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BIODEGRADABLE PACKING MATERIALS BASED ON WASTE COLLAGEN HYDROLYSATE CURED WITH DIALDEHYDE STARCH

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Hydrogels of collagen hydrolysate (H) of mean M_w 15–30 kDa obtained from waste collagen from meat casings manufacture, cross-linked with 15% (based on H) polymeric dialdehyde starch (DAS), have a marked tendency to ageing, which shows in hydrogel gradually increasing rigidity and decreasing thermo-reversibility. Methods of thermal analysis (DSC, TG) proved that ageing of hydrogels is not related with a non-equilibrium state of the cross-linking reaction but is rather given by increasing density of inter-chain hydrogen bonds between polypeptide segments of H. Plasticizing effect of DAS on H is not too pronounced but the difference between glass transition temperature of dry xerogel T_g=189.5±2.5°C and temperature of starting degradation (DAS component) 241.4±12.7°C offers certain space for processing these xerogels into biodegradable (edible) packaging material by usual plastics technologies. Films obtained from the reaction mixture by casting and drying at room temperature after thermal processing (105°C for 4 h) dissolve at room temperature only after 350 h. This effect can be employed for time-controlled releasing of active substances from such biodegradable (edible) packages.

Keywords: *collagen hydrolysate, cross-linking, dialdehyde starch, glass transition*

Introduction

In case we disregard application of collagen in food manufactures which are limited by its low nutritive value and deficit in essential amino acids, as well as manufacture of gelatin by boiling refined collagen till soft, its industrial application is practically always burdened with difficulty processable protein waste produced to a lesser or greater extent. Its utilization in the way of a secondary industrial raw material is complicated by the quite high density of irreversible cross-links introduced to stabilize collagen material against chemical and above all against microbial influences. The first step in procedures proposed for processing such waste usually consists in partial hydrolysis which is realised as acid hydrolysis, alkaline, or as the comparatively least energy demanding, and thus economically most interesting, enzymatic hydrolysis. That usually works with commercially available proteases of microbial origin. A survey of proposed working procedures was given, for example, by Heidemann [1], problems of enzymatic leather waste hydrolysis were described in detail by Kolomazník *et al.* [2]. However, industrial employment of obtained hydrolysates remains the weak point of suggested procedures.

In the course of time, it was recommended to utilize such hydrolysates as an agricultural fertiliser, protective colloid, rubber latex coagulant, or as liquefying agent for concrete and clays in manufacture of technical ceramics [3]. It was later proposed to use refined collagen hydrolysate as an efficient hydrating agent in cosmetic preparations for skin treatment [4] secondary raw material for producing surfactants of N-acylated amino acid type (Lamepon type) marked by outstanding dermatological properties [5] as component of urea-formaldehyde resins distinctly reducing formaldehyde emissions from their cured adhesive films [6, 7] and also curing agent for epoxide resins imparting a biodegradable character to cured epoxide systems [8]. None of these suggestions has been yet realised on an industrial scale, above all for not over strong final economic effects. Most produced collagen waste, despite declarations on importance of environmentally clean, no-waste technologies, has so far finished in landfills of industrial waste.

Environmental aspects of suitably utilised collagen waste have lately been accentuated by the ever growing production of packaging materials based on synthetic polymers, whose landfilling is highly problematic due to difficult degradation and for complicated recycling due to high costs. Biodegradability of packaging materials has become, lately in particular, a very closely watched field promoting application of collagen hydrolysates in industrial practice.

Hydrolysate of chrome-tanned leather waste does not meet requirements for packages of pharmaceutical and food products; packing considered rather more in this connection involves farming chemicals and fertilisers. The biodegradable character of such packages does not merely resolve the issue of used packages dis-

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posal. When suitably processed, such materials may provide time-controlled release of active substances. Hydrolysate of abattoir collagen waste, or of waste from manufacture of edible meat product casings, meets criteria of edible (and biodegradable) packages for pharmaceutical and food products, and possibilities of its industrial application are wider.

Processing collagen hydrolysates into edible packages has to take into account their lower molecular mass. That usually attains values around 15–30 kDa which, when compared with gelatin currently used for edible packages, is a level of 5–10 times lower. Higher water solubility and lower resistance of hydrolysate-based packaging films and foils to moisture has to be eliminated by increasing cross-link density in hydrolysate. Low-molecular aldehydes (formaldehyde, glyoxal, glutaraldehyde) are most usually employed to this purpose, their disadvantage is smaller or greater toxicity. For this reason, attention has lately rather focused on polymeric dialdehydes derived from various polysaccharides, particularly on commercially readily available dialdehyde starch (DAS), whose toxicity is very low [9] and which is even recommended as an adsorption agent for urea in treatment of chronic kidney diseases, uremia, etc. [10]. Some authors suppose that polymeric dialdehydes may also strongly plasticize proteinic films and foils, which conduces to the notion of utilising plastics technologies in their manufacture. Results of preliminary studies [11] lead to concluding the reaction of collagen hydrolysates with dialdehyde starch allows to prepare thermo-reversible gels unless hydrolysate concentration in reaction mixture exceeds 30% and dialdehyde starch concentration attains at most 15% based on hydrolysate.

Investigating the time course of rigidity of hydrogels prepared under these conditions enabled to detect their ageing, first connected with partial loss and eventually with complete loss of thermo-reversibility. Such properties may affect technology of making films from these hydrogels, and this work is focused on behaviour and properties displayed by these gels.

Table 1 Basic characteristics of enzymatic hydrolysate from collagen meat casings

Dry substance/%	92.99
Amide nitrogen in dry substance/%	14.85
Ash in dry substance/%	4.94
Ca in dry substance/ppm	27 456.62
Mg in dry substance/ppm	4 798.00
Primary amino groups in dry substance/ mmol $-NH_2$ g^{-1}	0.216
Avarage molecular mass (numerical mean, M_N)/kDa	17.75

Experimental

Materials

Collagen hydrolysate prepared by enzymatic hydrolysis of waste collagen meat casings (after [2]). Product characteristics are presented in Table 1.

Commercial dialdehyde starch (DAS) [CAS 9047/50/1] produced by oxidising starch: P-9265 – Sigma-Aldrich Biochemicals and Reagents 2004–2005, p. 1659.

Working procedure

Hydrolysate solution (100 mL of 27.5% solution) was prepared by dissolving hydrolysate in half the volume of water whose pH was adjusted to 11 with a 1 N solution of NaOH. To eliminate the buffer effect of hydrolysate, solution was left to stand overnight at laboratory temperature and after repeated pH adjustment was filled up with water to a final concentration of 27.5% *w*/*v*.

The prepared solution was heated to 60°C in a water bath and solid DAS in a quantity of 15% (based on hydrolysate content in reaction mixture) was gradually added in doses of 0.1–0.2 g under thorough stirring, always on complete dissolution of previous dose. Under these conditions, the reaction proceeded for 30 min and after adding all DAS, the reaction mixture was held (always under good stirring) at reaction temperature for another 30 min. After cooling to room temperature, a part of the reaction mixture was tempered at 10°C for 16–18 h in a standard vessel for measuring gel rigidity, and gel rigidity was measured by means of standard method (BS 757 1979 [12]) on standardized instrument (LFRA Texture Analyser by Stevens Dunmow, Essex, UK). Measured gel was then left to age at room temperature for a period presented in data of Table 2.

The quite high water content in prepared gels causes certain difficulties during DSC measurements (high intensity of endothermal DSC peak related with water evaporation, or plasticizing effect of residual moisture effecting a decrease in T_g of cross-linking xerogel). For that reason, DSC curves of gels were always measured in two stages. The first measurements, conducted in temperature interval 25–200°C (dT/dt=5°C min^{-1}) characterized ageing gel until dried out (formation of xerogel), the second measurements, which followed cooling of sample in apparatus to 25°C, were conducted in interval 25–350°C at same heating rate (dT/dt=5°C min^{-1}) and characterized behaviour of dry gel (xerogel). The second measurement mainly enabled to characterize plasticizing effect of DAS and define the beginning of xerogel thermal degradation. No significant differences between DSC curves of gels aged for various times were detected and DSC curves of both such measurements are shown in Fig. 3.

Table 2 Influence of ageing time of gels of collagen hydrolysate cross-linked with dialdehyde starch at room temperature on hydrolysate rigidity [°Bloom] and on melting temperature of gel [°C], estimated from first scan of DSC curves in interval 25–200°C

Gel ageing time/h	Gel rigidity/°Bloom	Thermo-reversibility	Gel–sol transition/°C from DSC
20	86	reversible	23.0
44	101	reversible	28.4
164	115	revesible	40.6
188	118	partly reversible	43.5
212	123	partly reversible	45.9
236	125	partly revesible	46.2
332	129	irreversible	47.9
356	130	irreversible	43.5
380	133	irreversible	47.1
404	135	irreversible	47.1
476	146	irreversible	50.0

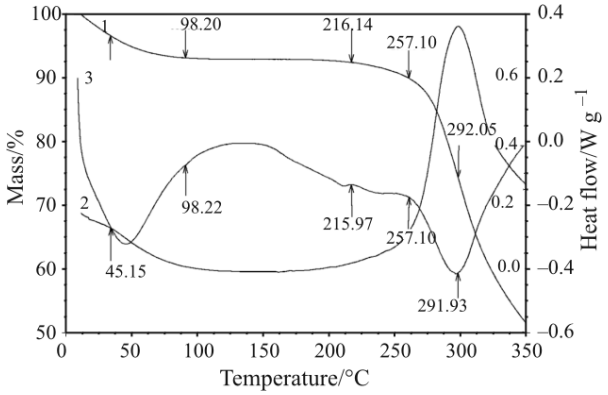

Fig. 1 TG, DTG and DSC curves of collagen hydrolysate (H) in 25–350°C range at heating rate dT/dt=5°C min^{-1}; 1 – TG, 2 – DTG, 3 – DSC curve

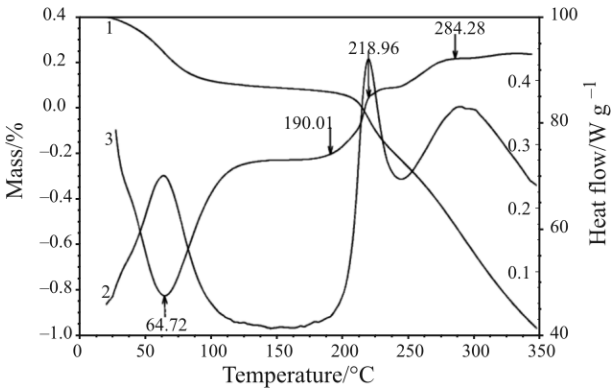

Fig. 2 TG, DTG and DSC curves of dialdehyde starch (DAS) in 25–350°C range at heating rate dT/dt=5°C min^{-1}; 1 – TG, 2 – DTG, 3 – DSC curve

Methods

Characteristics of both starting materials were extended with results of their thermogravimetric analysis (TG) (TGA Q 500 by TA Instruments, New Castle DE, USA) and differential scanning calorimetry (DSC) (DSC 2010 by TA Instruments, New Castle DE, USA) in temperature range 25–350°C at heating rate dT/dt=5°C min^{-1}. The courses of TG and DSC curves for starting hydrolysate are shown in Fig. 1, the course of DAS in Fig. 2.

Results and discussion

Gel ageing is a phenomenon studied quite keenly [13]. With gelatin gels, the principal cause of ageing is considered to be an increasing density of hydrogen bonds between polypeptide segments [14]. The ageing tendency of hydrogels based on H cross-linked with DAS is more obvious than with gelatin gels and is shown by the time dependence of gel rigidity (Fig. 4). In addition, ageing is accompanied with gradual loss in gel thermo-reversibility (compare data in Table 2) and hence the share of a non-equilibrium state of the cross-linking reaction in ageing cannot be well excluded.

Hydrogels under study were deliberately prepared (27.5% H and 15% DAS based on H) in such manner that they retained full thermo-reversibility for not less than 100 h at room temperature. In order to estimate the influence of contained water and also of cross-linking reaction on the strong ageing tendency displayed by these gels, the technique chosen for experimentally evaluating gel ageing was two-stage DSC measurement because starting gels contained approx. 68.5% water, which is a very good plasticizer for proteins and content of which somewhat decreases through ageing.

During first measurements (temperature interval 25–200°C, dT/dt=5°C min^{-1}), DSC curves of all gels under study exhibited two endothermal peaks of which the first, more intensive, is situated in the temperature region of 44.3±6.2°C (mean of 16 determina-

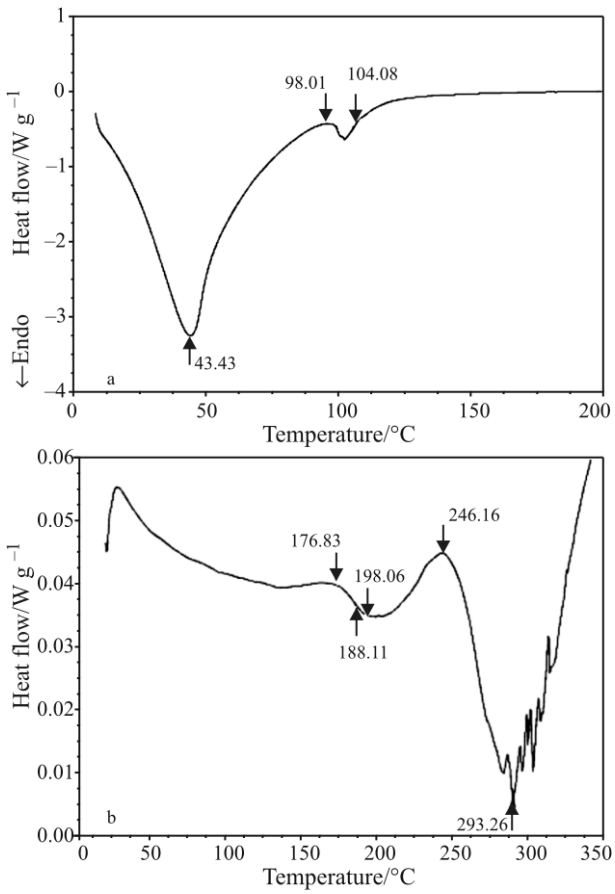

Fig. 3 Typical DSC curve of hydrogel of collagen hydrolysate – H cross-linked with polymeric dialdehyde – DAS (H 27.5 mass%, DAS 15 mass% based on H) in 25–350°C range at heating rate dT/dt=5°C min^{-1}; a – first scan: 25–200°C range, dT/dt=5°C min^{-1}, b – second scan: 25–350°C range, dT/dt=5°C min^{-1}

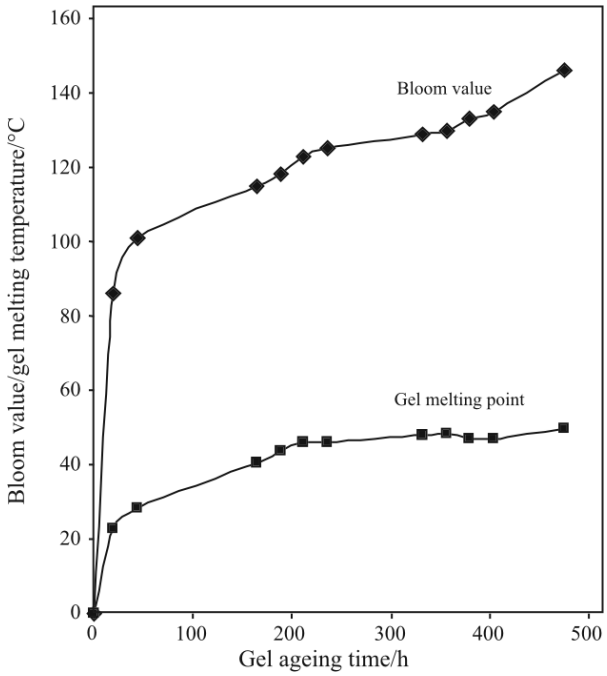

Fig. 4 Time dependence of rigidity (°Bloom) of hydrogels (27.5 mass% H+15 mass% DAS based on H) on their ageing at room temperature

tions). With gels, such an endothermal peak is usually attributed to gel→sol phase transition (gel dissolution) [15, 16]. According to respective TG curves recorded under same conditions, a mean 9.86±3.35% mass loss of measured samples occurs in this temperature interval so that this endothermal peak apparently does not correspond to a mere phase transition but is rather a gel→sol transition combined with evaporation of water. This conclusion is supported by the cited authors who found that temperature co-ordinates for the minimum of this endothermal peak (and also for its width) depend on dry matter concentration of measured gels. Application of this DSC endothermal peak to deriving conditions for processing of such hydrogels into biodegradable (edible) packing materials by procedures common in plastics technology is thus not too reliable, and considerations of this kind require more detailed study.

The second of the detected endothermal peaks is localized in the 97.5±6.16°C region. The cumulated mass loss of corresponding TG curves in it achieves a mean 69.6±6.78%, corresponding to virtually total evaporation of water from hydrogel. The first DSC measurements above this temperature cannot detect any marked drops or peaks on DSC curves. Cross-linking of H by means of DAS under the selected reaction conditions (27.5% H+15% DAS based on H, pH=11.0, T=60°C, t=90 min) is obviously finished and connection of gel ageing with a possible non-equilibrium condition of the cross-linking reaction cannot be proved. Increased tendency of synthetized hydrogels to ageing and gradual loss of thermo-reversibility is probably more related – with a view to structure and molecular mass of DAS – with its tendency to form inter-chain hydrogen bonds with H polypeptides.

Some authors [17] mentioned the plasticizing effect of dialdehyde starch on proteins. If the plasticizing effect of DAS were strong enough, it would enable applying usual plastics procedures in manufacture of biodegradable packing films based on H. With proteinic materials, however, it is quite difficult to separate the plasticizing effect of plasticizers from that of contained water. For that reason, following the first DSC measurement and cooling of sample to room temperature (without withdrawal from instrument), a second DSC measurement was performed (under otherwise same conditions, dT/dt=5°C min^{-1}, temperature interval 25–350°C). Curves obtained in repeated measurements confirm the sample in second measurement contains practically no water and the detected plasticizing effect (reduced T_g) may be regarded as the effect of sole DAS.

No marked drops or peaks may be seen on DSC curves of second measurement up to approx. 170°C. Their prominent drop (decrease in heat flow level) occurs only in interval 177±3.9–199.1±2.1°C. Such a decrease is typical for the glass transition region of polymeric materials (second-order transitions) and the point of inflection of DSC curve in this region is regarded as temperature of their glass transition – T_g.

With the measured samples (needless of ageing time of corresponding gels), T_g was found in these measurements in region 189.5±2.52°C. Starting powdery H (containing approx. 7% water) yielded in measurements repeated sixfold a virtually same glass transition value (T_g=189.75±2.52°C).

Some authors [18, 19] draw attention to the fact that increasing cross-link density of proteins (for example, gelatin) brings about an elevated glass transition temperature of such materials. Yannas and Tobolski [20], for example, quote T_g of uncross-linked gelatin as 175±10°C which grows for cross-linked gelatin to 196±3°C. A similar phenomenon was also detected by Sobral and Habitante [21] (T_g of dehydrated gelatin 220±8°C, Marshall and Petrie [22] (T_g of non-dehydrated gelatin 175–180°C). Other authors put such changes of T_g in connection with increased density of hydrogen cross-links between polypeptide chains during gelatin dehydration.

Removal of moisture in the first DSC measurement of samples admittedly minimizes participation of water in the detected plasticizing effect, nevertheless, it cannot be ruled out that a plasticizing effect produced by DAS and increased cross-link density due to cross-linking of H interfere to such extent that plasticizing effect of DAS does not show more distinctly.

With a number of polymers including proteins, an endothermal peak may be detected, as a rule immediately behind the T_g region, and is usually attributed to present low-molecular components (plasticizers) [23]. Such behaviour is described with gelatin of low water content [18]. As samples were practically rid of moisture in the first DSC measurement, such an endothermal peak (assumed in a region of 200–210°C) in second measurements is not detected.

Contrarily, in the 241.4±12.7°C region there is a distinct exothermal peak, whose position corresponds to the double exothermal DSC peak of polymeric dialdehyde (DAS) and which is connected on corresponding TG curves with an 11.5–12.5% mass loss of measured sample. Accordingly, thermal degradation (probably oxidative) of chains obviously occurs in this temperature region. It is followed practically immediately (at 255.4±8.1°C) by deep thermal degradation of hydrolysate.

Thus, temperatures above 230–240°C, with a view to processing studied materials by plastics technology, are a hazardous region. The difference between found value of T_g (189.5±2.5°C) and temperature of starting thermal degradation (241.4±12.7°C) seems to be, from the angle of such considerations, quite reasonable. Of course, flow properties of such materials in the 190–230°C region remain an open issue.

An interesting aspect of the studied materials is also their reduced water-solubility which, for example with gelatin containing less than 2% water, was discussed by Kozlov and Burdygina [14]. These authors attribute the cited effect to increased density of hydrogen bonds between polypeptide segments which leads not only to reduced water-solubility but also to increased brittleness of proteinic films.

In the case of synthesized hydrogels, ageing is admittedly accompanied with gradual loss of thermo-reversibility, but even after 400-h ageing, hydrogels display unlimited swelling in water and preserve their water-solubility. Dissolution rate, of course, somewhat decreases with prolonged ageing time of their gels.

That was confirmed by an additional orientation swelling test executed with films prepared by casting sol of hydrogels (composition – 27.5% *w*/*w* H+15% DAS based on H), drying at room temperature and subsequent thermal treatment in a forced-circulation drier for 4 h at 105°C. The objective was to simulate an extreme ageing time of prepared gels. Swelling curves (time dependence of mass increments of films thus processed related to mass of dry film) determined by technique after Bigi *et al.* [24] are shown for illustration in Fig. 5.

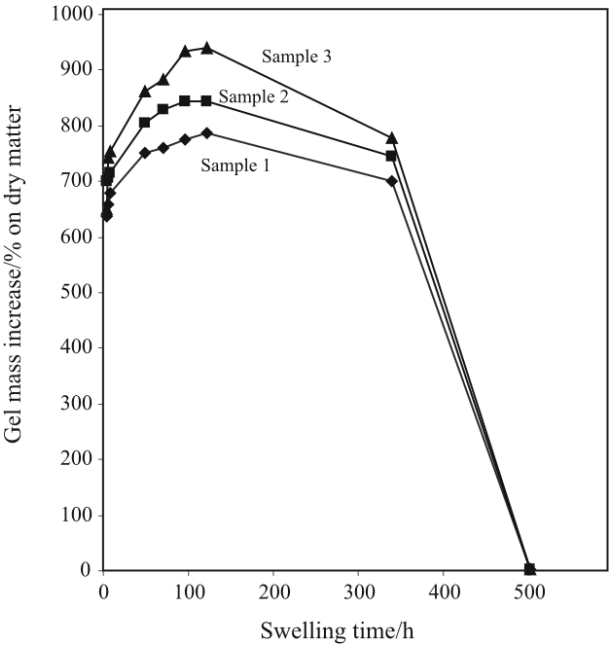

Fig. 5 Time dependence of swelling degree of gels (27.5 mass% H+15% DAS based on H) treated for 4 h at 105°C

While films merely dried at room temperature break up in an aqueous environment in a time whose length is proportionate to gel ageing time and does not exceed 2 h, films that were dehydrated for 4 h at 105°C first swell for about 120 h (increase in mass) and only later start dissolving (decrease in film mass – diagrams in Fig. 5). They break up only after approx. 500 h. We believe problems connected with ageing of hydrogels may be bypassed by thermal treatment and, moreover, film dissolution retarded by this process may be utilised for time-controlled releasing of active substances from such biodegradable packages.

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