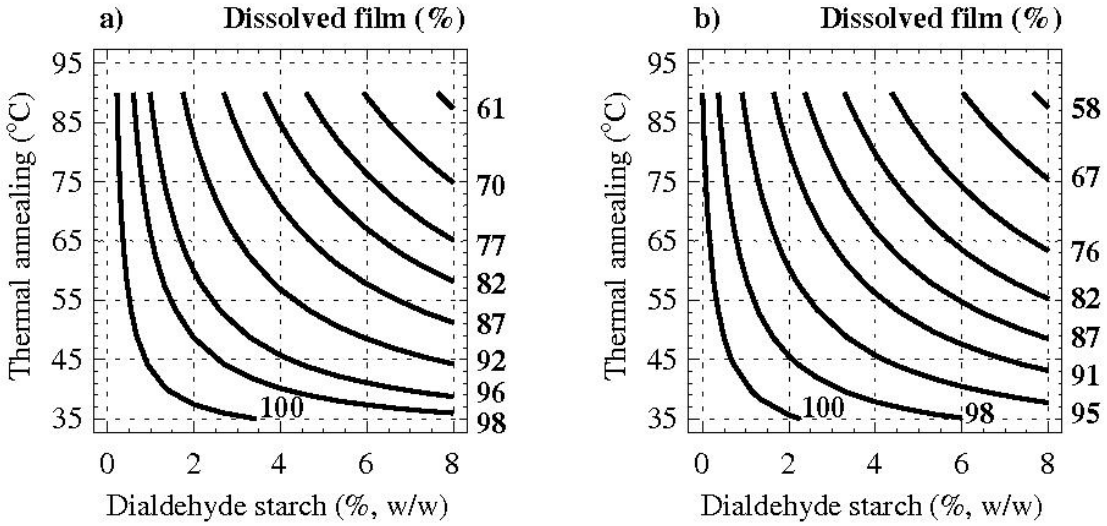

Figure 6. Contour graph of dissolved film (%) after 120 minutes dissolution:
a) film without glycerol; b) film with 10% glycerol (w/w).

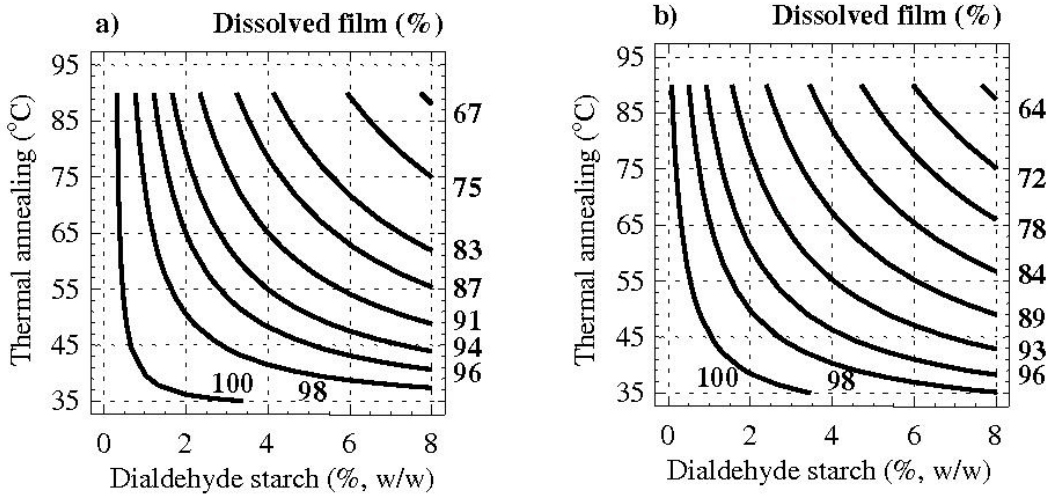

Figure 7. Contour graph of dissolved film (%) after 240 minutes dissolution:
a) film without glycerol; b) film with 10% glycerol (w/w).

Considering film without GLY (Fig. 3a), it can be seen that films with no added DAS and thermally unexposed almost completely (98%) dissolved in this time. On the contrary, with an 8% DAS addition and thermal annealing at 90°C, film solubility was reduced almost three-fold. A similar tendency concerning the amount of dissolved film is apparent with films containing 10% added GLY (Fig. 3b).

After longer dissolution times (30, 60, 120 and 240 minutes – Figures 4-7) the amount of dissolved film is higher. Despite that, a marked influence of DAS addition and thermal annealing is again obvious. After 30min dissolution (Fig. 4), conveniently combining added DAS and thermal annealing allows us to model the amount of dissolved film in a 40-100% interval (film without added GLY, Fig. 4a) or in a 35-98% interval (film containing 10% added GLY, Fig. 4b). After 60min dissolution (Fig. 5), the interval in which combining DAS addition with thermal exposure allows to model the amount of dissolved film is reduced to about 50-100%. After 120 minutes dissolution (Fig. 6), the amount of dissolved film increases but nevertheless may be reduced 1.5 times through higher DAS additions and higher thermal annealing. After 240 minutes exposure (Fig. 7), films with low DAS additions (up to about 4%) and low levels of thermal annealing (up to about 60°C) are dissolved to an extent more than 90%. When both studied factors are at their maximum, the amount of dissolved film is in the vicinity of 65%.

CONCLUSIONS

A 15% (w/v) solution of collagen hydrolysate (produced by enzymatic hydrolysis of chrome-tanned shavings) served to prepare biodegradable films by casting (35°C, 72 hours).

Factors under study were influence of added plasticizer (glycerol 0-10%, w/w), cross-linking agent (dialdehyde starch, 0-8%, w/w) and additional thermal annealing (62.5 and 90°C, 24 hours) on dissolution rate of films in water at 25°C. Amount of dissolved film was evaluated by gravimetry.

Evaluating statistical significance of factors under study (A – addition of glycerol, B – addition of dialdehyde starch and C – thermal annealing of produced films) on percentage of dissolved film by employing the Fisher test showed that addition of dialdehyde starch and additional thermal annealing of films quite prominently influence the dissolution course of these. Mutually combining these two factors, particularly with shorter dissolution times (up to 30 minutes), we may slow down (or accelerate) film dissolution rates threefold to fourfold.

Graphic models of film dissolution were produced by applying the statistical program Statgraphics.

Modeling dissolution rate of films produced (not only) from collagen hydrolysate in chosen media has great importance for their practical application. This path opens new possibilities for utilizing collagen hydrolysates, for example, for creating ways of oral or anal medicine dosage or feed complements in veterinary practice, or use in chemical industry as packing, for instance for stabilizers, catalysts, flame retarders, hardening agents, dyes, pigments, detergents, aromatic components, anticorrosive additives and others.

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References

1. Ambrosio, L., Netti, P., Santaniello, B. *et al.,* Composite Materials as Scaffolds for Tissue Engineering. *In* Chiellini, E., Sunamoto, J., Migliaresi, C. *et al.,* eds., Biomedical Polymers and Polymer Therapeutics. 1st ed., Kluwer Academic/Plenum Publishers, New York, 227-233, (2001).
2. Mora, P. C. and Baraldi, P. G., Dermocosmetic Applications of Polymeric Biomaterials. *In* Dumitru, S., ed., Polymeric Biomaterials. 2nd ed., CRC Press, New York, 459-490, (2001).
3. Brodsky, B., Werkmeister, J. A. and Ramshaw, J. A. M., Collagens and Gelatine. *In* Fahnenstock, S. R. and Steinbüchel, A., eds., Biopolymers: Volume 8 - Polyamides and Complex Proteinaceous Materials II. 1st ed., Wiley-VCH Verlag GmbH, Weinheim, 119-153, (2003).
4. Sander, E. A. and Barocas, V. H., Biomimetic Collagen Tissues: Collagenous Tissue Engineering and Other Applications. *In* Fratzl, P., ed., Collagen: Structure and Mechanics. 1st ed., Springer Science + Business Media, New York, 476-505, (2008).
5. Urry, D. W., McPherson, D. T., Xu, J., Gowda, D. C. and Parker, T. M., Elastic and Plastic Protein-Based Polymers: Potential for Industrial Uses. *In* Gebelein, Ch. G. and Carraher, Ch. E., eds., Industrial Biotechnological Polymers. 1st ed., Technomic Publishing Company, Lancaster, 259-280, (1995).
6. Li, S. T., Biologic Biomaterials: Tissue-Derived Biomaterials. *In* Park, J. B., and Bronzino, J. D., eds., Biomaterials: Principles and Applications. 1st ed., CRC Press, Boca Raton, 117-139, (2002).
7. Djagny, K. B., Wang, Z., and Xu, S., Gelatin: A Valuable Protein for Food and Pharmaceutical Industries: Review. *Crit. Rev. Food Sci. Nutr.*, 2001, **41**, 481-493.
8. Henrickson, R. L., Hide Collagen as a Food. *J. Amer. Leather. Chem. Ass.,* 1980, **75**, 464-470.
9. Kaplan, D. J., Mayer, J. M., Ball, D. *et al.,* Fundamentals of Biodegradable Polymers. *In* Kaplan, D. L., Thomas, E. L., and Ching, C. T. K., eds., Biodegradable Polymers and Packaging. 1st ed., Technomic Publishing Company, Lancaster, 1-42, (1993).
10. Miller, A. T., Current and Future uses of Limed Hide Collagen in the Food Industry. *J. Amer. Leather. Chem. Ass.*, 1996, **91**, 183-189.
11. Dean, K., and Yu, L., Biodegradable Protein-Nanoparticle Composites. *In* Smith, R., ed., Biodegradable Polymers for Industrial Applications. 1st ed., CRC Press, Boca Raton, 289-309, (2005).
12. Friess, W., Collagen - Biomaterial for Drug Delivery. *Eur. J. Pharm. Biopharm.*, 1998, **45**, 113-136.
13. Radwick, A. E. and Burgess, D. J., Proteins as Microencapsulating Agents and Microencapsulated Active Compounds in Pharmaceutical Applications. *In* Gennadios, A., ed., Protein-Based Films and Coatings. 1st ed., CRC Press, Boca Raton, 341-443, (2002).
14. Ockerman, H. W. and Hansen, C. L., Animal By-Product Processing & Utilization, 1st ed., CRC Press, Boca Raton, 205-210, (2000).
15. Schrieber, R. and Gareis, H., Gelatine Handbook: Theory and Industrial Practice, 1st ed., Wiley-VCH Verlag GmbH, Weinheim, 119-299, (2007).
16. Verhe, R., Industrial Products from Lipids and Proteins. *In* Stevens, Ch. and Verhe, R., eds., Renewable Bioresources: Scope and Modification for Non-Food Applications. 1st ed., John Wiley & Sons, Chichester, 208-249, (2004).
17. Aftab, M. N., Hameed, A., Haq, I. *et al.,* Biodegradation of Leather Waste by Enzymatic Treatment. *Chinese J. Process Eng.,* 2006, **6**, 462-465.
18. Gousterova, A., Braikova, D., Goshev, I. *et al.,* Degradation of Keratin and Collagen Containing Wastes by Newly Isolated Thermoactinomycetes or by Alkaline Hydrolysis. *Lett. Appl. Microbiol.*, 2005, **40**, 335-340.
19. Kolomaznik, K., Mladek, M., Langmaier, F. *et al.,* Experience in Industrial Practice of Enzymatic Dechromation of Chrome Shavings. *J. Amer. Leather. Chem. Ass.*, 2000, **95**, 55-63.
20. *Idem,* Collagenous Hydrolysates from Untraditional Sources of Proteins. *Int. J. Cosmet. Sci.*, 2001, **23**, 193-199.
21. *Idem*, Collagenous Hydrolysates from Untraditional Sources of Proteins: Reaction Condition and Yield of Enzymatic Hydrolysis of Short Cattle Tendons. *Int. J. Cosmet. Sci.*, 2001, **23**, 201-206.
22. Mokrejs, P., Janacova, D., Kolomaznik, K. *et al.,* Processing of Solid Leather By-Products from Shoemaking Industry. *Leather Sci. Eng.*, 2007, **17**, 3-9.
23. *Idem,* Processing of Solid Leather By-Products from Shoemaking Industry (To be continued). *Leather Sci. Eng.,* 2007, **17**, 3-7.
24. Mu, C., Lin, W., Zhang, M. *et al.,* Towards Zero Discharge of Chromium-Containing Leather Waste through Improved Alkali Hydrolysis. *Waste Manage.*, 2003, **23**, 835-843.
25. Tahiri, S., Albizane, A., Messaoudi, A. *et al.,* Thermal Behaviour of Chrome Shavings and of Sludges Recovered after Digestion of Tanned Solid Wastes with Calcium Hydroxide. *Waste Manage.*, 2007, **27**, 89-95.
26. Langmaier, F., Mladek, M., Kolomaznik, K. *et al.,* Hydrolysates of Chromed Waste as Raw Materials for the Production of Surfactants. *J. Amer. Leather. Chem. Ass.*, 2002, **39**, 47-51.
27. Mokrejs, P., Sukop, S., Langmaier, F. *et al.,* Properties of Collagen Hydrolysates Obtained from Leather Shavings. *Asian J. Chem.,* 2007, **19**, 1207-1216.
28. Semrl-Kosmac, V., Gantar, A. and Anzur, I., Modification of Collagen Hydrolysates. *Resour. Conserv. Recycl.*, 1995, **13**, 97-104.
29. Zhang, Z., Li, G. and Shi, B., Physicochemical Properties of Collagen, Gelatin and Collagen Hydrolysate Derived from Bovine

Limed Split Wastes. *J. Soc. Leather Technol. Chem.,* 2006, **90**, 23-28.
30. Langmaier, F., Mokrejs, P., Kolomaznik, K. *et al.,* Cross-Linking Epoxide Resins with Hydrolysates of Chrome-Tanned Leather Waste. *J. Therm. Anal. Calorim.*, 2007, **88**, 857-862.
31. Langmaier, F., Kolomaznik, K., Mladek, M. *et al.,* Curing Urea-Formaldehyde Adhesives with Hydrolysates of Chrome-Tanned Leather Waste from Leather Production. *Int. J. Adhes. Adhes.,* 2005, **25**, 101-108.
32. Vasileva-Tonkova, E., Nustorova, M. and Gushterova, A., New Protein Hydrolysates from Collagen Wastes. *Curr. Microbiol.,* 2007, **54**, 54-57.
33. Bigi, A., Bracci, B., Cojazzi, G. *et al.,* Drawn Gelatin Films with Improved Mechanical Properties. *Biomaterials*, 1998, **19**, 2335-2340.
34. *Idem,* Structural and Mechanical Properties of Cross-linked Drawn Gelatin Films. *J. Therm. Anal. Calorim.*, 2000, **61**, 451-459.
35. *Idem*, Stabilization of Gelatin Films by Cross-linking with Genipin. *Biomaterials*, 2002, **23**, 4827-4832.
36. de Carvalho, R. A., and Grosso, C. R. F., Characterization of Gelatin Based Films Modified with Transglutaminase, Glyoxal and Formaldehyde. *Food Hydrocolloids*, 2004, **18**, 717-726.
37. Hara, M., Various Cross-Linking Methods for Collagens: Merit and Demerit of Methods by Radiation. *J. Oral Tissue Eng.*, 2006, **3**, 118-124.
38. Hernandez-Munoz, P., Villalobos, R. and Chiralt, A., Effect of Thermal Treatments on Functional Properties of Edible Films Made from Wheat Gluten Fractions. *Food Hydrocolloids*, 2004, **18**, 647-654.
39. Keyong, T., Jian, C., Jintao, W. *et al.,* Modification of Collagen Hydrolysate by EDAC. *J. Soc. Leather Technol. Chem.*, 2008, **92**, 23-29.
40. Kim, K. M., Weller, C. L., Hanna, M. A. *et al.,* Heat Curing of Soy Protein Films at Atmospheric and Sub-Atmospheric Conditions. *J. Food Sci.: Food Eng. Phys. Prop.*, 2002, **67**, 708-713.
41. Marqui, C., Chemical Reactions in Cottonseed Protein Cross-Linking by Formaldehyde, Glutaraldehyde, and Glyoxal for the Formation of Protein Films with Enhanced Mechanical Properties. *J. Agr. Food Chem.,* 2001, **49**, 4676-4681.
42. Soliman, E. A., Eldin, M. S. M. and Furuta, M., Biodegradable Zein-Based Films: Influence of γ-Irradiation on Structural and Functional Properties. *J. Agr. Food Chem.*, 2009, **57**, 2529-2535.
43. Vaz, C. M., de Graaf, L. A., Reis, R. L. *et al.,* Effect of Cross-linking, Thermal Treatment and UV Irradiation on the Mechanical Properties and *in vitro* Degradation Behavior of Several Natural Proteins Aimed to be Used in the Biomedical Field. *J. Mater. Sci.: Mater. Med.,* 2003, **14**, 789-796.
44. Bucevschi, M. D., Chirita, G., Colt, M. *et al.,* Chemical Modification of Collagen Hydrolyzates. *J. Amer. Leather. Chem. Ass.,* 1999, **94**, 89-95.
45. Langmaier, F., Mokrejs, P., Karnas, R. *et al.,* Modification of Chrome-Tanned Leather Waste Hydrolysate with Epichlorhydrin. *J. Soc. Leather Technol. Chem.*, 2005, **90**, 29-34.
46. Statistical Graphics System – Version: 6.0; Manugistic, Inc. and Statistical Graphics Corporation, USA, 1992.
47. Strange K., Angewandte Statistik: Mehrdimensionale Probleme, 1st ed., Springer Verlag, Heidelberg, (1971).

Legend

DAS	dialdehyde starch
DSC	differential scanning calorimetry
GLY	glycerol
HGC	hard gelatin capsules
SGC	soft gelatin capsules
TGA	thermogravimetric analysis
T1, T2, T3, T4	characteristic peaks and transitions of film on DSC record
T_{SW}	temperature related to evaporation of remaining sorbed water from film
T_g	glass transition temperature of film
T_m	melting temperature of film
T_c	start of thermal degradation of film
$-\Delta m$	cumulated mass loss of film on TG curve