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Heat-treated biodegradable films and foils of collagen hydrolysate crosslinked with dialdehyde starch

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Abstract Gels of collagen hydrolysate (H) crosslinked with dialdehyde starch (DAS) are marked by a strong tendency to aging, which means a certain problem during their processing into biodegradable packaging materials. Applying casting technology and drying these materials by heating air-dry films and foils for a limited time (1–4 h) at 105 °C may eliminate the aging problem. Solubility of heat-treated films in an aqueous environment remains preserved, but depending on how long this temperature acts and on the DAS content in the film, time of film disintegration prolongs from 1–1.5 h to 1300 h ($\approx$54 days). It is probably caused by the functional groups initially blocked by sorbed water, which get released to produce hydrogen inter-chain crosslinks. The decrease in glass transition temperature (T_g) of such films varies with content of water sorbed in films in an interval of 90.2–189 °C.

Keywords Collagen hydrolysate · Dialdehyde starch · Film curing · Film disintegration · Thermal treatment

Introduction

Processing collagen proteins is accompanied with considerable quantity of produced collagen waste whose partial hydrolysis is precondition of its industrial employment. The energetically most convenient procedure is enzymatic hydrolysis by proteases of microbial origin leading to collagen hydrolysates of mean molecular weight 20–30 kDa [1, 2]. These hydrolysates behave like gelatin but owing to their lower molecular weight gel formation requires approximately tenfold higher concentration of dry matter than gelatin. Lower mean molecular weight of hydrolysates also increases their hydrophilic character. Making biodegradable packaging materials from these hence requires increasing their mean molecular weight by crosslinking, best with polymeric dialdehydes lacking the toxicity of lower aldehydes. The agent frequently recommended to this purpose is dialdehyde starch (DAS) [3].

Gels of collagen hydrolysate crosslinked with polymeric dialdehydes (particularly with DAS) display an increased tendency to aging, which shows through a time-dependent increase in the rigidity of prepared gels, accompanied as a rule with a loss in their thermo-reversibility [4]. Aging does not lead to loss of gel solubility in an aqueous environment (typical of chemo-gels), the dissolution process is merely slowed down. This supports the view that aging of such gels is associated with formation of a network of inter-chain hydrogen bonds [5]. Processing into biodegradable (packaging) materials by dipping technology that is usual, for example, when manufacturing hard or soft gelatin capsules (HGC or SGC) thus gets complicated [6, 7].

Preparation of biodegradable films (or foils) by casting and drying, frequently recommended for some other protein substrates, is a more practicable path to processing such hydrogels. With protein films of various types obtained by casting and drying at lower and higher temperatures, differences were detected in solubility in an aqueous environment as well as in permeation characteristics [8]. Kozlov and Burdygina attribute such behavior of gelatin films to growth in hydrogen bond density, increasing with a decrease in film water content. If water content drops below $\sim$2%, gelatin behaves like a fragile, almost water-insoluble, tightly crosslinked polymer [9].

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Water content of air-dry films of collagen hydrolysate crosslinked with DAS ranges around 40% (w/w). Behavior of such films comes close to behavior of starting hydrogels [10]. During limited action (1–4 h) by a temperature of 105 °C, connected with water content in films getting reduced to ∼1–4%, the principal change taking place relates to dissolution rate of films in an aqueous environment [11].

Experimental

Starting materials

Collagen hydrolysate (H) was prepared by controlled enzymatic hydrolysis of collagen waste from collagen casings manufacture after Kolomaznik et al. [1]. Basic characteristics of H are presented in Table 1.

DAS, CAS 9047-50-1, produced by oxidizing starch; Sigma-Aldrich Biochemicals and Reagents, 2004–2005, p. 1659, no. P 9265.

Table 1 Basic characteristics of enzymatic hydrolysate of collagen waste from meat casings manufacture

Parameter	Value
Dry substance/%	92.99
Amide nitrogen (in dry substance)/%	14.85
Ash (in dry substance)/%	4.94
Ca content (in dry substance)/%	2.75
Mg content (in dry substance)/%	0.48
Primary amino groups (in dry substance)/mmol $–NH_2$ g^{-1}	0.216
Molecular mass-numerical mean/kDa	17.75

Hydrogels were prepared by crosslinking H with DAS so as to cover the range of thermo-reversible and also thermo-irreversible gels [3]. Composition of the corresponding reaction mixtures is shown in Table 2.

Working procedure

Weighed quantity of H, corresponding to 25–30% solution of 20-mL volume, was dissolved in 10 mL water, and the solution pH was adjusted to 11.0 (for the reason of good DAS solubility) with 1 N NaOH. The solution was left to rest overnight to overcome the buffer effect of H. Its pH level was then checked and adjusted if necessary. The solution was filled to 20 mL and, under good stirring in water bath heated to 60 °C, the corresponding quantity of DAS (see Table 2) was gradually added in doses of 0.1 g. A following dose of DAS was always added only after complete dissolution of previous dose. On adding the complete quantity of DAS, reaction mixture was kept at a temperature of 60 °C for another 60 min to ensure perfect progress of the crosslinking reaction.

Reaction mixture was evenly distributed into silicone dishes, and drying in a forced-ventilation thermostat at 35 °C enabled o prepare air-dry films of 0.25-mm thickness.

Thermogravimetric analysis (TGA Q500, TA Instruments, New Castle, DE, USA) in temperature interval 25–300 °C at $dT/dt = 5$ °C min^{-1}, nitrogen stream 150 mL min^{-1} mainly offered information on water content in films, or on their thermal stability. Differential scanning calorimetry (DSC) (DSC 2000, TA Instruments, New Castle, DE, USA) conducted under same conditions then provided information on glass transition temperature of films and foils and on temperature of their thermal breakdown. Typical TG and DSC curves of air-dry films

Table 2 Scheme of factor experiment of 2^3 type for evaluating effect of thermal treatment at 105 °C of air-dry films crosslinked with DAS on their properties

Run	Reaction mixture composition		Time of film treatment at 105 °C/h	Water content in film/%		T_g/°C	Film disintegration time in water environment/h	
	Factor A/% H	Factor B/% DAS	Factor C	Sorbed	Structural		pH = 4.8	pH = 7.4
1	27.5	15.0	2.5	1.65	0.80	106.7	384	504
2	25.0	20.0	4.0	0.43	1.28	189.6	1344	1344
3	30.0	10.0	1.0	1.18	1.12	130.5	96	196
4	25.0	10.0	4.0	0.50	1.50	185.7	120	120
5	30.0	20.0	1.0	2.29	0.93	89.3	360	312
6	30.0	10.0	4.0	0.30	0.92	186.7	366	336
7	25.0	10.0	1.0	1.85	0.75	124.6	240	192
8	30.0	20.0	4.0	0.70	2.10	191.6	1008	1176
9	25.0	20.0	1.0	2.65	1.10	100.8	336	336
10	27.5	15.0	2.5	1.66	0.82	90.2	288	240

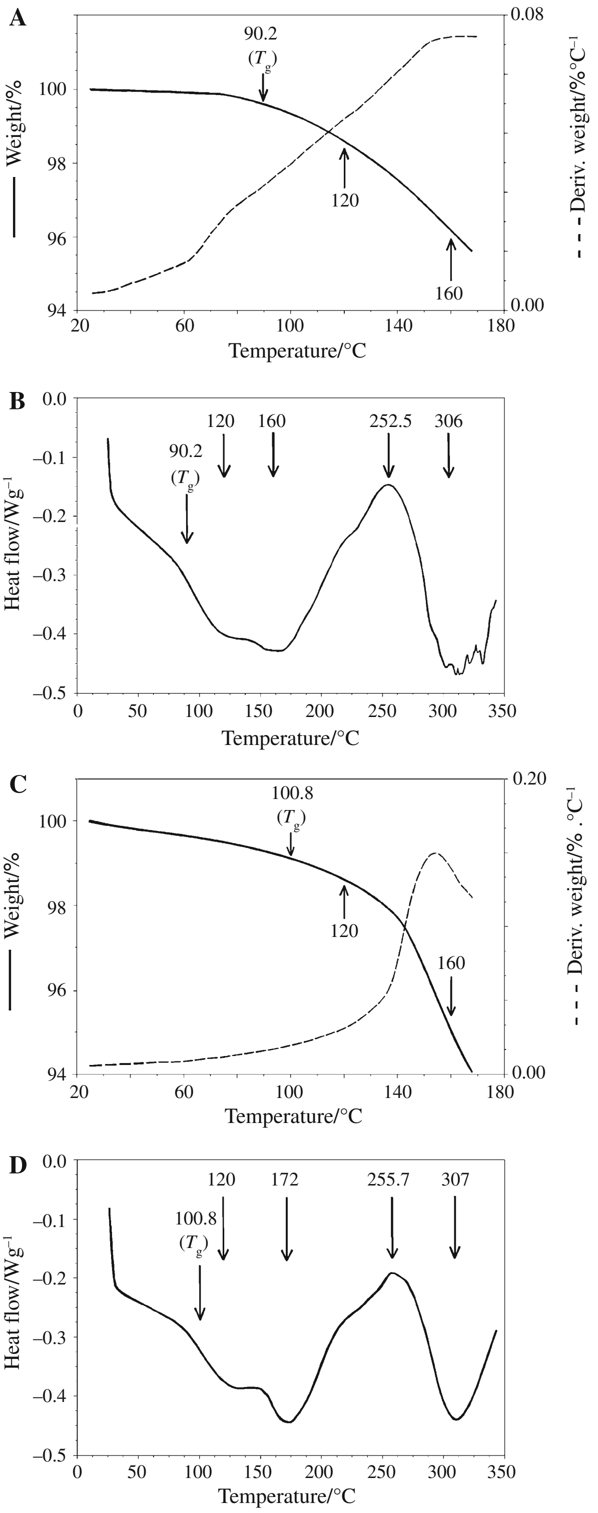

Fig. 1 Typical TG and DSC curves of films treated for definite time at 105 °C. **a** TG and DTG curves of film treated 2.5 h at 105 °C. Starting reaction mixture: 27.5% H + 15% DAS. **b** DSC curve of film treated 2.5 h at 105 °C. Starting reaction mixture: 27.5% H + 15% DAS. **c** TG and DTG curves of film treated 1 h at 105 °C. Starting reaction mixture: 25% H + 20% DAS. **d** DSC curve of film treated 1 h at 105 °C. Starting reaction mixture: 25% H + 20% DAS

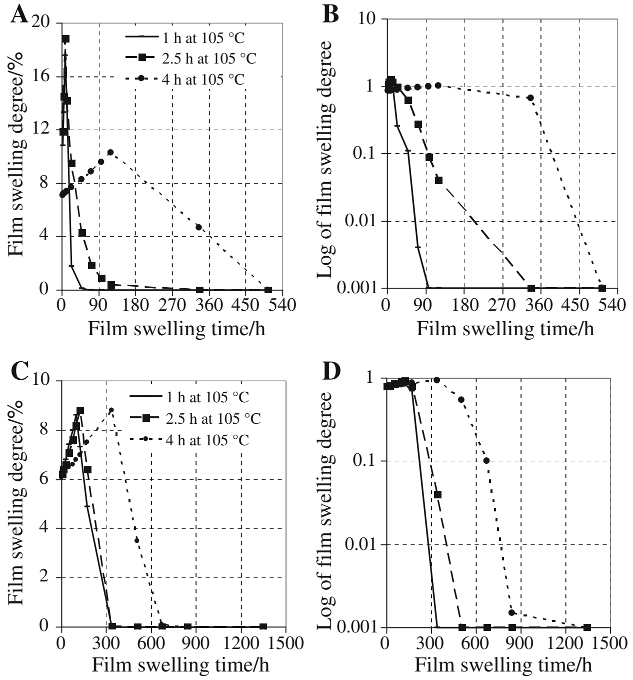

Fig. 2 Typical swelling degree versus swelling time of thermally treated films. **a** Swelling degree versus swelling time for air-dry films, treated 1–4 h at 105 °C. Starting reaction mixture: 25% H + 10% DAS. **b** Logarithms of swelling degree versus swelling time for air-dry films, treated 1–4 h at 105 °C. Starting reaction mixture: 25% H + 10% DAS. **c** Swelling degree versus swelling time for air-dry films, treated 1–4 h at 105 °C. Starting reaction mixture: 25% H + 20% DAS. **d** Logarithms of swelling degree versus swelling time for air-dry films, treated 1–4 h at 105 °C. Starting reaction mixture: 25% H + 20% DAS

thermally treated at 105 °C for 1 and 2.5 h are presented for illustration in Fig. 1a–d.

Disintegration of thermally treated films in an aqueous environment at pH levels 4.8 and 7.4 (values typical for gastro-intestinal tract) was studied by the technique of Einerson et al. [12]. Weighed-in quantity 0.2–0.5 g film was placed in 10 mL water of pH adapted to a level of 4.8 or 7.4 with added 0.1 N HCl or NaOH. At convenient time intervals, the sample under study was withdrawn, dried with filter paper, and weighed. The change in weight of sample in % (related to weight of starting film ≈ [M/M_0]) indicates the swelling degree of air-dry film. At a certain time, swelling degree attains a maximum after which control of process kinetics is taken over by film dissolution. This region is characterized by a quite fast drop in the value of swelling degree of film under study (compare Fig. 2a, c). A more demonstrative expression of these dependencies is in semi-logarithmic coordinates (log[M/M_0] vs. t [h]), when both stages of the process may be fairly reliably approximated by linear branches: the first of them—rising—characterizes swelling course of thermally treated film, the second—descending—dissolution course of swelled film (compare Fig. 2b, d). Intersection of both branches specifies the logarithm of maximal degree of film swelling ($\log M_{\max}$). The gradient of both linear branches is obviously proportional to the apparent diffusion coefficient of both processes (swelling and/or dissolution).

Characterizing disintegration of the investigated films by means of (apparent) diffusion coefficient, the same as determining maximal swelling degree or its time coordinates, is (due to the employed gravimetric technique) linked with some uncertainty and greater measuring time demand. Anyway, the informative potential of such an expression is essentially equivalent to the informative potential of the more simply monitored "film disintegration time" (t_D), defined for purposes of this work as the time (in hours) after which the presence of the studied film in water can be no longer visually detected.

It may be assumed that disintegration time of thermally treated films, the same as their glass transition temperature, will be affected by sorbed water content, but at the same time we cannot rule out the influence exerted by crosslinking degree of DAS protein. The fastest way to resolving similar issues is in applying mathematical statistics methods—above all the technique of factor experiments.

As determining content of water sorbed by thermally processed films is in itself somewhat difficult and, moreover, this factor does not enable to choose equidistant levels necessary for orthogonal factor experiments, the time (hours) during which temperature of 105 °C acted on air-dry films served here to substitute the influence of sorbed water in applied factor experiments.

Assessing the influence exerted by composition of films on their glass transition temperature and on time of disintegration in aqueous environment utilized space-centered factor experiments of 2^3 type with two repetitions in centre (for independent estimate of variance).

The monitored factors were as follows:

- Concentration of H in reaction mixture: factor A—upper limit 30% (w/w) and lower limit 25% (w/w).
- Concentration of DAS—crosslinking agent: factor B (%w) related to H content in reaction mixture—lower limit 10% and upper limit 20%.
- Time of action (hours) at 105 °C on air-dry films: factor C—upper limit 4 h and lower limit 1 h.

The monitored quantities were as follows:

- Time of film disintegration (hours) in an aqueous environment at pH 4.8 (t_D^{H+}) and 7.4 (t_D^{OH-}).
- Glass transition temperature of films after defined thermal treatment (T_g/°C).

For completeness, the monitored quantities moreover included:

- Percentage of contained water (w/w) sorbed by films that were thermally treated in various manner (water volatilizing up to 120 °C during TG of films under mentioned conditions—in Table 2, H_2O_{sorb}).
- Percentage of water bound structurally by films under study (volatilizing in temperature interval 120–160 °C during TG—in Table 2, H_2O_{struct}).

A survey showing the organization of this factor experiment together with the attained results is summarized in Table 2.

Statistical significance of particular factors for monitored quantities was evaluated by standard procedure applying the Fisher test of significance [13, 14]. Results of evaluation are listed for ready survey in Table 3.

Discussion

Air-dry films of collagen hydrolysate crosslinked with DAS contain about 40% water, whose content decreases by

Table 3 Results of Fisher test of statistical significance of particular factors for monitored characteristics of thermally treated films from Table 2

Factor	Water content in film/%		T_g/°C	Film disintegration time, t_D/h	
	Sorbed	Structural		pH = 4.8	pH = 7.4
A: % H in reaction mixture	1.22	0.10	0.19	0.21	0.00
B: % DAS in reaction mixture	6.67	0.64	0.03	18.60[a]	26.13[a]
C: duration of action at 105 °C	48.49[a]	1.85	12.54[a]	11.53[a]	18.21[a]
Interactions					
AB	0.81	0.38	0.06	0.54	0.82
AC	1.61	0.00	0.25	0.00	0.02
BC	3.32	0.33	0.12	18.62[a]	15.74[a]
Critical value $F_{(1,3)}^{95\%}$			10.13		

[a] Factors affecting given characteristics of thermally treated film in statistically significant manner

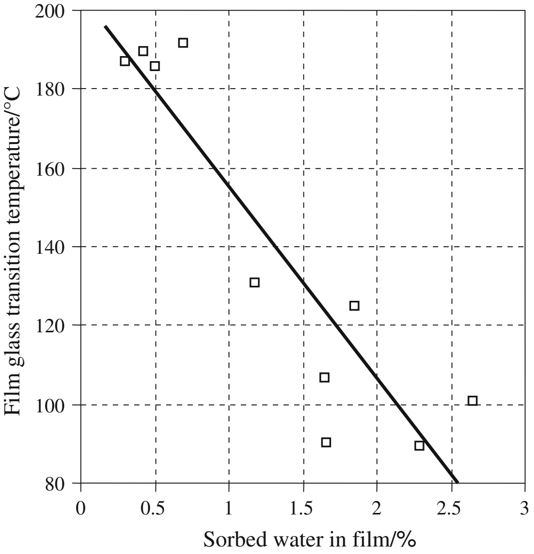

Fig. 3 Dependence of films glass transition temperature (T_g/°C) versus % (w/w) of sorbed water in thermally treated films

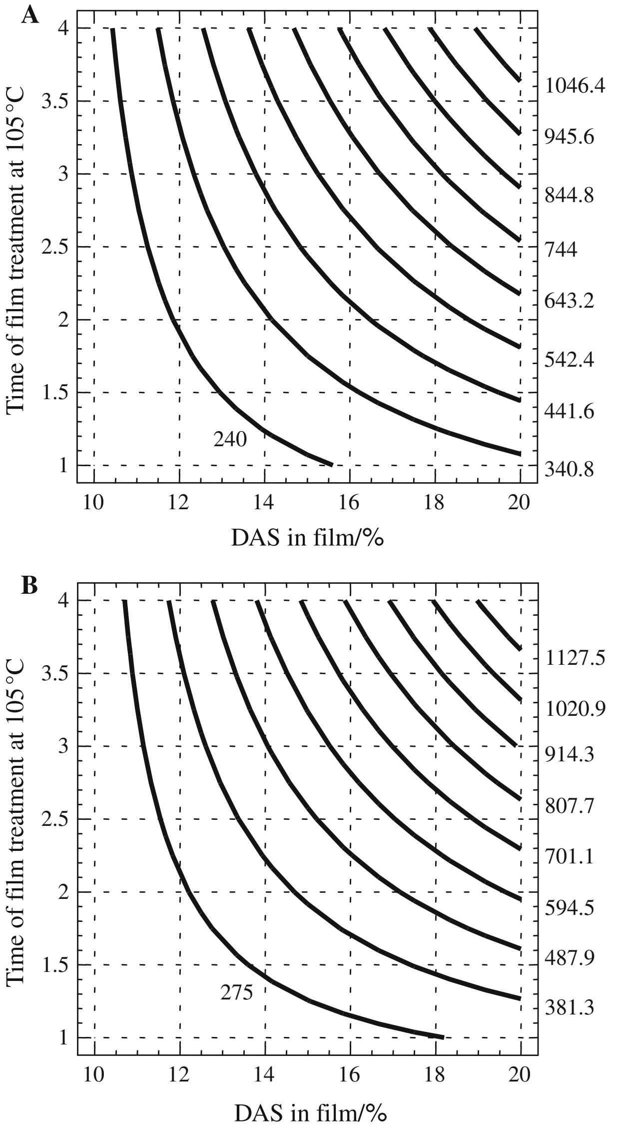

Fig. 4 Contour diagrams of disintegration time (t_D/h) of thermally treated films in aqueous environment. Influence of air-dry film treatment at 105 °C, of various time and % of DAS (w/w) in starting reaction mixture. pH of **a** aqueous environment 4.8 and **b** water environment 7.4

heating to 105 °C (in a forced-ventilation thermostat) depending on the duration of this action (1–4 h) to ~4–1.2%. With gelatin films, such water content corresponds to the region where striking changes in properties take place: films turn fragile and frangible with a glass transition temperature of $T_g \approx 180$–190 °C, and are poorly soluble in an aqueous environment. These changes are associated with ceased blocking of sorption centers of water due to its reduced concentration in gelatin [9].

Setting an equidistant interval of water content in films to acceptable reliability (conditioning applicability of statistical schemes in factor experiments) is altogether difficult. For this reason, the parameter was replaced by the time for which a temperature of 105 °C was allowed to act on air-dry films in a forced-ventilation thermostat (compare Table 2). This factor (within framework of executed tests) exerts statistically significant influence also on the glass transition temperature of air-dry films treated in this manner (compare Table 3).

Based on the course of TG curves of films under study, we may conclude water in films is bound at least in two ways. More loosely bound water, which evaporates during TG analysis in a temperature interval up to 120 °C, is often designated "sorbed" water and is more important for reducing the glass transition temperature of monitored films than the fraction of more strongly bound water (released from films during TG in a temperature interval of 140–160 °C), usually designated "structurally bound" water. Release of that takes place only on exceeding glass transition temperature of films and so it very probably participates only to minimum extent in reducing the glass transition temperature of films. Within the scope of performed experiments this is in accord with the 95% confidence interval of mean sorbed ($I^{95\%} = 1.32 \pm 44.7\%$) and structural ($I^{95\%} = 1.13 \pm 26.1\%$) water: mean content of structurally bound water displays (within scope of performed experiment) roughly half the variability of sorbed water content. Correlating glass transition temperature of films to their sorbed water content (within scope of performed experiments) allows to estimate that approximately 91% observed variability of their T_g can be linked with altered content of sorption-bound water in them. Such dependency, graphically presented in Fig. 3, evidences the significance of sorbed (more loosely bound) water for plasticizing proteinic films.

Solubility of thermally treated films in an aqueous environment, monitored by the modified method of Einerson et al. in a weakly acid ($\text{pH} = 4.8$) and weakly alkaline ($\text{pH} = 7.4$) environment which roughly corresponds to conditions in the human gastro-intestinal tract, is not affected to statistically significant extent by altered pH, $t_{\mathrm{D}}^{\mathrm{H}+} \approx t_{\mathrm{D}}^{\mathrm{OH}-}$ [12]. In both cases, factors possessing statistical significance are time during which a temperature of 105 °C acts on air-dry films, and degree of film crosslinking—characterized by percentage of DAS crosslinking agent (related to H content in starting reaction mixture). A higher level of both factors tends to slow down dissolution of films (compare data in Table 2). Moreover, both factors are in mutual interaction. This is evidenced both by results summarized in Table 3 as well as by contour diagrams indicating same times of film disintegration—dependently on the duration of action by 105 °C and on DAS percentage—in Fig. 4a for a weak acid environment (pH 4.8) and in Fig. 4b for a weak alkaline environment (pH 7.4).

Conclusions

Both heat-treatment and the dosage of DAS increase the degree of crosslinking of films. Prolonged time (4 h) of films treatment at 105 °C significantly reduces (70–84%) more loosely bound (sorbed) water. It can be assumed that functional groups, initially blocked by sorbed water, get release to produce hydrogen bonds contributing to polypeptides inter-chain crosslinking. The amount of sorbed water in films is not influenced by the dosage of DAS. This fact cannot be precisely explained.

The distinct inclination to aging displayed by gels of collagen hydrolysate crosslinked with DAS, posing a certain limitation when applying dipping technology, may conversely mean a certain advantage when applying technology for manufacturing packaging materials by casting and drying. Controlled thermal treatment of air-dry films (combined with suitably chosen crosslinking degree) allows to regulate their rate of disintegration in an aqueous environment to the extent of almost 1300 h (≈54 days). This fact can be utilized in a number of practical applications for such biodegradable (or biocompatible) packaging materials. Achieved results can also be significant for potentially applying methods, common when processing synthetic thermoplastics that combine effects of pressure and temperature, to this type of biodegradable (and/or biocompatible) compounds.

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