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Impregnation of Poly(lactic Acid) with Polyphenols of Plant Origin

Abstract
The aim of the research was the solvent-based impregnation of poly(lactic acid) (PLA) with polyphenols of plant origin. The process is a scientific novelty because quercetin, rutin, xanthone and green tea extract had not been previously used to stabilise PLA as a result of the solvent-based impregnation of a polymer. As part of the work, the extruded poly(lactic acid) was impregnated with ethanol solutions of polyphenols. Samples after impregnation were subjected to tests: determination of mechanical properties, differential scanning calorimetry, melt flow index and SEM microscopy. In addition, the properties of the samples, such as the Vicat softening temperature and change in colour, before and after controlled weathering as well as thermal and UV aging were investigated. On the basis of the results presented, the effectiveness of the impregnation of poly(lactic acid) with natural compounds was confirmed as a new method of stabilisation of the biodegradable polyester selected.

Key words: *poly(lactic acid), PLA, natural polyphenol, antioxidants, impregnation.*

RESEARCH & DEVELOPMENT

Introduction

Poly(lactic acid) (PLA) is a more and more commonly used biodegradable polymer. It is an aliphatic, biodegradable polyester, and can be derived from 100% renewable resources. The first time this polymer was described was in 1932 by Carothers (DuPont), who produced a low molecular weight product by heating lactic acid under a vacuum. Due to its availability, high cost of manufacture, and low molecular weight, the initial uses of PLA were limited to medical and pharmaceutical applications such as sutures, resorbable implants and controlled drug-release applications [1-3]. Developed PLA synthesis techniques for polycondensation and ring-opening polymerisation reported in the last decade, on the basis of industrial technique modifications and advanced laboratory research, have allowed to lower its price and increase availability [4-5]. Currently, poly(lactic acid) has many areas of application. In addition to medical applications, it is gaining popularity as a packaging material [6-7]. Due to the fact that it is biodegradable, it can be used for disposable packaging, such as loose-fill packaging, compost bags, food packaging, and disposable tableware. PLA can also be applied in the form of fibres. Fibres made of poly(lactic acid) have good moisture-wicking properties, and clothing, textiles and non-woven fabrics can be successfully made from them [8-10].

Depending on the potential applications, poly(lactic acid) can be modified by the addition of other substances. The modifications are aimed at improving the properties of the polymer or obtaining specific features of the finished product.

Due to the very favourable properties, substances of plant origin are increasingly used as additives to polymers.

One of the methods of PLA modification is the impregnation of its surface. Literature describes the supercritical impregnation of poly(lactic acid), as well as its composite and nanocomposite with two plant substances: thymol and cinnamaldehyde [11-14]. Thymol is one of the most abundant components of thyme and oregano. It has received considerable attention because of its antimicrobial activity and antioxidant capacity [15-19]. Cinnamaldehyde is a biologically active compound present in the essential oil of the genus Cinnamomun, which is responsible for the distinctive flavour and aroma of cinnamon [20]. The purpose of introducing these compounds by supercritical impregnation was to obtain materials with antibacterial properties. Materials with such properties are especially dedicated as active packaging for food.

The aim of the study was the solvent-based impregnation of poly(lactic acid) with natural polyphenols as a new method of its stabilisation. The solvent-based impregnation of PLA (in EtOH) with quercetin, rutin, xanthone and polyphenol extract from green tea has not yet been described in the literature and is a scientific novelty of the work. These natural compounds were chosen for their advantageous properties that ensure the stabilisation of the polymer. In turn, ethanol is a good solvent for plant polyphenols. These natural substances have strong antioxidant properties that result from their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, natural phenolic compounds have a metal chelation potential [21-25]. Quercetin is a plant flavonol from the flavonoid group of polyphenols. It is found abundantly in almost all edible vegetables and fruits [26] and has antioxidant, anti-inflammatory, anti-bacterial, anti-coagulative, and anti-hypertensive properties. Rutin, like quercetin, has significant antioxidant activity and anti-inflammatory properties [27]. It is also called quercetin-3-rhamnosyl glucoside, a natural flavone derivative. Rutin was first discovered in buckwheat but is widespread in vegetables and fruits [28]. Another selected compound – xanthone, also shows good antioxidant and antibacterial properties. Xanthones have been isolated from 62 families of higher plants, fungi, and lichens [29]. For impregnation, in addition to individual plant substances, a mixture of polyphenols obtained from green tea was also used. HPLC analysis showed that the composition of the mixture is rich in antioxidant substances: gallic acid, (–)-epigallocatechin, (+)-catechin, (–)-epigallocatechin gallate, (–)-epicatechin, (–)-epicatechin gallate, flavonols and procyanidine B1. Due to the very complex composition and the presence of strong antioxidant substances, Polyphenone 60 is characterised by very good ability to reduce free radicals [30].

The combination of biodegradable polilactide and natural compounds allows to obtain a new generation of polymeric materials which are fully composed of natural substances, easily degradable and friendly to both the environment and health.
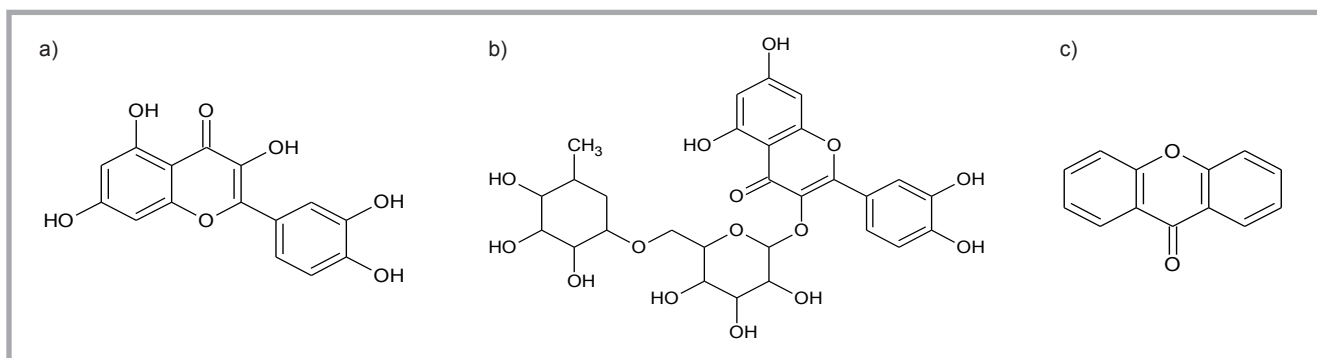
Figure 1. Chemical structure of a) quercetin, b) rutin and c) xanthone.

■ Experimental

Materials

The object of the research was poly(lactic acid) (PLA) Ingeo™ Biopolymer 4043D from Nature Works™ (USA) with the following properties: T_g = 55-60 °C; T_m = 145-160 °C; MFI = 6 g/10 min. Dried (12 h, 50 °C) granulate of PLA was extruded using a laboratory extruder. Striped samples of 1.6-1.8 mm thickness were obtained. The temperature of the working chamber of the extruder was 180 °C.

Different polyphenols of plant origin were used as a stabiliser for PLA. Quercetin (2-(3,4-Dihydroxyphenyl)-3,5,7-trihy-droxy-4H-1-benzopyran-4-one; 3,3′,4′,5,6-Pentahydroxyflavone, ≥95%, HPLC), rutin (Quercetin-3-rutinoside hydrate, Vitamin P hydrate, 95%, HPLC), xanthone (9-Xanthenone, 97%) and Polyphenon 60 from green tea were obtained from Sigma-Aldrich (Germany). (*Figure 1*) Polyphenon 60 is an extract containing a mixture of polyphenolic compounds (minimum 60% total catechins).

Impregnation of poly(lactic acid) (PLA) with polyphenols of plant origin

Extruded poly(lactic acid) strips were weighed and dried to a constant weight at 70 °C. The samples prepared were placed in sulphonation flasks equipped with a magnetic stirrer and thermocouple. 4% ethanolic polyphenol solutions were poured into the flasks. The reference sample was PLA impregnated only with ethanol without natural compounds, carried out for 4 hours at 50 °C. After 4 hours, the flask and its contents were left for 24 hours at room temperature. After this time the samples were dried in a dryer (70 °C) to a constant weight. The impregnation efficiency, % was calculated from the changes in the mass of the samples.

Measurement methods

Mechanical properties

Mechanical properties were determined using a Zwick Roell Z005 strength testing device (manufacturer Zwick Roell, Germany), according to Standard ISO 527. Extruded polymer strips with a length of 10 cm and thickness of 1.4-1.5 mm were subjected to testing. The test was performed at an initial force of 0.1 N and test speed of 50 mm/min. The following values were determined: T_{Fmax} – the maximum stress transferred by the sample, MPa, E_{Fmax} the maximum elongation of the sample at the time of break, %; TS the tensile strength, MPa; and E_b the elongation at break of the sample, %.

Differential scanning calorimetry (DSC)

Samples for DSC measurements were small pieces of the extruded strip after impregnation weighing 5-6 mg, which had a visible layer of polyphenols on the surface. PLA samples were taken from the bulk, not as a surface layer. The glass transition temperature (T_g), crystallisation temperature (T_{cc}), and melting temperature of the crystalline phase (T_m) as well as the oxidation temperature (T_o) were determined using a Mettler Toledo DSC analyser (Greifensee, Switzerland). The phase changes were accompanied by heat changes (ΔH), which were also determined. The DSC assay method comprised four segments. Samples in open, aluminium crucibles were heated from 0 to 200 °C at a rate of 20 °C/min in an argon atmosphere. After 10 min at 200 °C, the samples were cooled to 0 °C. Then, the gas was switched from argon to air (flow rate 50 ml/min), and the samples were heated to 350 °C.

Melt flow index

The melt flow rate index (MFR), cm^3/10 min and melt volume rate index (MVR), g/10 min were determined using a Melt-Flow-Index-Tester, MeltFlow@ on plus (KARG Industriatechnik), according to the ISO 1133D standard. The melt index was measured at 190 °C with a weight of 2.16 kg and preheating time of 240 seconds.

Vicat softening temperature

The Vicat softening temperature is that at which a hardened steel needle with a circular cross-section of 1 mm² penetrates the sample under load of 10 N to a depth of 1 mm. The test was done according to the standards ISO 306 (method A120) and ASTM D 1525, using D-Vicat. HDT/3/400FA apparatus. The measurement parameters were as follows: 10 N load, inductive displacement gauges with 0.001 mm resolution, temperature gradient of 120 °C/h, initial temperature of 45 °C, final temperature of 150 °C, and a displacement range of up to 15 mm.

Change in colour

Colour measurements were performed in the CIE-Lab space, the change in colour in a system of three coordinates: L, a and b was determined, where L is the lightness parameter (the maximum value of 100 represents the colour white and the minimum value of zero – the colour black). Parameter a is the axis of red–green and b is that of yellow–blue. The a and b axes have no specific numerical limits. The colour measurements were carried out using a Konica Minolta CM-3600d spectrophotometer, according to Standard PN-EN ISO 105-J01. The change in colour [31-32] (dE*ab) after UV, thermooxidation and weathering ageing was calculated as follows (1):

$$dE*ab = \sqrt{(\Delta a^2) + (\Delta b^2) + (\Delta L^2)} \quad (1)$$

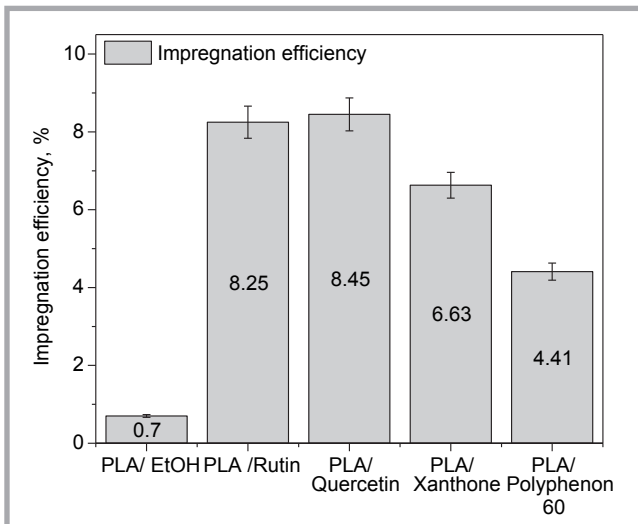Colour parameters such as the whiteness index, chroma and hue were cal-

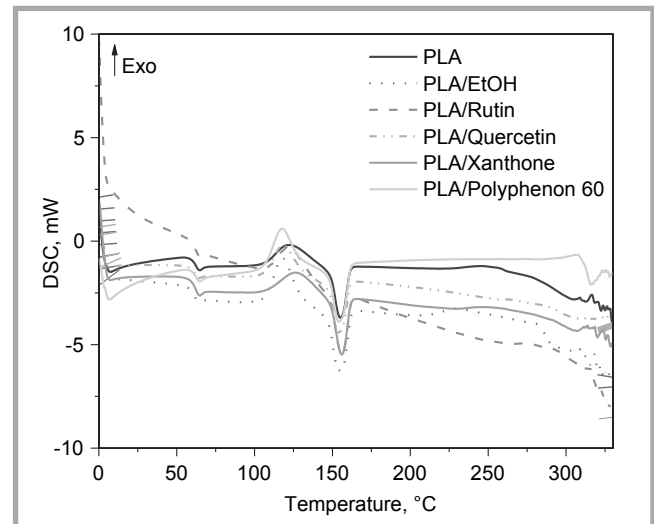*Figure 2. Impregnation efficiency of PLA with natural polyphenols.*

Figure 3. DSC thermograms of PLA samples.

culated according to the following equations:

Whiteness index:

$$Wi = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (2)$$

Chroma: $\quad C_{ab}^* = \sqrt{a^2 + b^2} \quad (3)$

Hue:

h*ab = arc tg (b/a); where a > 0 and b > 0 (4)

h*ab = 180 + arc tg (b/a); where a < 0 and b > 0 (5)

Accelerated ageing

UV ageing was carried out using a UV 2000 chamber (Atlas). The measurement lasted 100 h and was composed of two segments: the daily segment (radiation intensity 0.78 W/m^2, temperature 60 °C, duration 8 h), and the night segment (no UV radiation, temperature 50 °C, duration 4 h).

Thermooxidation ageing was determined according to the PN-82/C-04216 standard. The samples were exposed to air at 70 °C for 100h in a dryer with thermo-circulation.

Weathering was performed using a Weather-Ometer (Atlas; Ci 4000). Material weathering tests were carried out according to the ISO 4892-2 standard, and samples were subjected to two cycles: the daily cycle (radiation intensity 0.4 W/m^2, temperature 60 °C, duration 240 min, humidity 80%, rain water on), and the night cycle (no radiation, temperature 50 °C, humidity 60%, duration 120 min). The duration of the test was 100 h.

■ Results and discussion

The first step was to determine the efficiency of the impregnation process. The impregnation efficiency was calculated on the basis of the mass difference of the samples before and after impregnation. Before and after the impregnation, the samples were dried and weighed to a constant weight. *Figure 2* shows the efficiency of the impregnation process, %. The highest impregnation efficiency (around 8%) was obtained for samples with quercetin and its glycoside – rutin. Quercetin and rutin have a lot of OH groups in their structure, hence they can have a higher affinity to hydrophilic poly(lactic acid). Polyphenone 60, according to the manufacturer, has about 60% catechins. Due to the large number of hydroxyl groups of catechins, Polyphenon 60 will have a strong affinity to PLA. The impregnation efficiency of the PLA/EtOH sample was 0.7%. Compared to samples impregnated with natural polyphenols, this value was very low, and may be the result of the hydrophilic nature of poly(lactic acid).

Table 1 shows the mechanical properties of PLA before impregnation and of samples after impregnation with the reference ethanol and 4% EtOH polyphenol solutions. The impregnation of natural compounds on the PLA surface apparently caused changes in the tensile strength TS, MPa and elongation at break of the sample, E$_b$ [%]. Compared to the PLA/EtOH sample, the addition of polyphenols caused an increase in the tensile strength (14.4% for PLA/Xanthone and 99.1% for PLA/Rutin) and a decrease in the elongation at break value (22.1% for PLA/Quercetin and 63.6% for PLA/Rutin). Analysis of the PLA and PLA/EtOH samples shows that the impregnation of poly(lactic acid) with ethanol causes a decrease in the tensile strength TS and an approximate 4-fold increase in the elongation at break value. The impregnation of PLA with pure alcohol and 4% ethanol solution of polyphenols caused an increase in E$_{Fmax}$ and E$_b$, which may indicate greater polymer chain flexibility and mobility of polymer segments as well as less interaction between PLA chains in samples after impregnation. The impregnation of poly(lactic acid) with ethanol could cause a slightly plasticising effect in materials.

The next step of the study was differential scanning calorimetry (DSC), the test results of which are summarised in

Table 1. Mechanical properties of PLA impregnated with substances of plant origin.

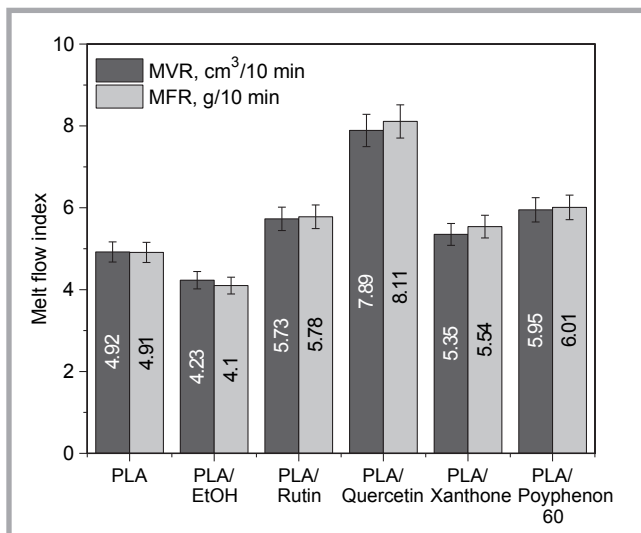| Sample	T$_{Fmax}$, MPa	E$_{Fmax}$, %	TS, MPa	E$_b$, %
PLA	54.0	3.5	51.0	4.2
PLA/EtOH	50.1	7.2	35.5	18.1
PLA/Rutin	70.7	6.6	70.7	6.6
PLA/Quercetin	63.6	10.5	57.2	14.1
PLA/Xanthone	42.8	8.1	40.6	8.3
PLA/Polyphenon 60	62.5	6.5	58.6	9.5

Figure 4. MFR index and MVR index of PLA samples impregnated with natural polyphenols.
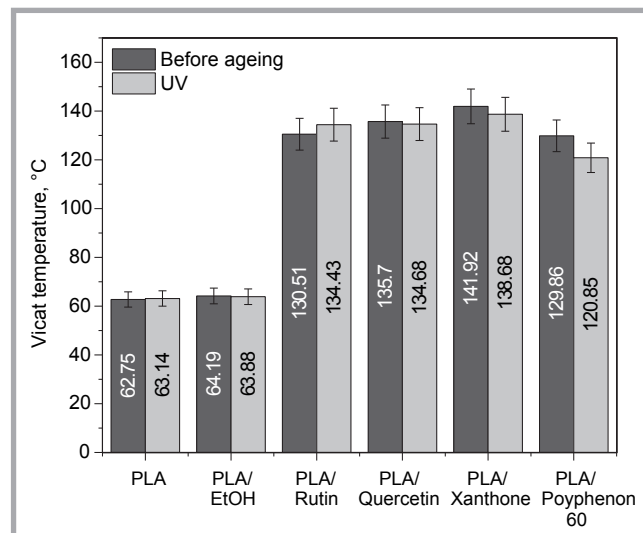

Figure 5. Vicat softening temperature of PLA samples impregnated with natural compounds

Table 2 and **Figure 3**. The addition of polyphenols did not significantly affect the glass transition temperature (T_g), crystallization temperature (T_{cc}) or melting temperature (T_m). However, the addition of all plant substances clearly increased the initial and final oxidation temperatures of the samples. The oxidation temperature is that at which an exothermic oxidation peak appears on the DSC curve. **Table 2** gives the initial (onset) and final (endest) temperatures of the oxidation peaks. The impregnation of poly(lactic acid) with Polyphenone 60 resulted in the highest increase in the oxidation temperature of the sample – in the initial temperature of about 55 °C and in the final temperature of about 16 °C. Higher temperatures of oxidation of PLA with all polyphenols can result from the chemical reactions of hydroxyl groups of natural compounds occurring during DSC measurement, in the middle of the

heating of samples. Oxidation temperatures shifted to higher values should improve samples' resistance to oxidation and increase the stability of the materials.

The crystallinity of polymeric materials can be determined by differential scanning calorimetry and knowledge of the melting enthalpy of 100% crystalline 100% polymer. The degree of crystallinity X_c was calculated as follows: X_c (%) = $\Delta H_m / \Delta H_0 \times 100$, where ΔH_m is the melting enthalpy of the test polymer and ΔH_0 is that of 100% crystalline 100% PLA. The melting enthalpy estimated for 100% crystalline PLA is from 97.3 J/g to 148 J/g [33]. 97.3 J/g was used for calculations. The impregnation of PLA with EtOH and 4% ethanol solution of polyphenols caused an increase in the degree of crystallinity X_c of samples. The temperature of the impregnation process (50 °C) could cause the appearance

of a higher content of the crystalline phase in the samples. An exception is PLA/Xanthone, for which the degree of crystallinity before and after impregnation has a similar value. This may be due to the heterogeneity of samples and the analysis of fragments of samples with a lower content of the crystalline phase. The results may be subject to error due to the assumed values of 100% crystallinity of polymers.

The melt flow index is used in industrial practice to evaluate the rheological properties of plastics. **Figure 4** shows changes in the melt flow rate index (MFR) and melt volume rate index (MVR) of the samples. The impregnation of poly(lactic acid) with all plant substances resulted in an increase in the MFR and MVR indices. Higher values of the MFI index (MVR about 25-85% and MFR about 35-95%) indicate a decrease in the viscosity of samples with polyphenols. Polyphenols, especially quercetin, behaved like polymeric plasticisers. These natural compounds may weaken the intermolecular interactions between poly(lactic acid) molecules through their separation from one another in space, or by direct interaction with them. A reaction between polyphenols and the polymer matrix could have occurred during the test in the middle of plasticising the sample. During this segment of the MFI test, there was a mix of polyphenols impregnated on the surface of the mass of poly(lactic acid).

In contrast to the MFI test, Vicat's softening temperature assay does not mix the adsorbed polyphenols with the polymer

Table 2. DSC analysis of PLA samples impregnated with substances of plant origin. **Note:** T_g – glass transition temperature, ΔH_{cc} – enthalpy of crystallisation, T_{cc} – crystallisation temperature, ΔH_m – enthalpy of melting, T_m – melting temperature, ΔH_o – enthalpy of oxidation, T_o – initial (onset) and final (endest) oxidation temperature, X_c – degree of crystallinity.

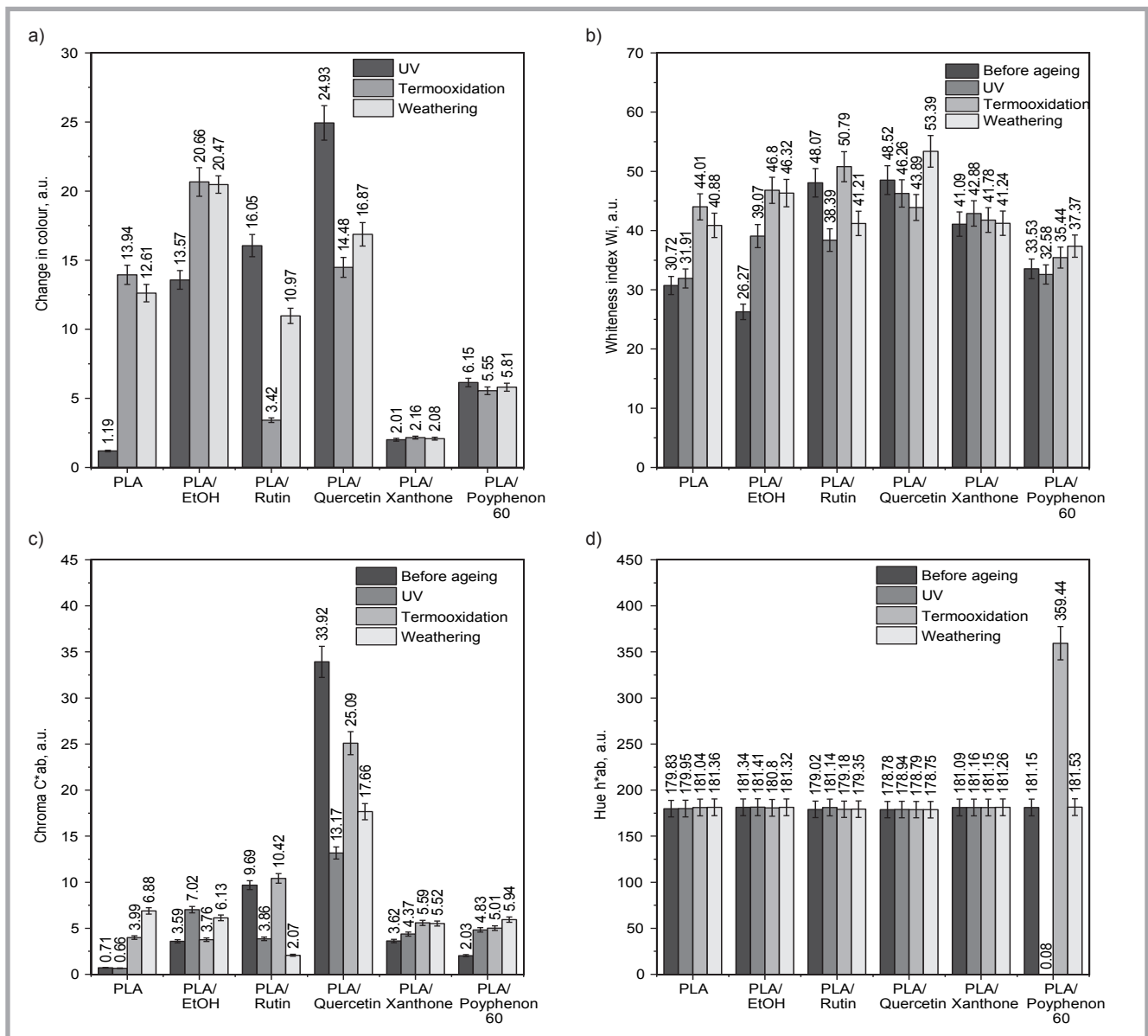| Sample	T_g, °C	ΔH_{cc}, J/g	T_{cc}, °C	ΔH_m, J/g	T_m, °C	ΔH_o, J/g	T_o, °C	X_c, %
PLA	59.6	18.9	108.1	16.8	148.6	21.6	229.4 298.2	17.27
PLA/EtOH	58.4	23.0	107.3	25.2	146.7	15.9	210.9 283.1	25.93
PLA/Rutin	58.9	21.6	108.7	20.1	148.1	12.5	261.2 307.8	20.66
PLA/Quercetin	59.4	25.4	106.6	23.1	147.9	9.2	253.0 300.4	23.74
PLA/Xanthone	58.7	19.2	106.4	14.8	148.8	11.5	231.0 297.4	15.21
PLA/Polyphenon 60	59.1	25.1	106.3	20.9	147.7	15.5	285.3 315.0	21.48

Figure 6. Change in colour (a) and changes in colour parameters (the whitenes index (b), chroma (c) and hue (d)) of PLA samples impregnated with natural polyphenols after ageing.

matrix. This test concerns a shallow sample layer (1 mm). The impregnation of poly(lactic acid) with plant compounds very clearly increased the softening temperatures of the samples, which may be related to the strong interactions between hydroxyl groups from polyphenols and the hydrophilic surface of poly(lactic acid). Polyphenols may have formed on the polymer thin layer, having a higher thermal stability than the poly(lactic acid) itself. Moreover, controlled UV aging does not cause major changes in both the reference PLA and samples with natural substances. These results demonstrate good poly(lactic acid) resistance to this type of aging (***Figure 5***).

An important parameter, being the first sign of aging polymeric materials, is the change in colour. ***Figure 6.a*** shows changes in the colour of samples after UV irradiation, weathering and thermooxidation ageing. The figure also shows changes in colour parameters, such as the whiteness index (***b***), chroma (***c***) and hue (***d***). The reference PLA is slightly affected by UV aging. A colour change factor of 1.19 [a.u.] means that only an experienced observer will notice the difference in colour of a sample before and after aging. However, UV aging causes a very pronounced change in the colour of the PLA/rutin and PLA/quercetin samples. A thin layer of polyphenols impregnated into samples can be sensitive to UV radiation, which can cause changes in the structure of natural compounds or their degradation. Such reactions are accompanied

by a change in the colour of polyphenols. The smallest colour changes were found for PLA/xanthone. Small colour changes in the xanthone and Polyphenone 60 impregnated samples mean that these polyphenols effectively protect the surface of poly(lactic acid) from adverse changes caused by various degradation factors. In the CIE Lab colour space, hue can be calculated together with chroma by transforming coordinates a and b from a rectangular to a polar form. Parameter hue is an angular component of the polar representation, whereas chroma is a radial component. The largest change in parameter chroma was found for the PLA/Quercetin sample, which can be the result of the intense colour change of quercetin caused by aging factors.

Conclusions

Biodegradable poly(lactic acid) is a polymer with a wide range of applications. Suitable modifications, such as impregnation, allow to obtain the material properties expected. Thanks to the method of impregnation proposed, plant polyphenols can be easily introduced onto the PLA surface. Solvent-based impregnation does not require advanced equipment or special experimental conditions. The method presented uses a low temperature in the impregnation process, which does not destroy the natural compounds. Moreover, ethanol is a common and cheap solvent that can be regenerated after impregnation. The layer of polyphenols of plant origin applied effectively increases the resistance of poly(lactic acid) to oxidation. Xanthone and Polyphenon 60 protect PLA against colour changes well under the influence of UV aging, thermooxidation and weathering. In contrast, quercetin and rutin change their colour intensively under the influence of various degrading factors, which is why they can potentially be used as indicators of the aging time of polymers.

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