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Plasticizing collagen hydrolysate with glycerol and low-molecular weight poly(ethylene glycols)

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Abstract

The plasticizing efficiency of glycerol (GLY)and poly(ethylene glycols) of molecular mass 300–3350 Da (PEG 300–PEG 3350) for films of collagen hydrolysate (H) was assessed on the basis of the glass transition temperature depression (ΔT_g). The plasticizing effect of hydrophilic plasticizers was separated from the plasticizing effect of water absorbed to H films by means of two-stage differential scanning calorimetry (DSC). The first stage of DSC, conducted in a temperature interval of 25–150 °C led to removal of absorbed water. The T_g values of un-plasticized films estimated in second DSC stage (after cooling the measured sample to 25 °C without withdrawal the sample from the instrument and running it again in the temperature range of 25–350 °C) correspond to value of dehydrated gelatin films and H films contain only structurally (more firmly) bound water (approximately 2.5% mass). With plasticized H films a decrease in T_g depending on the type and concentration of employed plasticizer was observed. With glycerol, T_g decreases with the film increasing glycerol concentration in film, with poly(ethylene glycols) it attains a certain limit level at their approximately 20% concentration in film.

At 20% concentration the plasticizers under study gave a T_g depression (ΔT_g) – corresponding their plasticizing efficiency for H films – was found as follows: GLY = −66.8 °C, PEG 300 = −48.8 °C, PEG 400 = −47.1 °C, PEG 600 = −50.3 °C and PEG 3350 = −9.6 °C.

Keywords: DSC; Collagen hydrolysate; Glass transition temperature; Hydrophilic plasticizers

1. Introduction

Collagen, industrially produced as a secondary product in the meat industry, finds merely limited application in foodstuffs manufacture due to its low nutritive value and deficient essential amino acids. It is more a starting material for edible meat product casings. Gelatin, its partial hydrolysate, has certain significance as a thickening agent of food dispersion systems (gelation agent) and is used as a biodegradable and also edible packaging material for food additives, encapsulating medicines as well as cosmetic products. Properties appreciated most with gelatin films are low permeability for oxygen, carbon dioxide, aromatic substances and some other substances, and ready water solubility, which may be regulated in quite wide limits by increasing cross-link density.

The high density of cross-links in collagen (due to natural ageing with native collagen, and to the manufacturing method with industrial products) does not always influence the properties of collagen materials and their products in a positive manner. In the case of native collagen it reduces the yield as well as quality of obtained gelatin [1], in industrial products it often causes unfavourable (mechanical) properties, which may easily result in the production of difficult to apply industrial collagen waste.

In the prevailing industrial application of collagen – manufacturing large-area materials (leathers) for clothing and footwear mass production – the quantity of such collagen waste, according to some authors [2,3], may attain 60–70% of the mass of starting collagen raw material; data from casings manufacture or other branches are available with difficulty and often incomplete.

The production of difficulty to utilize collagen waste has stimulated interest in no-waste (clean) manufacturing technologies, but results achieved so far are not too encouraging. For this reason, attention has lately focused on using collagen waste as a secondary industrial raw material, mostly for the production of packing materials.

Owing to the high cross-link density of industrial collagen waste, suggested procedures virtually always comprise its controlled (partial) hydrolysis as the first step. A particularly

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advantageous procedure – from an energetic as well as economic point of view – is controlled enzymatic hydrolysis by means of commercially available proteases of microbial origin [4]. Collagen hydrolysates thus obtained (of mean molecular mass approximately 15–30 kDa) approach gelatin [5] in behaviour and properties and can be easily utilized for manufacturing biodegradable (potentially even edible) packaging materials [6].

The fragility of proteinous biodegradable packages, particularly noticeable at lower relative humidities is usually overcome by adding plasticizers of polyol type (glycerol, poly(ethylene glycols), sorbitol, maltitol or also poly(vinyl alcohol) and others [7]. Plasticizing of proteinous films even stimulates attempts at applying traditional plastics technologies (blow moulding) in their industrial production.

The usual mechanism for interpreting action of hydrophilic plasticizers leads usually to a reduction in their binding to polar centres of proteinous chains which leads to reducing inter-chain attractive (van der Waals) forces [8,9] and thus to increasing mutual mobility of proteinous chains [10].

Hydrophilic plasticizers simultaneously act as humectants for proteinous films, retaining in them moisture and affecting their water vapour sorption isotherms. As a rule, it is difficult to decide to which extent plasticizer alone shares in the achieved plasticizing effect and to which plasticizer-bound water does [11]. The significance of data by particular authors on the effect of plasticizers, determined empirically on the basis of tensile strength and elongation at break of plasticized films conditioned at a certain relative humidity, is certainly limited [12].

Hydrophilic plasticizers are usually applied in proteinacious films in concentrations of 15–50% (based on mass of protein), the one being recommended for this function most often is glycerol (GLY) which, however, has a marked tendency to migrate [13]. Migration of plasticizers of poly(ethylene glycol) type in proteinacious films is suppressed in proportion to their molecular mass. Alcoholic sugars or other mono- and disaccharides are recommended as plasticizers less frequently.

Proteinacious films are usually prepared by casting film-forming solutions and drying. In industrial practice, this technique is associated with certain problems [14], which increases interest in the use of "dry" plastics procedures combining the effect of pressure and temperature (extrusion, thermo-moulding, etc.) [15,16]. This also increases the significance of more reliably assessing the plasticizing effect on proteinacious films by hydrophilic plasticizers, mostly utilizing their effect on depressing glass transition temperature (T_g) which is usually determined by DSC technique [17–19]. The technique was also employed when evaluating the plasticizing effect of glycerol and poly(ethylene glycol)s of mean molecular mass 300–3350 Da on films of enzymatic collagen hydrolysate (H).

The data of hydrophilic plasticizers efficiency and their action mechanisms presented in this work may be useful for the production of soft capsules on collagen hydrolysate basis.

2. Experimental

Starting collagen hydrolysate (H) was prepared by controlled enzymatic hydrolysis of collagen waste from casings production by a protease of microbial origin in such manner that 50 kg water, 0.5 kg MgO and 10 kg collagen waste from collagen casings (minced to pieces of approximately 2 cm × 2 cm) manufacture were introduced into a reactor vessel Under good stirring, 0.3 kg commercial protease of bacterial origin ALCALASE DNL (Novonordisk A/S Bagsvaerd, Denmark) was added to the reaction mixture and that was heated for 5 h to 70 °C under constant good stirring. The vacuum-filtered reaction mixture yielded a solution of collagen hydrolysate in the form of a clear liquid and a filter cake containing approximately 5% non-degraded collagen waste. The clear collagen hydrolysate solution was vacuum-thickened to a content of approximately 30% dry matter and dried to powder in a spray drier (see also ref. [4]). Table 1.

Table 1
Basic characteristics of collagen enzymatic hydrolysate

Dry substance (%)	92.99
Amide nitrogen in dry substance (%)	14.85
Ash in dry substance (%)	4.94
Ca content in dry substance (ppm)	27 456.6
Mg content in dry substance (%)	4 798.0
Primary amino groups in dry substance (mmol NH_2 g^{-1})	0.216
Average molecule. mass (numerical mean, M_N) (kDa)	17.75

The studied plasticizers were commercial products glycerol (GLY) and poly(ethylene glycols) (PEGs) of mean molecular mass 300–3350 Da, specified by the supplier (Sigma–Aldrich) as follows:

GLY Boiling point$_{(760)}$ = 290 °C, ζ = 1.25 g/ml (G7893 Aldrich Handbook of fine chemicals 2007–2008, p. 1340.

PEG 300 Poly(ethylene glycol) M_N 300 Da: viscous liquid, m.p. = −15 to −8 °C (202371 Aldrich Handbook of fine chemicals 2007–2008, p. 2021).

PEG 400 Poly(ethylene glycol) M_N 400 Da: viscous liquid, m.p. = 4 to 8 °C (202398 Aldrich Handbook of fine chemicals 2007–2008, p. 2021).

PEG 600 Poly(ethylene glycol) M_N 600 Da: damp waxy substance, m.p. = 20 to 25 °C (20240 Aldrich Handbook of fine chemicals 2007–2008, p. 2021)

PEG 3350 Poly(ethylene glycol) M_N 3350 Da: solid waxy substance, m.p. = 54 to 58 °C (20244 Aldrich Handbook of fine chemicals, p. 2021.

For better characteristics, the selected plasticizers were evaluated by DSC (differential scanning calorimetry) and TGA (thermogravimetric analysis). DSC measurements were performed on instrument DSC 2010 (TA Instruments, New Castle, DEL/USA), TGA measurements on instrument TGA 500 from same manufacturer. Measuring conditions were identical in both cases (temperature range ΔT = 25°–350 °C, dT/dt = 5 °C min^{-1}, N_2 flow = 150 ml min^{-1}). Thermal co-ordinates of detected endothermal peaks and the corresponding cumulated mass loss in TGA measurements of studied plasticizers are overall summarized in Table 2.

Table 2
Characteristics of thermal behaviour of plasticizers under study

Plasticizer	Peak	T (°C) (DSC)	$-\Delta m$ (%mass) (TGA)
Glycerol			0.0
	$ENDO_{1\ start}$	120	4.0
	$ENDO_{1\ peak}$	192.2	82.4
	$ENDO_{1\ end}$	213	90.0
PEG 300	$ENDO_{1\ start}$	203.9	4.8 ± 1.7
	$ENDO_{1\ peak}$	248.5	17.6 ± 1.13
	$ENDO_{1\ end}$	301.3	52.0 ± 1.0
		348	92.17 ± 1.38
PEG 400	$ENDO_{1\ start}$	218.1 ± 22.7	5.3 ± 0.9
	$ENDO_{1\ peak}$	290.8 ± 23.1	27.3 ± 0.9
	$ENDO_{1\ end}$	333.6 ± 5.0	62.3 ± 1.4
		348	74.5 ± 0.3
PEG 600	$ENDO_{1\ start}$	256.6 ± 1.5	3.47 ± 0.04
	$ENDO_{1\ peak}$	302.4 ± 7.4	10.25 ± 0.77
	$ENDO_{1\ end}$	342.7 ± 5.5	$29.1 \pm .98$
PEG 3350	$ENDO_{1\ start}$	50.8 ± 1.0	0
	$ENDO_{1\ peak}$	60.2 ± 0.56	0
	$ENDO_{1\ end}$	66.8 ± 0.14	0
		348	<1.0

Temperature co-ordinates of detected endothermal DSC peaks (°C) and related average cumulated mass loss on TGA curves ($-\Delta m$, %mass).

Films of collagen hydrolysate (non-plasticized, also plasticized with glycerol and poly(ethylene glycols) of the above mentioned mean molecular masses) were prepared by casting 30% (w/w) aqueous solutions of hydrolysate containing incorporated plasticizer (0–50% based on protein dry matter) on flat silicone dishes, and drying films at 35 °C in a forced ventilation drier. Thickness of obtained films was 0.2 ± 0.05 mm.

The efficiency of plasticizers was evaluated on the basis of depressed glass transition temperature (T_g) which depended on their concentration in a given film (0, 10, 30, 40 and 50 mass% based on protein dry matter). The thermal co-ordinate in the middle of the interval of descending basic line of the DSC curve is regarded as the glass transition temperature (T_g) [20].

Films of collagen hydrolysate are dehydrated with difficulty even when dried at 105 °C in a forced ventilation drier and moisture, which is a natural plasticizer for proteinous films in general, makes assessing efficiency of actual plasticizers rather complicated. The influence of water content on thermal co-ordinates of characteristic peaks (or descent) of non-plasticized H films, as determined by TGA and DSC measurements of H films under same conditions (temperature interval $\Delta T = 25°–350$ °C, $dT/dt = 5$ °C min^{-1}, nitrogen flow 150 ml min^{-1}) is demonstrated in Table 3. Data of Table 3 are complemented with DSC and/or TGA data of non-plasticized H film dried in a forced circulation drier for 4 and 12 h at 105 °C (see Fig. 1A–D).

To isolate plastication effect of water and hydrophilic plasticizers themselves, the DSC measurements were conducted in open pans (instrument DSC 2000, TA Instruments, New Castle, DE/U.S.A.) in two stages. In the first stage (measurement in temperature interval 25–150 °C, $dT/dt = 5$ °C min^{-1}, N_2 flow = 150 ml min^{-1}), a single wide endothermal peak could be detected on DSC curves in the 30–120 °C range and that was clearly associated with evaporation of moisture. Hence, adsorbed moisture was eliminated from films during the first measurement. Following measurement, after cooling the sample to 25 °C without withdrawal from the instrument (temperature

Table 3
Effect of drying on temperature co-ordinates of DSC characteristics (°C) and related cumulated mass loss TGA curves (%w/w) of non-plasticized collagen hydrolysate films

Designation	1	2	3*	4	5	6**
Dried (°C)	25	35	105	105	105	105
Hours	72	72	4	6	8	12
Water (%)						
Adsorbed	11.8	11.6	4.8	3.6	2.7	2.0
Structural	3.6	3.8	3.6	3.4	3.7	2.4

	DSC (°C)	TGA (%w/w)	DSC (°C)	TGA (%w/w)	DSC (°C)	TGA (%w/w)	DSC (°C)	TGA (%w/w)	DSC (°C)	TGA (%w/w)	DSC (°C)	TGA (%w/w)
$E_{1\ start}$	38.4	2.2	36	3.2	30.3	1.0	30.3	0.3	28.7	0	28.8	0
$E_{1\ peak}$	62.9	8.1	56.6	7.6	37.9	2.0	37.9	1.2	36.4	1.1	37.8	1.0
$E_{1\ end}$	126.2	11.7	114.7	10.8	112.1	4.8	101.5	3.6	104.5	2.7	101.5	3.0
$T_{g\ start}$	137.7	11.7	175	11.8	177.3	5.2	180.3	4.4	175.8	2.7	177.3	3.0
$T_{g\ mean}$	150.8	11.7	188.5	11.8	187.9	5.8	187.8	4.6	187.8	2.7	195.4	3.0
$T_{g\ end}$	163.9	11.7	198.3	11.8	200.7	6.4	196.9	4.8	201.5	2.7	200.1	3.5
$E_{2\ start}$	180.3	11.8	199.3	11.9	204.5	7.2	204.5	5.6	203	3.7	200.5	4.9
$E_{2\ peak}$	189	14.1	209.8	13.0	212.7	8.0	212.1	7.4	214	5.3	209.1	5.5
$E_{2\ end}$	204.9	15.2	219.7	15.7	219.7	9.4	133.3	10.6	219.7	6.4	216	5.9
$E_{decomp.\ start}$	255	21.1	249.2	20.5	244.7	13.2	248.5	13.2	245	10.1	248.5	11.9
E_{peak}	292.4	35.4	290	35.7	290.9	30.0	295.7	33.6	292.4	27.7	292.4	29.2
E_{end}	341.8	56.2	348	56.2	348	54.4	348	54.8	348	51.5	348	49.7

* For corresponding DSC traces see Fig. 1A.
** For corresponding DSC traces see Fig. 1C.

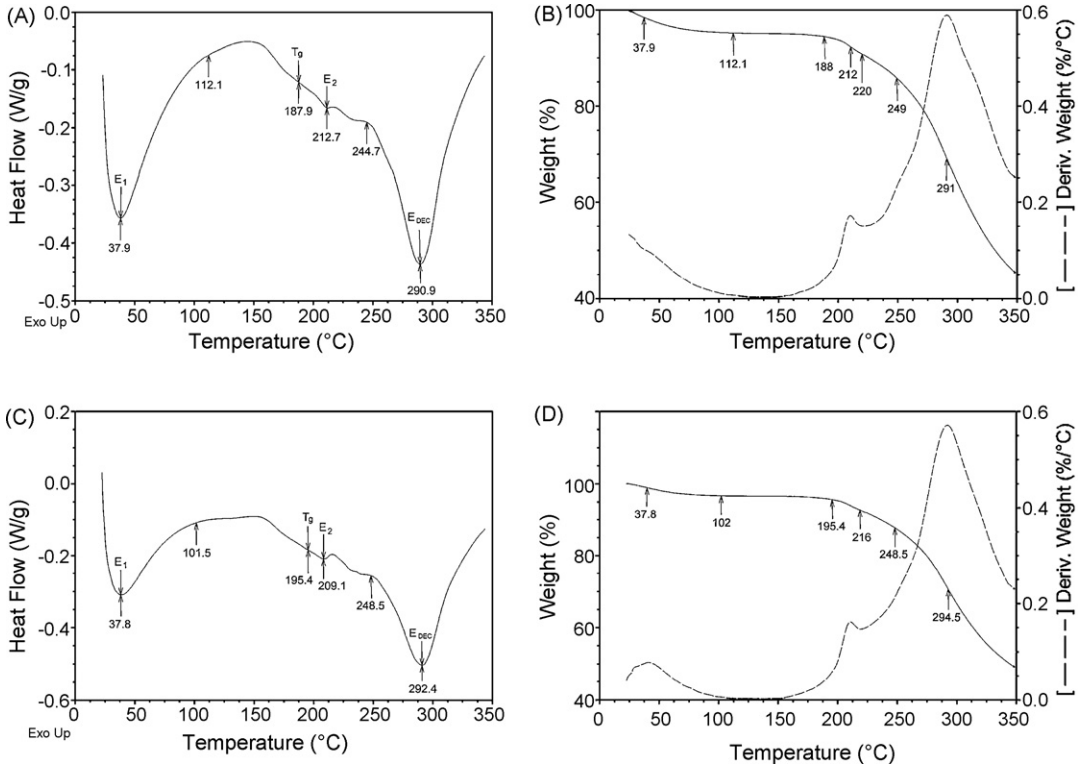

Fig. 1. Thermal analysis of non-plasticized H films dried at 105 °C in forced ventilation air drier for various time. (Thermal analysis condition: ΔT = 25–350 °C, dT/dt = 5 °C min^{-1}, nitrogen flow rate = 150 ml min^{-1}): (1A) DSC curve of film dried for 4 h (see also Table 3, sample 3); (1B) TGA curve of film dried for 4 h (a = TGA curve, b = 1st derivative of TGA curve;(1C) DSC curve of film dried for 12 h (see also Table 3, sample 6); (1D) TGA curve of film dried for 12 h (a = TGA curve, b = 1st derivative of TGA curve).

interval 25–350 °C under otherwise conditions as previous), produced curves corresponding to films disposed of adsorbed water. Results of both measurements of non-plasticized H film are arranged in tabular manner for facilitated orientation in Table 4, corresponding DSC traces for illustration are shown in Fig. 2. Fig. 3A–D then shows typical DSC curves of the second scan illustrating both of H films plasticized with 30% studied plasticizers.

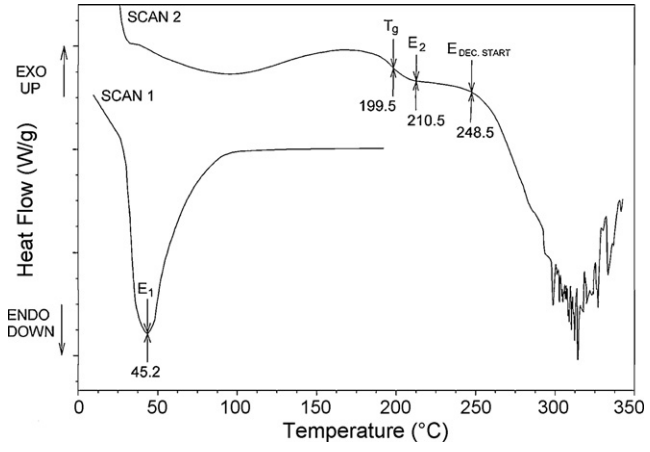

Fig. 2. DSC traces of characteristic two-stage scanning of H film prepared by casting and drying in forced ventilation drier for 24 h at 35 °C. (1) first scan, temperature range 25–150 °C and (2) second scan, temperature range 25–350 °C.

Table 4
DSC (T, °C) and TGA ($-\Delta m$, %w/w) characteristics of two-stage scanning of H films prepared by casting and drying in forced ventilation drier for 24 h at 35 °C

	1. Scan (25–150 °C)		2. Scan (25–350 °C)	
Water sorbed (%)	9.8%		–	
Water structural	–		2.3	
	DSC T(°C)	TGA Δm (%w/w)	DSC (°C)	TGA Δm (%w/w)
$E_{1\ start}$	29.8	1.9		0.0
$E_{1\ peak}$	45.2	7.9	Missing	0.0
$E_{1\ end}$	114.7	9.8		0.0
$T_{g\ start}$	–	–	187.1	0.0
$T_{g\ mean}$	–	–	199.5	0.8
$T_{g\ end}$	–	–	204.5	1.0
$E_{2\ start}$	–	–	206.1	1.8
$E_{2\ peak}$	–	–	210.5	2.1
$E_{2\ end}$	–	–	219.7	3.3
$E_{Decomp.\ start}$	–	–	248.5	9.2

1. Scan: ΔT = 25–150 °C, dT/dt = 5 °C min^{-1}, N_2 flow rate = 150 ml min^{-1}, corresponding DSC trace 1 in Fig. 2. 2. Scan: ΔT = 25–350 °C, dT/dt = 5 °C min^{-1}, N_2 flow rate = 150 ml min^{-1}, corresponding DSC trace 2 in Fig. 2-2.

Temperature co-ordinates of characteristic peaks (or descent) on DSC curves of H films in dependency on type and concentration of studied plasticizers are arranged in Table 5.

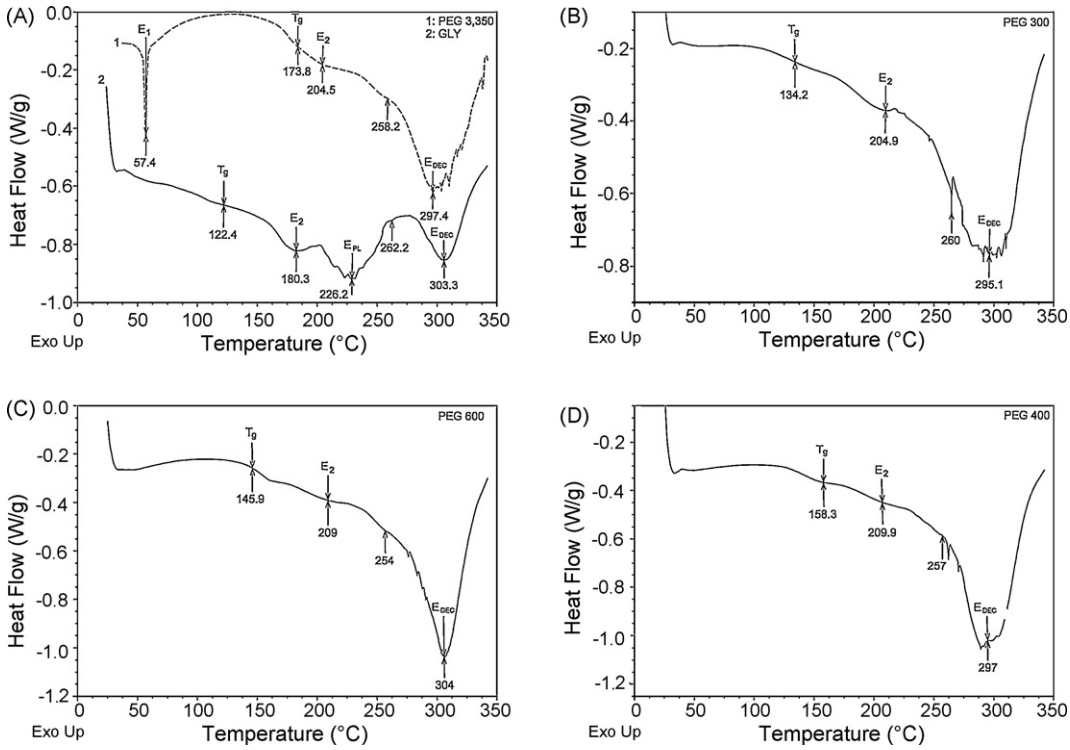

Fig. 3. Typical DSC curves of second measurement of collagen hydrolysate films plasticized with 30% plasticizers under study. (See corresponding data in Table 5.) (A) 1-GLY, 2-PEG 3350; (B) PEG 300; (C) PEG 600 and (D) PEG 400.

3. Discussion

DSC curves of H films are similar to DSC curves of gelatin and their course (the same as with gelatin) is strongly affected by moisture content. The wide endothermal peak (peak E_1 in Table 3) in the 30–120 °C region with a minimum around 38 °C is often linked with a gel → sol phase transition [21]. Its width is undoubtedly associated with moisture content in the given film. Cumulated mass loss in this temperature interval, according to TGA curves, ranges within limits 11–3% mass depending on drying degree, which indicates the peak is related to a potential gel → sol transition and, above all, to evaporating adsorbed moisture from films (see also refs. [22,23]).

Water, as a natural and very efficient plasticizer for proteins, chiefly affects [24] the glass transition temperature of H films. The earlier frequently employed method for evaluating T_g of proteinacious films, which involved their conditioning under constant relative humidities, is influenced both by conditioning prerequisites as well as by content of hydrophilic plasticizers that alter their water sorption characteristics. Thus, evaluating efficiency of hydrophilic plasticizers properly by that procedure has somewhat limited informative quality. The double DSC scanning of H films technique leads to more unambiguous results and permits separating the plasticizing effect of hydrophilic plasticizer from the plasticizing effect of film-adsorbed moisture.

Data in Table 4 show that films obtained by drying at 35 °C for 24 h merely lose adsorbed water during the first DSC measurement (temperature interval 25–150 °C). In film thus dehydrated, there remains only approximately 2% water bound structurally (more firmly, see DSC peak E_2 in Table 4, DSC trace 2 in Fig. 2). This result is otherwise obtained only by drying the sample at 105 °C for at least 12 h. During the second measurement (after cooling the measured sample to 25 °C without withdrawal from apparatus), conducted in the temperature interval of 25–350 °C under otherwise identical conditions ($dT/dt = 5\,°C\,min^{-1}$, N_2 flow = 150 ml min^{-1}), non-plasticized H film displays a glass transition temperature of 199.5 °C. This is in good agreement with the T_g value of dehydrated gelatin quoted, e.g., by Yannas ($T_g = 196 \pm 3$ °C, see ref. [25]), so that elimination of the plasticizing effect of moisture may be assumed.

The plasticizing effect of hydrophilic plasticizers alone, as evaluated on the basis of depressed T_g (see Table 5), depends on their type and concentration. For facilitated survey, the dependencies (T_g vs. % plasticizer in film) are presented graphically in Fig. 4.

Experimental data reveal (see also Table 5) that T_g of H films is most strongly depressed by glycerol ($\Delta T_g \approx -66.5$ °C). Moreover, the depression achieved has a tendency to increase with an increasing concentration of plasticizer in film. On the contrary, plasticizers of poly(ethylene glycol) type achieve a certain limit depression of T_g at a concentration of approximately 20% (with PEG 300 $\Delta T_g \approx -48.8$ °C; PEG 400 $\Delta T_g \approx -47.1$ °C; PEG 600 $\Delta T_g \approx -50.3$ °C). With a mean molecular mass of 3350 Da with poly(ethylene glycol), a steep decrease in depressed T_g appears ($\Delta T_g \approx -9.6$ °C).

The achieved results are in accord with the relatively small plasticizing effect of starch dialdehyde on films of collagen hydrolysate cross-linked with this substance [23], or of poly(vinyl alcohol) which is discussed in association with

Table 5
Temperature co-ordinates of significant peaks and T_g (°C) region of 2nd DSC scanning of collagen hydrolysate films plasticized with plasticizers under study

Plasticizer		Temperature co-ordinates of peaks minima in (°C)					
Type	(%w/w)	E_1	T_g	E_2	$E_{Plasticizer}$	$E_{Decomp.\ start}$	$E_{Decomp.\ peak}$
0	0	37.9	199.5	210.5	–	248.5	291.0
GLY	10	–	135.0	186.1	227.9	265.6	301.6
	20	–	125.9	181.9	225.4	262.3	303.3
	30*	–	122.4	180.3	226.2	262.2	303.3
	40	–	119.8	168.8	221.3	263.9	306.5
	50	–	116.4	163.9	219.6	263.9	304.9
PEG 300	10	–	145.9	203.0	–	263.9	301.5
	20	–	135.4	204.0	–	264.0	295.2
	30**	–	134.2	204.9	–	260.0	295.1
	40	–	133.3	208.0	–	255.7	295.1
	50	–	133.0	196.7	–	257.3	288.5
PEG 400	10	–	170.9	203.3	–	255.7	291.8
	20	–	162.3	206.2	–	253.3	295.3
	30***	–	158.3	209.9	–	257.0	297.0
	40	–	153.9	204.9	–	255.7	290.2
	50	–	152.4	211.5	–	249.2	293.4
PEG 600	10	–	157.5	203.3	–	268.0	304.9
	20	–	146.9	208.2	–	265.6	301.6
	30****	–	145.9	209.0	–	254.0	304.0
	40	–	145.9	204.9	–	262.0	303.1
	50	–	146.7	208.1	–	254.2	306.0
PEG 3350	10	57.4	183.6	218.0		267.2	304.9
	20	59.0	177.0	208.9	–	267.2	309.8
	30[a]	57.4	173.8	204.5	–	258.2	297.4
	40	55.8	173.2	200.0	–	254.9	303.1
	50	59.0	173.8	209.8	–	252.4	298.3

[a] See trace 1 in Fig. 3A.
* See trace 2 in Fig. 3A.
** See Fig. 3B.
*** See Fig. 3D.
**** See Fig. 3C.

gelatin by Sarti and Scandola [7]. The reduced plasticizing efficiency of starch dialdehyde may be associated with its cross-linking of H film; with poly(vinyl alcohol), the above mentioned authors relate the reduced plasticizing effect to its limited compatibility with gelatin.

The more firmly bound part of structural moisture, in the case of non-plasticized H films evident on DSC curves as an endothermal peak in the 180–220 °C region (peak E_2, see Table 3), immediately follows the glass transition region. According to TGA curves, it corresponds to a mass loss of 2.7–3.6% (depending on drying mode, compare Table 3) and may be attributed to the loss of moisture structurally bound in film. Comparing the content of adsorbed and structurally bound water with glass transition temperature of films in this table demonstrates that glass transition temperature is much more strongly affected by adsorbed water than by structurally bound water.

With gelatin, the removal of structurally bound water is linked with markedly retarded swelling of its films and their lower water solubility [26], hence also with their retarded biodegradation. Our experiments (not yet fully finished) confirm this trend also with H films. The usefulness of expelling structurally bound water from these films appears therefore to be somewhat problematic.

The endothermal DSC peak, attributed to structurally bound water, also appears with films plasticized with glycerol (see peak E_2 in Table 5) and poly(ethylene glycols). Films plasticized with poly(ethylene glycol) indicate a certain tendency shifting the endothermal minimum of such a DSC peak to a somewhat higher temperature range (approximately 200–210 °C). That may be associated with water binding more strongly to plasticizer than to protein, or also with evaporation of lower-molecular components of poly(ethylene glycols) under study. Cumulated mass loss values of films on corresponding TGA curves of these films rather favour the first of the mentioned alternatives.

Admissible thermal stress of H films is limited by the onset of their thermal breakdown, which may be registered with non-plasticized films in the 245–250 °C range (see DSC peak $E_{decomp.}$ in Table 3). This value agrees with thermal stability data by some earlier authors [25,27]. In addition, H films plasticized with glycerol exhibit a well developed endothermal peak with a minimum at 225–226 °C, which on TGA curves of glycerol corresponds to its 96% cumulated mass loss. Tabulated values of glycerol boiling point (bp_{760} = 290 °C, with degradation (see ref. [28]) are considerably higher and we may thus conclude that the detected peak reveals evaporation rather than thermal breakdown of glycerol. Obvious thermal decom-

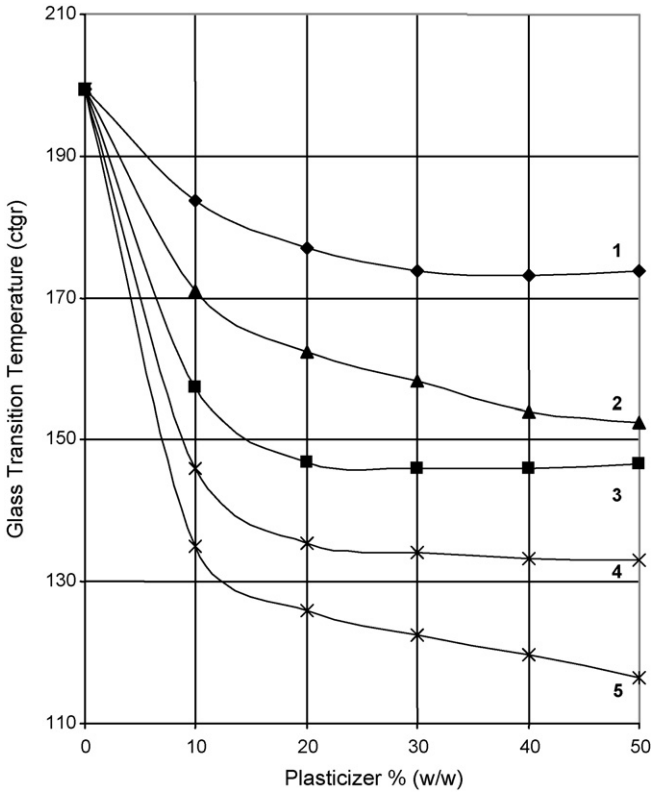

Fig. 4. Dependency of T_g (°C) of collagen hydrolysate plasticized films on concentration of hydrophilic plasticizers under study. (1) PEG 3350, (2) PEG 400, (3) PEG 600, (4) PEG 300 and (5) GLY.

position of glycerol-plasticized films then comes about in the region of 250–260 °C, the same as with films plasticized with poly(ethylene glycols), and it seems hydrophilic plasticizers do not influence the degradation of H films in a more pronounced manner.

The temperature regions of T_g (even with plasticized films), regions where structurally bound water gets lost and finally also regions of temperature breakdown for H films are quite close, which may somewhat limit potential application of procedures well known in thermoplastics processing which combine the effect of pressure and temperature. Dipping technique applied in the manufacture of soft (SGC) and also hard (HGC) gelatin capsules will hence probably maintain a dominant role even when collagen hydrolysates are processed into edible and biodegradable packaging.

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