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BIODEGRADABLE POLYMERS FROM RENEWABLE RESOURCES: EFFECT OF PROTEINIC IMPURITYON POLYCONDENSATION PRODUCTS OF 2-HYDROXYPROPANOIC ACID

BIORAZGRADLJIVI POLIMERI IZ OBNOVLJIVIH VIROV: VPLIV PROTEINSKIH NEČISTOT NA PRODUKTE POLIKONDENZACIJE 2-HIDROKSIPROPANOJSKE KISLINE

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The goal of the presented work is to describe the proteinic impurity (as possible residual component from biotechnological lactic acid production from whey) in the reaction mixture with 2-hydroxypropanoic acid (lactic acid) for its direct polycondensation into poly(lactic acid). The impurity presence study was carried out in the range from mass fractions 0 % up to 2 %. For an evaluation of the impurity effect on the values of molecular weight, melting and glass transition temperatures the measurements of intrinsic viscosity, gel permeation chromatography and differential scanning calorimetry were used. Results show slight reduction of molecular weight and increase in polydispersity with rising amount of the impurity. The thermal properties investigation shows noticeable reduction of melting temperature and moderate decrease in glass transition temperature. The results reveal considerable role of lactose on formation of structural irregularities in lactic acid polycondensates.

Keywords: poly(lactic acid), whey protein, polycondensation, impurity effect

V prispevku je predstavljena študija vpliva proteinskih nečistot, ki so v reakcijski mešanici, na polikondenzacijo 2-hidroksipropanojske kisline (mlečne kisline) do polimlečne kisline. Koncentracijsko območje nečistot (komponente, ki ostanejo pri biotehnološki pridelavi mlečne kisline iz sirotke) je bilo od 0 % do 2 % glede na monomer. Z meritvami lastne viskoznosti in z gelsko prepustnostno kromatografijo ter diferenčno dinamično kalorimetrijo smo preučevali vpliv nečistot na povprečno molsko maso, na temperaturo tališča in na temperaturo steklastega prehoda nastalih polimerov. Z naraščajočo koncentracijo nečistot vrednost povprečnih molskih mas pada, narašča pa indeks polidisperznosti polimernih molekul. Rezultati raziskave termičnih lastnosti kažejo znižanje temperature tališča in tudi zmerno znižanje temperature steklastega prehoda z naraščajočo koncentracijo nečistot. Iz rezultatov študije je razvidna pomembna vloga laktoze na tvorbo strukturnih nepravilnosti pri polikondenzaciji mlečne kisline.

Ključne besede: polimlečna kislina, protein sirotke, polikondenzacija, vpliv nečistot

1 INTRODUCTION

Biodegradable and biocompatible polymers have attracted increasing interest over the past two decades both in the fundamental research and also in practical use.[1–3] Among them, the polymers from renewable resources are paid a special attention due to environmental concerns as well as sustainability factors. Poly(lactic acid) (PLA) is of the polymers, which is considered as biodegradable and obtainable form the not petroleum based raw materials. PLA belongs in a group of biodegradable polyesters and it represents its applicability in packaging technology but also in highly complex medical field as materials for drug delivery, orthopaedics, sutures and scaffolds due to its biocompatible properties.[3–18]

Production of the monomer for PLA preparation, 2-hydroxypropanoic acid, (known under trivial name lactic acid) can be carried out through both synthetics and biotechnological route. The latter way mostly includes microbial fermentation of saccharidic substrates into lactic acid and its subsequent polymerization. One of the promising renewable resources for lactic acid production is by-product of dairy industry – whey. Liquid whey appears in large quantities and its annual production rises continuously. Despite of its using in several applications (e.g. food supplements), a lot of whey is still wasted. Consequently, there is significant interest in finding new utilization methods for that.[19,20]

The polymerization step represents the most difficult part of PLA preparation process due to necessity of high

monomer purity. However, biotechnologically prepared lactic acid contains various impurities (co-products, residual lactose, protein and inorganic components)[21–25]. Purification of lactic acid represents additional economical loads, which reduce a competitiveness of this polymer with petroleum based plastics. In spite of extended research in the field of PLA synthesis, the effect of impurities has not been studied.

This work is focused on investigation of the effects of a residual proteinic impurity in lactic acid as monomer for PLA production via direct polycondensation reaction. This factor was quantified by observation of the changes in molecular weight distribution, thermal properties of the resulting product of the polycondensates.

2 EXPERIMENTAL PART

2.1 Materials

L-lactic acid (LA) $C_3H_6O_3$, 80 % water solution, optical rotation α = 10.6° (measured by the polarimeter Optech P1000 at 22 °C, concentration of 10 %) was purchased from Lachner Neratovice, Czech Republic. Stannous 2-ethylhexanoate ($Sn(Oct)_2$) (≈95 %) was supplied by Sigma Aldrich, Steinheim Germany. The solvents acetone C_3H_6O, chloroform $CHCl_3$ andmethanol CH_4O (all analytical grade) were bought from IPL Lukes, Uhersky Brod, Czech Republic. Whey protein concentrate (PROT) with a certified composition with mass fractions of 75 % protein, 17.5 % lactose and 7.5 % minerals was provided by Milcom a. s. Prague, Czech Republic.

2.2 Polycondensation

A typical procedure was as follows: relevant portions of LA and PROT (0, 0.25, 0.5, 0.75, 1.5 and 2) % – related to LA. Samples are designed as PLAPROT *X*/%, where *X* represents concentration of the PROT in the reaction mixture) were added into a double necked flask (250 mL) equipped with a Teflon stirrer. Total mass of the mixture at the beginning of reaction was 50 g (water is not included). The flask was then placed in an oil bath heated by magnetic stirrer with heating and connected to a laboratory apparatus for distillation under reduced pressure. The dehydration step followed at 160 °C, reduced pressure 15 kPa for 4 h. After that, the reactor was disconnected from the vacuum pump and the relevant amount (0.5 %, related to initial mass of the reactants) of the catalyst ($Sn(Oct)_2$) was added dropwise under continuous stirring. The flask with dehydrated LA/PROT/catalyst mixture was connected back to the source of vacuum (100 Pa) and the reaction continued for 24 h at the temperature 160 °C. The resulting product was allowed to cool down at room temperature and then dissolved in acetone. The polymer solution was precipitated in a mixture of chilled methanol/distilled water 1 : 1 (volume fraction) The obtained product was filtrated, washed with methanol and dried at 45 °C for 48 h. The dissolving-precipitation procedure was repeated three times.

2.3 Characterization methods

2.3.1 Intrinsic viscosity determination

Viscosity measurements were performed in chloroform at 30 °C in an Ubbelohde viscometer with capillary 0a. The intrinsic viscosity η was calculated using the equation (1):

$$[\eta] = \lim_{c \to 0} \frac{\eta_{rel} - 1}{c} \quad (1)$$

where η_{rel} is the relative viscosity, which is equal to the ratio of polymer solution and pure solvent viscosities and c is the concentration of the polymer solution (0.4, 0.8 and 1.2) %.

2.3.2 Determination of molecular weight by gel permeation chromatography (GPC)

GPC analyses were performed using a chromatographic system Breeze (Waters) equipped with a PLgel Mixed-D column ((300 × 7.8) mm, 5 μm) (Polymer Laboratories Ltd). For detection, a Waters 2487 Dual absorbance detector at 239 nm was employed. Analyses were carried out at room temperature with a flow rate of 1.0 mL min^{-1} in chloroform. The column was calibrated using narrow molecular weight polystyrene standards with molar mass ranging from 580 g mol^{-1} to 480 000 g mol^{-1} (Polymer Laboratories Ltd). A 100 μL injection loop was used for all measurements. The sample concentration ranged from 1.6 mg mL^{-1} to 2.2 mg mL^{-1}. Data processing was carried out using the Waters Breeze GPC Software (Waters). The weight average molar mass M_w, number average molar mass M_n and polydispersity (M_w/M_n) of the tested samples were determined.

2.3.3 Differential scanning calorimetry (DSC)

For the determination of glass transition temperature T_g, melting point T_m and crystallinity χ_c of the polycondensates the differential scanning calorimetry was used. Approximately 8 mg of the sample were placed in an aluminium pan, sealed and analyzed on NETZSCH DSC 200 F3, calibrated in terms of temperature and heat flow, using indium. The experiments were performed under nitrogen atmosphere (60 mL min^{-1}) in two scans in the temperature range of (0–180) °C and at the heating rate of 10 °C min^{-1}.

3 RESULTS AND DISCUSSION

The basic characteristics of the samples prepared by melt polycondensation of LA and *PROT* are shown in **Figures 1 and 2**. The value of η decreases with increasing impurity content. While pure PLA shows η 0.47 dL g^{-1}, PLAPROT 0.25 % proves almost 45 % reduction (0.26 dL g^{-1}). Interestingly, η of PLAPROT 0.5 % and 0.75 % fluctuates around that value. Further addition of the PROT into the reaction mixture does not influence η significantly and only slight decrease of η can be noticed (**Figure 1**). It corresponds to results from GPC analysis. The pure PLA sample has M_w 47.0 kg

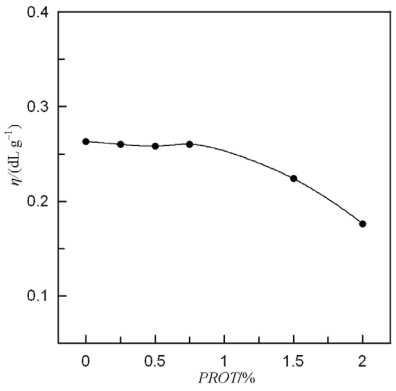

Figure 1: Dependence of intrinsic viscosity on PROT impurity concentration

Slika 1: Odvisnost lastne viskoznosti od koncentracije *PROT*-nečistot

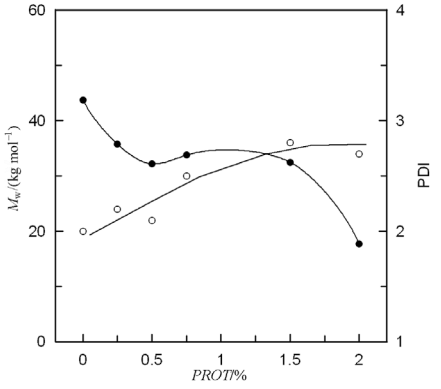

Figure 2: *PROT* concentration versus M_w (filled symbols)) and PDI (empty symbols)

Slika 2: Odvisnost masnega povprečja molskih mas M_w, (polni simboli) in indeksa polidisperznosti, PDI, (prazni simboli) od koncentracije PROT-nečistot

mol^{-1} and polydispersity index ($PDI = M_w/M_n$) 2.08. The presence of PROT impurity is attended by slight M_w reduction and rising PDI. The sample designated PLAPROT 2 % (M_w = 23.4 kg mol^{-1}, PDI = 3.0) can be still considered as polycondensates (Figure 2). The yield of the all polycondensations was over 60 % in all cases. However, it should be mentioned that it represents pure yield of the polycondensation products after purification process described in the experimental part above. These facts reveal that the presence of proteinic components do not disturb the polycondensation reaction as significantly as, for instance, other simple organic acids as we reported in our previous works.[26,27] The reason could be found in, firstly, lactose presence and secondly proteins denature at temperatures higher that 40 °C. The polycondensation was carried outat 160 °C. It means that all proteinic ingredients had denatured even before polycondensation reaction started. The question is how the low molecular products of protein denaturation influences (if even) the polycondensation reaction.

The effect of *PROT* on thermal properties is presented in **Figure 3**. Pure PLA has a melting temperature, T_m, positioned at 151.2 °C and glass transition temperature T_g at 53.8 °C (**Figure 3**). Increasing content of

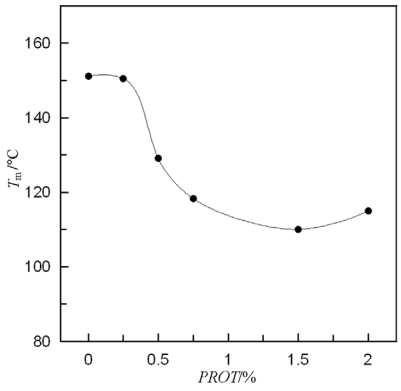

Figure3: Dependence of melting temperature of PLA and PLAPROT samples on concentration of the impurity

Slika 3: Odvisnost temperature tališča PLA- in PLAPROT-vzorcev od koncentracije nečistot

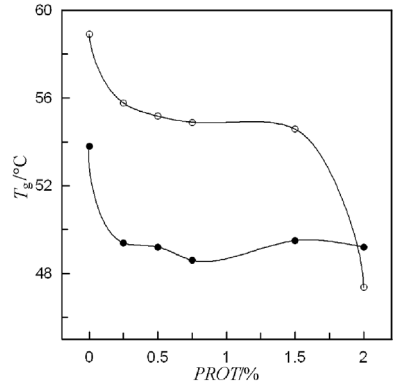

Figure 4: Glass transition temperature versus PROT concentration, full symbols represents experimental data, empty symbols represents Fox-Flory prediction of glass transition temperature (T_g^∞ = 58 °C and $K = 5.5 \cdot 10^4$)[17]

Slika 4: Odvisnost temperature steklastega prehoda od koncentracije PROT-nečistot; polni simboli pomenijo eksperimentalno pridobljene vrednosti, prazni simboli pa vrednosti, izračunane s Fox-Floryjeve enačbo za napoved temperatur steklastega prehoda (T_g^∞ = 58 °C in $K = 5{,}5 \cdot 10^4$)[17]

PROT in the reaction mixture leads to reduction of both T_m and T_g. The sample with the highest impurity content, PLAPROT 2 %, shows T_m lower about almost 23 °C. Reduction in T_g is about 4.6 °C, which is not as significant as in case of T_m. It means that the impurity presence effects mostly ability of the polycondensates chains to organize into highly uniform structures. It can be the result of molecular weight reduction.[28] However, the structural changes cannot be excludes due to low difference between T_m values of pure PLA and PLA-PROT 0.25 % whose M_w varies noticeably (**Figure 2**). The same assumption can be done on the basis of T_g measurements, where Fox-Flory (T_g^∞ = 58 °C and $K = 5.5 \cdot 10^4$)[29] for theoretical prediction of T_g from M_n fails (**Figure 4**).

These facts can led to an assumption that the impurity presence causes a non-selective branching of the PLA chain as well as inhibition of the PLA chain growth. On the basis of the fact that proteins are supposed to undergo denaturation at high temperatures,

the lactose can play important role here. This is supported by our preliminary results when the effect of pure lactose was studied[30,31].

4 CONCLUSIONS

The aim of this work was to investigate the effect of the proteinic impurities in the reaction mixture on resulting properties of the lactic acid polycondensates. The protein concentrate originating from the real biotechnological production was used as a source of impurities. The obtained results indicates that protein components potentially presented in the reaction mixture as residuals (up to the mass fraction 2 % related to lactic acid) from biotechnological lactic acid production do not effect polycondensation reaction significantly by means of molecular weight reduction. However, slight decrease was observed. On the other hand, noticeable decreases were observed in melting temperatures of the samples. Glass transition temperatures were reduced moderately with increasing content of the whey protein concentrate. It reveals possible structural modification of polycondensates chains (random branching). The presence of disaccharide seems to play important role here. Detailed studies of this factor will be accomplished in our future work.

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