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Fractionation of technical lignins as a tool for improvement of their antioxidant properties

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ABSTRACT

The modern biorefinery concept is aimed at the elaboration of sustainable processes with the most profitable utilization of all biomass products obtained at the technological cycle. Lignin separated as by-product in the lignocellulosic chemical processing is recognized as an important component of polymer systems. The presence of sterically hindered phenolic hydroxyl groups in the lignin macromolecule opens the possibility of its application as antioxidant for composites, e.g. polyurethanes (PU), which are considered as one of the most versatile polymeric materials. In the present work, the object of investigations was lignin obtained as a product, so-called BIOLIGNIN^{TM}, of wheat straw organosolv processing for pulp and fuel ethanol production (CIMV pilot plant, France). However, heterogeneity of the lignin obtained negatively influences its applicability that can be overcome by fractionation. Three soluble fractions were isolated from CIMV lignin by a sequential extraction with dichloromethane (A fraction), methanol (B fraction) and mixture of both the solvents (C fraction) and characterized in terms of their composition, functionality and structure using analytical pyrolysis (Py-GC/MS), ^{31}P NMR and Size Exclusion Chromatography (SEC) methods. Antioxidant properties of BIOLIGNIN^{TM} fractions were assessed in the tests with free radicals $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. Application of Py-GC/MS for characterization of the fractions opened an opportunity to found some novel "structure–activity" correlations needed for understanding and tuning of antioxidant properties of lignins. The antioxidant activity of the fractions under investigation was tested by their influence on thermo-oxidative destruction of prepared model PU films. The data of TGA method (oxidative conditions) clearly testified the antioxidant effect of all three fractions with the most prominent activity for C fraction. The shifting of the exothermal maxima connected with oxidizing of volatile products of PU destruction to the higher temperature region by 20–30 K and 30–40 K was registered.

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1. Introduction

The biorefinery concept is aimed at the elaboration of sustainable processes with the most profitable utilization of all biomass products obtained at the technological cycle. Lignins separated as by-products in lignocelluloses chemical and bio-chemical processing, despite their good recognized potentials for usage as an important component of polymer systems, is mainly (approximately up to 98%) burned for the improvement of energy balance of the main production processes and only a small portion (1–2%) is processed into value-added products [1,2].

In addition to traditional avenues for lignin application, e.g. binder, plasticizer, sorbent, filler of composite materials, polymeric carrier of biologically active agents, additive for animal food preparations [3,4], lignins are considered as prospective antioxidants. Hindered phenolic groups of lignin can act as stabilizers in reactions induced by oxygen and its reactive species and condition slowdown of ageing of composites and biological systems. The well documented antioxidant properties of lignins open variety of fields for their topical application in industry, healthcare and agriculture [5,6]. Lignin is characterized by higher thermal and biological stability than compounds with low molecular weight and could be applied in those fields in which the employment of a single molecule with antioxidant activity will be inefficient. However, the well-known polydispersity and chemical heterogeneity along MMD ask for the detailed characterization of the lignins composition and structure for determination of their applicability as antioxidants in targeted systems

The antioxidant efficiency of lignin is both related to its solubility in protecting material and structural characteristics [5]. Due to

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the molecular complexity of lignins, it is difficult to assign their antioxidant efficiency to specific structural elements, compared to the activities of synthetic antioxidants or naturally originated chemically defined polyphenols, e.g. tannins and flavonoids [7]. Researches carried out on the lignin model compounds indicate that free phenolic hydroxyl groups and ortho-methoxy substitution in aromatic ring are essential for antioxidant activity, but conjugated carbonyl group in the side chain has a negative effect on the antioxidant activity [8,9]. High molecular weight, polydispersity and heterogeneity in terms of functionality of lignin are factors that decrease radical scavenging activity [10,11]. Since lignins in situ are embedded in a complex network containing several types of polymers (polysaccharides, protein or other macromolecules – according to biological origins), isolated lignins contain admixtures, which may influence the efficiency of the lignins as antioxidants by changing the polarity (e.g. at the presence of polysaccharides that are more polar than lignins) and by decreasing the concentration of the reactive phenolic functions [5].

In the present work, the object of investigations was a novel type of technical lignins – BIOLIGNIN™, obtained as a product of a recently developed biomass refinery technology (CIMV, France) [12], which realizes efficient plant biomass fractionation with production of three main biomass components – cellulose, hemicelluloses and lignin that will themselves be marketable commodity chemicals and raw materials for further processing as well. Fractionation of technical lignins by extraction with organic solvents seems to be attractive opportunity for obtaining of more uniform by molecular mass distribution (MMD), component composition and, respectively, by properties, value added products [13,14].

The aim of the present work was the evaluation of suitability of fractionation of BIOLIGNIN™ as a tool for obtaining on its basis the products with good antioxidant properties for polymeric composite materials, specifically for polyurethanes (PU) on the basis of polyethers-polyols and aromatic isocyanates, e.g. elastomers. With this purpose, BIOLIGNIN™ was fractionated by sequential extraction using two organic solvents of different polarity and their mixture. Each soluble fraction obtained was characterized in terms of structure of lignin macromolecule and the presence of carbohydrate and other admixtures, using analytical pyrolysis, functionality, MMD, and radical scavenging activity. The characteristics obtained were compared with the data on effect of lignin addition in low quantity on the thermo-oxidative destruction of model PU elastomer used as indications of antioxidant efficiency of lignin fractions.

2. Experimental

2.1. Materials

BIOLIGNIN™ was extracted at pilot scale from wheat straw using a mixture of acetic acid/formic acid/water at the CIMV pilot plant (Pomacle, France). Purity of the lignin was about 95% [12].

Chemicals used for the analyses, including solvents, were of analytical grade (Sigma–Aldrich). All tests solutions were prepared freshly before use.

The parent BIOLIGNIN™ sample was dried in vacuum at 313 K (a vacuum drying oven VACIOTEM-T, Spain) to moisture content below 1%. The dried sample was grinded in a Retsch Mixer Mill MM200 (Retsch, Germany) at the frequency 30 s^{-1} for 30 min and fraction with particle size $d < 0.5$ mm was used for the further extraction with organic solvents and analyses.

2.2. Solvent fractionation

Lignin was fractionated in duplicate by successive extraction with dichloromethane, methanol and the mixture of methanol with dichloromethane (7/3, v/v). Lignin (225 g) was suspended in 1000 mL of the respective solvent and continuously stirred at room temperature for 2 h. The undissolved material was filtered off and resuspended for a second identical extraction. The fractions from both steps were combined. Collected dissolved material was filtered and vacuum dried. The yields of the fractions were shown in % on dry ash-free non-extracted lignin.

2.3. Chemical analysis

All analyses were done in triplicate. All results are expressed on a dry-weight and ash free basis. The dry weight was determined by separate oven drying of samples at 378 K for 18 h. Ash content was determined by the combustion of samples at 973 K for 3 h in a Carbolite furnace ELF 11/6B (UK).

The methoxyl group (OCH_3) content in lignin samples was determined according to the Viebock–Schwappach method in a Zeisel apparatus (domestic glassware). The procedure is described in detail in [15].

The contents of phenolic and aliphatic hydroxyl groups (OH_{phen} and OH_{aliph}, respectively) and carboxylic groups (OH_{COOH}) were determined in the derivatizated lignin samples by ^{31}P NMR with Bruker 400 MHz (30° pulse angle, inverse gated proton decoupling, a delay time of 5 s and 256 scans). The procedure is described in detail in [14]. Signal assignment was performed as described by [16].

The molar mass distribution (MMD) of the fractions obtained was analysed by alkaline SEC method in accordance with procedure described in [14]. Lignin samples of 1 mg mL^{-1} dissolved in 0.5 M NaOH were injected into a manually packed column (4.6 cm × 30 cm) with ethylene glycolmethacrylate copolymer TSK gel Toyopearl HW-55F (thermostated at 298 K), eluted with the same solvent and detected at 280 nm. Standards for calibration of the molar mass distribution: sodium polystyrene sulfonates (M_w range: 891–976,000 Da) and phenol.

2.4. Py-GC/MS

The Py-GC/MS analysis was performed using a Frontier Lab (Japan) Micro Double-shot Pyrolyser Py-2020iD (pyrolysis temperature 773 K, heating rate 600 K s^{-1}) directly coupled with the Shimadzu GC/MS – QP 2010 apparatus (Japan) with capillary column RTX-1701 (Restec, USA), 60 m × 0.25 mm × 0.25 μm film (the injector temperature 523 K, ion source 523 K with EI of 70 eV, the MS scan range m/z 15–350, carrier gas helium at the flow rate of 1 mL min^{-1} and the split ratio 1:30). The mass of a sample probe (residual moisture content < 1%) was 1.00–2.00 mg. The oven program was 1 min isothermal at 333 K, then 6 K min^{-1} to 543 K, and finally held at 543 K for 10 min. The apparatus was modified by installation of the splitter of gas-carrier flow Vitreous Silica Outlet Splitter VSOS (SGE, Australia) in order to operate FID and MS detectors simultaneously. The mass spectrometer was operated in the electron impact mode using 70 eV electron energy. Fluoranthene is used as an inner standard for quantification of content of individual phenols. The identification of the individual compounds was performed on the basis of GC/MS chromatogram using Library MS NIST 147.LI13, whereas the relative area of the peak of individual compounds was calculated using the Shimadzu software on the basis of GC/FID data. The summed molar areas of the relevant peaks were normalized to 100% and the data for five repetitive pyrolysis experiments were averaged.

2.5. Assessment of radical scavenging activity

Testing radical scavenging activity was performed against stable radicals 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic cation

radical ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), and superoxide anion-radical ($O_2^{\bullet-}$).

In the $DPPH^{\bullet}$ radical scavenging assay the method described in [10] was adopted with minor modification. A sample solution in DMSO (0.03 mL) was mixed for 15 min with 3.0 mL of a 1×10^{-4} mol L^{-1} $DPPH^{\bullet}$ methanol solution, and then the absorbance at 517 nm of the mixture was immediately measured using a Perkin Elmer Lambda 25 UV/VIS spectrometer.

$ABTS^{\bullet+}$ was produced by reacting 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) with potassium persulfate ($K_2S_2O_8$) [17]. A stock solution of ABTS (2 mM) was prepared by dissolving in 50 mM of phosphate buffered saline (PBS), consisting of 8.18 g NaCl, 0.27 g KH_2PO_4, 3.58 g $NaHPO_4 \times 11H_2O$ and 0.15 g KCl in 1 L of distilled water. The pH of the solution should be 7.4; otherwise it was adjusted with 0.1 M NaOH. The $ABTS^{\bullet+}$ solution was produced reacting 50 mL of stock solution with 200 μL of 70 mM $K_2S_2O_8$ water solution. The mixture was left to stand in the dark at room temperature for 15–16 h before use. For the evaluation of the antioxidant capability, the $ABTS^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. 0.03 mL of the sample solution in DMSO were mixed with 3 mL of the $ABTS^{\bullet+}$ solution in the 1 cm path length microcuvette. The absorbance at 734 nm was read at ambient temperature after 10 min. PBS solution was measured as a blank sample.

The characterization of the antioxidant activity against $O_2^{\bullet-}$ was carried out using the hypoxantine–xanthine oxidase system and $O_2^{\bullet-}$ was detected by the NBT (nitroblue tetrazolium) method [18], where the formazan formed upon NBT oxidation was controlled. The generation of $O_2^{\bullet-}$ was measured in a reaction mixture containing 250 μM hypoxanthine, 150 μM NBT, and 4 mU mL^{-1} of xanthine oxidase in $NaHCO_3$–Na_2CO_3 buffer (pH 10.2). The reduction of the NBT concentration was measured by the change in absorbance at 560 nm for 12 min. Test solutions were prepared in DMSO and added to the reaction mixture to give a final concentration of 0.2% (v/v) DMSO. The solution without xanthine oxidase was used as a blank sample.

For all tests performed, the inhibition percentage (IP) of the radical species was calculated as follows:

$$\mathrm{IP}\,(\%) = \frac{(A_B - A_A)}{A_B} \times 100,$$

where A_B is absorbance in blank probe (antioxidant was omitted) and A_A is absorbance in the sample after 15, 10 and 12 min for $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ assays, respectively. Using different concentrations of antioxidants, the dependence of the IP values on the antioxidant concentration was established and used for the calculation of IC_{50} (the concentration of the tested sample required for a 50% inhibition of radical species). To calculate the IC_{50} values, the linear regression analysis was made, using SPSS Statistics 17.0 (level of significance 0.05). According to the definition, higher antioxidant activity results in lower value of IC_{50}.

Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (water soluble derivative of vitamin E) was tested as a reference antioxidant.

2.6. Obtaining model polyurethane (PU) elastomer films

The model PU elastomers in the form of films were obtained in accordance with [19] using a system included PEG (polyethylene glycol) with M_n of 400 g mol^{-1}, PMDI (commercial polymeric diphenylmethane diisocyanate Voratec SD 100) with the content of isocyanate groups (NCO) of 31% and DBTD (dibutyltin dilaurate) as a catalyst. The films were prepared by pre-polymerization of constituents followed by preparation of films by solution casting. PEG, PMDI and DBTD were dissolved each in extra dry THF (tetrahydrofuran), solutions obtained were combined and a system was pre-polymerized at 20 °C under argon atmosphere. The NCO/OH ratio in the composition was 1.05. In order to avoid bubbles appearance in PU films, the solutions were pre-treated in the ultra sound bath. The pre-polymerized mixture was then cast into the disk mold with flat bottom covered by polytetrafluorethylene (Teflon) for curing. After 24 h the cast films were peeled and dried for 7 days in desiccators above P_2O_5 at 20 °C following by thermal curing for 8 h at the temperature of 90 °C in air atmosphere. The films containing 5% of lignin fractions were prepared in the same manner, adding their solutions in extra dry THF on the pre-polymerization step.

The cross-link density of the PU elastomers obtained was measured as an effective number of cross-linked chains per volume unit of a polymer network, mol cm^{-3} using Flory–Rehner method as described in [19]. Dimethyl formamide was used as a swelling agent.

2.7. The effect of lignin fractions on thermo-oxidative destruction of PU

The thermo-oxidative destruction of the model PU elastomer films was studied in the temperature range 293–973 K by thermogravimetric analysis in air atmosphere (flow rate 50 mL min^{-1}) using the Metler Toledo Star System TGA/ADTA 851e device at a heating rate of 10 K min^{-1}. The sample size of 8–10 mg was used. The data for five parallel experiments were averaged.

3. Results and discussion

3.1. The fractionation of BIOLIGNIN™ and functionality of the fractions obtained

The weak solubility of technical lignins in organic solvents usually used in PU chemistry (e.g. THF, glycol, dioxane) restricts their application in PU systems. The solvent fractionation is one of the pathways for obtaining more uniform completely soluble products for PU production.

Three soluble fractions were isolated from BIOLIGNIN™ by a sequential extraction with dichloromethane (A fraction), methanol (B fraction) and mixture of both the solvents (C fraction). The fractionation procedure in total solubilized about 40% of the lignin. Among soluble fractions, C fraction was dominating with the yield of (18.2 ± 0.4)% (from parent lignin), while A and B fractions were obtained with the yields of (7.4 ± 0.2) and (14.5 ± 0.3)%, respectively.

The results of SEC analysis (Table 1) showed that the fractionation yielded fractions of increasing molecular weight. The A and B fractions had rather close molecular weights, whereas both M_n and M_w of C fraction were substantially higher. All soluble fractions obtained had about twice lower polydispersity index (M_w/M_n) than that for parent BIOLIGNIN™.

The comparison of the results of functional analysis (Table 1) shows that the fraction obtained slightly differs by the content of phenolic hydroxyl groups, which have the important influence on the lignins antioxidant activity [10]. The difference in the content of metoxyl group, which is the diagnostic group for lignin, is more prominent. The OCH_3 group content is the highest in the fraction soluble in the CH_3OH/CH_2Cl_2 mixture (C fraction) and it was considerably higher than that in the parent lignin. For the same fraction the highest content of phenolic hydroxyl groups included in the so called condensed phenolic units, i.e. biphenyl- and diphenylmethane lignin substructures, was observed. This could indicate that the lignin yielded in C fraction has more condensed structure of lignin extracted by the mixture of solvents. The last assumption was confirmed by the data obtained using Py-GC/MS.

Table 1
Molar masses and functional groups contents of BIOLIGNIN™ and its fractions: A–F – CH_2Cl_2 soluble fraction; B–F – CH_3OH soluble fraction; C–F – CH_3OH/CH_2Cl_2 soluble fraction.

Index	Lignin sample			
	Unfractionated BIOLIGNIN™	A–F	B–F	C–F
Average M_n, Da	1051 ± 22	334 ± 2	541 ± 5	1094 ± 80
Average M_w, Da	11,143 ± 146	1973 ± 46	2201 ± 27	5295 ± 430
M_w/M_n	10.6 ± 0.3	5.9 ± 0.1	4.1 ± 0.1	4.8 ± 0.1
OCH_3 content, mmol g^{-1}	2.62 ± 0.03	2.67 ± 0.04	2.89 ± 0.02	3.22 ± 0.01
$OH_{aliphatic}$ content, mmol g^{-1}	1.51 ± 0.01	0.94 ± 0.02	2.51 ± 0.08	1.68 ± 0.02
$OH_{phenolic}$ content in condensed substructures,[a] mmol g^{-1}	0.50 ± 0.01	0.40 ± 0.01	0.43 ± 0.01	0.54 ± 0.01
$OH_{phenolic}$ content in syringyl substructures, mmol g^{-1}	0.31 ± 0.01	0.45 ± 0.01	0.33 ± 0.01	0.36 ± 0.01
$OH_{phenolic}$ content in guaiacyl substructures, mmol g^{-1}	0.54 ± 0.01	0.71 ± 0.01	0.67 ± 0.02	0.63 ± 0.01
$OH_{phenolic}$ content in p-hydroxy-phenyl substructures, mmol g^{-1}	0.22 ± 0.01	0.12 ± 0.01	0.31 ± 0.01	0.27 ± 0.01
Total $OH_{phenolic}$ content, mmol g^{-1}	1.58 ± 0.03	1.68 ± 0.04	1.74 ± 0.03	1.80 ± 0.01
COOH, mmol g^{-1}	0.69 ± 0.02	0.67 ± 0.01	0.81 ± 0.02	0.48 ± 0.01
Total OH groups content, mmol g^{-1}	3.77 ± 0.06	3.29 ± 0.08	5.10 ± 0.13	3.96 ± 0.01

[a] Biphenyl- and diphenylmethane substructures.

Evaluation of the phenolic hydroxyls composition of the fractions (Table 1) shows that the dichloromethane soluble fraction (A fraction) is characterized with the highest content of phenolic hydroxyl groups attached to syringyl and guaiacyl substructures. At the same time, this fraction is characterized by the much lower content of aliphatic hydroxyl groups as compared with parent BIOLIGNIN™ and other two fractions that could be connected with increased content of lipophilic admixtures in A fraction.

3.2. Characterization of BIOLIGNIN™ soluble fractions using Py-GC/MS

The distribution of lignin and carbohydrates related products detected by analytical pyrolysis for the parent BIOLIGNIN™ and its fractions is shown in Table 2. Lignin is the predominant component for parent BIOLIGNIN™ and its fractions, although noticeable amounts of compounds originated from carbohydrates as well as lipophilic admixtures are also present in the pyrolysates (Fig. 1). Application of the mixture of solvents allowed separating the C fraction with the highest molecular mass and the highest portion of the lignin derived compounds (Table 2, compounds 20–66) in the volatile pyrolysis products, which was higher than that for the parent BIOLIGNIN™. The latter is in conformity with the highest content of methoxyl groups detected for this fraction (Table 1). It could be explained by better affinity of hydrophilic/hydrophobic sites of lignin macromolecule to the combination of non-polar and polar solvents in the mixture used. At the same time, the lignin fraction soluble in CH_2Cl_2 (A fraction) differed from the parent lignin and other fractions by multi-times increased portion of aliphatic compounds (Fig. 1), which could originate from waxes and paraffins intrinsic to wheat straw. The volatile products from pyrolysis of B fraction are most enriched with the carbohydrates derived products. This is in good conformity with the results of ^{31}P NMR showing the highest content of aliphatic hydroxyl groups in this fraction (Table 1).

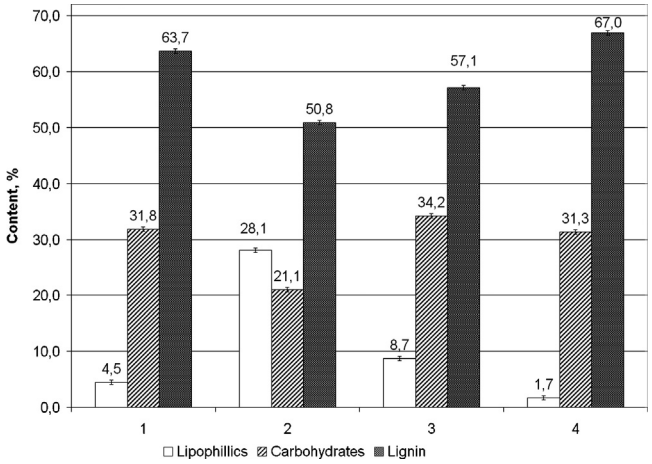

Fig. 1. Relative content (%) of lignin (L) derived compounds, carbohydrates (C) derived compounds and aliphatic compounds in the BIOLIGNIN™ and its fractions pyrolysis products. (A) Fraction – CH_2Cl_2 soluble fraction; (B) Fraction – CH_3OH soluble fraction; (C) Fraction – CH_3OH/CH_2Cl_2 soluble fraction.

In order to determine the variations in the chemical structure of the lignin containing in the fractions differed by molecular mass, special attention was paid to lignin derived pyrolysis products. With this purpose, peak areas of lignin pyrolysis products were normalized to 100% and peak areas of lignin-derived phenols (individual phenols) were calculated as relative percentages (Table 3). The lignin component of the A fraction contains the highest amount of syringyl sub-structures (Table 3, compounds 14–25) in comparison with other soluble fractions and, simultaneously, the highest portion of the substructures with α-carbonyl groups (Table 3, compounds 9–13 and 21–25). The fraction soluble in CH_3OH (B fraction) is characterized with the highest portion of lignin guaiacyl substructures (Table 3, compounds 1–13). Lignin components of both B and C fractions are characterized by higher aromaticity than parent lignin and lignin in the A fraction.

The highest ratio of the sum of portions of phenol and benzene derivatives to the sum of guaiacol and syringol derivatives in the pyrolysis products from the C fraction (0.24 ± 0.02 against 0.15 ± 0.02; 0.10 ± 0.01 and 0.16 ± 0.01, respectively, for parent lignin, A fraction and B fraction) indicates the most high degree of condensation of the lignin contained in this fraction that coincided with its highest content of phenolic hydroxyl groups in condensed lignin units (Table 1). The significantly decreased portion of 4-vinyl-2-metoxyphenol (Table 3, compound 4) in the pyrolysis products from C fraction, as compared with other fractions and the parent lignin, also confirmed indirectly the increased degree of condensity of lignin contained in C fraction, because this compound is formed as the result of destruction of the aryl–alkyl ether bonds in lignin during analytical pyrolysis. Therefore, decreasing relative portion of 4-vinyl-2-metoxyphenol could reveal the lower relative portion of ether interunit bonds in lignin of the A fraction and, respectively, the higher portion of C—C bonds in its macromolecule.

3.3. Radical scavenging activity of BIOLIGNIN™ fractions

In the present study, the antioxidant properties of the soluble fractions of BIOLIGNIN™ were characterized using three

Table 2
Peak assignments and relative abundance (%) of lignin (L) and carbohydrates (C) derived pyrolysis products detected in parent BIOLIGNIN™ and its fractions: A–F – CH_2Cl_2 soluble fraction; B–F – CH_3OH soluble fraction; C–F – CH_3OH/CH_2Cl_2 soluble fraction.

Number	Compound	Origin	Compound proportion in the volatiles, %			
			BL[a]	A–F	B–F	C–F
1	Acetic acid	C	9.13 ± 0.05	10.41 ± 0.05	7.71 ± 0.05	9.52 ± 0.05
2	Octyl 2-methyl-2-propenoate	C	n.d[b]	0.22 ± 0.01	0.07 ± 0.01	n.d.
3	Prop-2-enal	C	Trace	Trace	Trace	Trace
4	Propanone	C	0.53 ± 0.01	0.16 ± 0.01	0.51 ± 0.01	0.72 ± 0.03
5	2-Methylpropanal	C	0.09 ± 0.02	0.02 ± 0.005	0.10 ± 0.01	0.10 ± 0.01
6	Butane-2,3-dione	C	0.18 ± 0.01	0.02 ± 0.005	0.17 ± 0.01	0.19 ± 0.01
7	3-Methylbutanal	C	0.13 ± 0.01	0.03 ± 0.005	0.18 ± 0.01	0.14 ± 0.01
8	2-methylbutanal	C	0.08 ± 0.01	0.04 ± 0.005	0.08 ± 0.005	0.09 ± 0.01
9	Hexanal	C	Trace	0.06 ± 0.005	0.05 ± 0.005	Trace
10	1-(Acetyloxy)-2-propanone	C	0.07 ± 0.01	Trace	0.05 ± 0.005	0.07 ± 0.01
11	Furan	C	0.01 ± 0.005	Trace	Trace	0.06 ± 0.01
12	2-Methylfuran	C	0.46 ± 0.03	0.32 ± 0.01	0.26 ± 0.01	0.47 ± 0.01
13	Oxolane	C	n.d.	0.21 ± 0.01	n.d.	n.d.
14	2,5-Dimethylfuran	C	0.08 ± 0.01	0.05 ± 0.01	0.06 ± 0.005	0.11 ± 0.01
15	2-Ethyl-5-methylfuran	C	Trace	Trace	Trace	0.04 ± 0.005
16	Furan-2-carbaldehyde	C	0.38 ± 0.02	0.15 ± 0.02	0.39 ± 0.01	0.33 ± 0.02
17	2,3-Dihydro-1-benzofuran	C, L	3.68 ± 0.07	1.36 ± 0.02	5.76 ± 0.03	2.78 ± 0.05
18	5-Acetoxymethyl-2-furaldehyde	C	Trace	0.14 ± 0.02	Trace	Trace
19	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	C	0.07 ± 0.01	Trace	0.09 ± 0.005	0.09 ± 0.01
20	Benzene	L	0.04 ± 0.005	Trace	0.03 ± 0.005	0.03 ± 0.005
21	Methylbenzene	L	0.39 ± 0.01	0.30 ± 0.02	0.34 ± 0.02	0.31 ± 0.02
22	1,4-Dimethylbenzene	L	0.08 ± 0.005	0.04 ± 0.005	0.09 ± 0.005	0.10 ± 0.01
23	1,2-Dimethylbenzene	L	0.09 ± 0.005	0.12 ± 0.005	0.07 ± 0.005	0.08 ± 0.005
24	1,3- Dimethylbenzene,	L	Trace	0.11 ± 0.005	n.d.	n.d
25	Ethenylbenzene	L	0.10 ± 0.005	0.05 ± 0.005	0.09 ± 0.005	0.06 ± 0.005
26	1,2,3-Trimethylbenzene	L	Trace	0.07 ± 0.005	0.07 ± 0.005	0.08 ± 0.01
27	1,2,4-Trimethylbenzene	L	0.05 ± 0.005	0.07 ± 0.005	Trace	0.04 ± 0.002
28	1-Methoxy-2-methylbenzene	L	0.10 ± 0.01	0.01 ± 0.001	0.09 ± 0.005	0.09 ± 0.01
29	Pentylbenzene	L	n.d	0.04 ± 0.005	n.d	n.d
30	Phenol	L	0.85 ± 0.05	0.40 ± 0.02	1.15 ± 0.02	1.11 ± 0.03
31	2-Methylphenol	L	0.23 ± 0.02	0.22 ± 0.01	0.27 ± 0.01	0.31 ± 0.01
32	2,3-Dimethylphenol	L	0.03 ± 0.005	Trace	0.02 ± 0.005	Trace
33	4-Methylphenol	L	1.07 ± 0.05	0.65 ± 0.02	1.05 ± 0.02	1.23 ± 0.02
34	2-methoxy-3-methylphenol	L	0.04 ± 0.01	0.04 ± 0.005	0.06 ± 0.005	0.07 ± 0.005
35	3,4-Dimethylphenol	L	0.21 ± 0.02	0.22 ± 0.01	0.18 ± 0.02	0.26 ± 0.02
36	1,2-Dimethoxy-3-methylbenzene	L	0.08 ± 0.005	0.07 ± 0.005	0.05 ± 0.005	0.07 ± 0.005
37	4-Ethylphenol	L	0.33 ± 0.02	0.22 ± 0.01	0.60 ± 0.005	0.68 ± 0.02
38	3′,5′-Dihydroxy-1-phenylethanone	L	0.12 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.16 ± 0.01
39	4-Ethyl-1,2-dimethoxybenzene	L	0.09 ± 0.01	0.05 ± 0.005	0.04 ± 0.005	0.07 ± 0.005
40	3-methoxy-5-methylphenol	L	0.19 ± 0.01	0.05 ± 0.005	0.14 ± 0.01	0.24 ± 0.01
41	4-Allylphenol	L	Trace	Trace	0.08 ± 0.005	0.09 ± 0.005
42	2-Methoxyphenol	L	3.06 ± 0.04	2.88 ± 0.05	3.56 ± 0.05	3.97 ± 0.05
43	2-Methoxy-4-methylphenol	L	3.27 ± 0.05	2.96 ± 0.05	3.19 ± 0.01	3.87 ± 0.04
44	4-Ethyl-2-methoxyphenol	L	1.14 ± 0.05	1.04 ± 0.01	1.47 ± 0.01	1.99 ± 0.04
45	4-Vinyl-2-metoxyphenol p-vinylguaiacol	L	8.29 ± 0.05	7.57 ± 0.05	9.46 ± 0.05	5.25 ± 0.05
46	4-Allyl-2-methoxyphenol	L	0.23 ± 0.01	0.24 ± 0.01	0.19 ± 0.01	0.23 ± 0.01
47	2-Methoxy-4-propylphenol	L	0.15 ± 0.005	0.06 ± 0.005	0.15 ± 0.01	0.21 ± 0.01
48	2-Methoxy-4-[(Z)-prop-1-enyl]phenol	L	0.22 ± 0.01	0.19 ± 0.01	0.21 ± 0.005	0.29 ± 0.01
49	2-Methoxy-4-[(E)-prop-1-enyl]phenol	L	1.23 ± 0.02	0.77 ± 0.01	1.05 ± 0.02	1.18 ± 0.02
50	4-Hydroxy-3-methoxybenzaldehyde vanillin	L	0.27 ± 0.01	1.56 ± 0.03	0.30 ± 0.01	0.20 ± 0.01
51	1-(4-Hydroxy-3-methoxyphenyl)ethanone acetoguaiacon	L	0.19 ± 0.005	0.51 ± 0.01	0.18 ± 0.01	0.29 ± 0.02
52	1-(4-Hydroxy-3-methoxyphenyl)propan-2-one guacylacetone	L	0.17 ± 0.005	0.09 ± 0.01	0.15 ± 0.01	0.19 ± 0.01
53	1-(4-Hydroxy-3-methoxyphenyl)propan-1-one propioguaiacone	L	0.09 ± 0.005	0.07 ± 0.01	0.05 ± 0.005	0.07 ± 0.01
54	1-(4-Hydroxy-3-methoxyphenyl)propan-1-one-1-oxy propioguaiacone, alpha-oxy-	L	0.37 ± 0.01	1.40 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
55	2,6-Dimethoxyphenol syringol	L	1.90 ± 0.03	2.43 ± 0.05	2.40 ± 0.05	2.38 ± 0.05
56	2,6-Dimethoxy-4-methylphenol syringol, 4-methyl-	L	1.65 ± 0.05	2.00 ± 0.05	1.95 ± 0.03	2.25 ± 0.05
57	4-Ethyl-2,6-dimethoxyphenol syringol, 4-ethyl-	L	0.28 ± 0.03	0.32 ± 0.01	0.31 ± 0.01	0.50 ± 0.01
58	4-Vinyl-2,6-dimethoxyphenol syringol, 4-vinyl-	L	0.92 ± 0.02	0.91 ± 0.02	0.82 ± 0.01	0.74 ± 0.01
59	4-allyl-2,6-dimethoxy-phenol and 2,6-dimethoxy-4-propylphenol	L	0.35 ± 0.005	0.26 ± 0.01	0.31 ± 0.01	0.37 ± 0.01
60	2,6-Dimethoxyphenol derivate	L	0.07 ± 0.01	0.11 ± 0.01	0.06 ± 0.005	0.07 ± 0.005
61	2,6-Dimethoxy-4-[(E)-prop-1-enyl]phenol	L	0.95 ± 0.01	0.67 ± 0.02	0.77 ± 0.03	0.91 ± 0.01
62	4-Hydroxy-3,5-dimethoxybenzaldehyde	L	0.09 ± 0.01	1.50 ± 0.05	0.10 ± 0.01	0.14 ± 0.02
63	1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone	L	0.52 ± 0.02	0.46 ± 0.02	0.30 ± 0.01	0.63 ± 0.02
64	1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-2-one	L	0.08 ± 0.01	0.03 ± 0.005	0.11 ± 0.01	0.15 ± 0.01
65	1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-1-one	L	0.04 ± 0.005	0.05 ± 0.005	0.05 ± 0.006	0.06 ± 0.005
66	1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-1-one-1-oxy	L	0.18 ± 0.01	0.86 ± 0.01	0.21 ± 0.01	0.12 ± 0.005

[a] BIOLIGNIN™.
[b] n.d., not detected.

Table 3
The distribution of lignin-derived methoxylated phenols in pyrolysates of BIOLIGNIN™ and its fractions normalized to 100% of lignin derived products.

No.	Compound	Compound proportion in the lignin-derived volatiles, %			
		BIOLIGNIN™	A–F	B–F	C–F
1	2-Methoxyphenol	10.31 ± 0.01	9.08 ± 0.08	11.11 ± 0.08	12.69 ± 0.06
2	2-Methoxy-4-methylphenol	10.97 ± 0.05	9.33 ± 0.06	9.90 ± 0.07	12.31 ± 0.11
3	4-Ethyl-2-methoxyphenol	3.83 ± 0.08	3.27 ± 0.02	4.56 ± 0.03	6.33 ± 0.02
4	4-Vinyl-2-metoxyphenol	27.82 ± 0.07	23.85 ± 0.09	29.43 ± 0.04	16.73 ± 0.12
5	4-Allyl-2-methoxyphenol	0.78 ± 0.05	0.75 ± 0.02	0.59 ± 0.01	0.73 ± 0.04
6	2-Methoxy-4-propylphenol	0.50 ± 0.03	0.19 ± 0.01	0.47 ± 0.02	0.67 ± 0.03
7	2-Methoxy-4-[(*Z*)-prop-1-enyl]phenol	0.74 ± 0.03	0.60 ± 0.04	0.65 ± 0.01	0.92 ± 0.02
8	2-Methoxy-4-[(*E*)-prop-1-enyl]phenol	4.13 ± 0.04	2.42 ± 0.06	3.26 ± 0.08	3.75 ± 0.06
9	4-Hydroxy-3-methoxybenzaldehyde	0.91 ± 0.05	4.90 ± 0.03	0.93 ± 0.04	0.64 ± 0.02
10	1-(4-Hydroxy-3-methoxyphenyl)ethanone	0.64 ± 0.01	1.60 ± 0.07	0.56 ± 0.03	0.92 ± 0.02
11	1-(4-Hydroxy-3-methoxyphenyl)propan-2-one	0.57 ± 0,02	0.28 ± 0.04	0.47 ± 0.01	0.60 ± 0.03
12	1-(4-Hydroxy-3-methoxyphenyl)propan-1-one	0.30 ± 0.02	0.22 ± 0.04	0.16 ± 0.02	0.22 ± 0.02
13	1-(4-Hydroxy-3-methoxyphenyl)propan-1-one-1-oxy	1.24 ± 0.03	4.43 ± 0.08	0.96 ± 0.05	0.95 ± 0.03
14	2,6-Dimethoxyphenol	6.38 ± 0.09	7.70 ± 0.10	7.45 ± 0.05	7.57 ± 0.05
15	2,6-Dimethoxy-4-methylphenol	5.54 ± 0.08	6.28 ± 0.08	6.05 ± 0.07	7.16 ± 0.05
16	4-Ethyl-2,6-dimethoxyphenol	0.94 ± 0.02	1.01 ± 0.03	0.96 ± 0.02	1.59 ± 0.06
17	4-Vinyl-2,6-dimethoxyphenol	3.09 ± 0.06	2.86 ± 0.08	2.55 ± 0.02	2.35 ± 0.05
18	4-Allyl-2,6-dimethoxy-phenol and 2,6-dimethoxy-4-propylphenol	1.17 ± 0.01	0.82 ± 0.08	0.96 ± 0.01	1.18 ± 0.04
19	2,6-Dimethoxyphenol derivate	0.23 ± 0.02	0.35 ± 0.02	0.19 ± 0.02	0.22 ± 0.02
20	6-Dimethoxy-4-[(E)-prop-1-enyl]phenol	3.19 ± 0.07	2,10 ± 0.06	2.39 ± 0.05	2.89 ± 0.08
21	4-Hydroxy-3,5-dimethoxybenzaldehyde	0.30 ± 0.02	4.74 ± 0.08	0.31 ± 0.03	0.45 ± 0.03
22	1-(4-Hydroxy-3,5-dimethoxyphenyl) ethanone	1.75 ± 0.08	1.54 ± 0.06	0.93 ± 0.03	2.00 ± 0.04
23	1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-2-one	0.30 ± 0.02	0.10 ± 0.02	0.34 ± 0.03	0.48 ± 0.02
24	1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-1-one	0.12 ± 0.01	0.16 ± 0.04	0.16 ± 0.01	0.19 ± 0.02
25	1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-1-one-1-oxy	0.60 ± 0.06	2.70 ± 0.08	0.65 ± 0.03	0.38 ± 0.01

A–F – CH_2Cl_2 soluble fraction; B–F – CH_3OH soluble fraction; C–F – CH_3OH/CH_2Cl_2 soluble fraction.

antioxidant capacity assays frequently used to estimate antioxidant capacity of polyphenols. The results of the $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and $O_2^{\bullet-}$ tests are presented in Table 4 in terms of IC_{50} (the concentration of the tested antioxidant sample required for a 50% inhibition of radical species). The lower is this value the higher is radical scavenging activity of the compounds tested.

Radical scavenging activity against stable free $ABTS^{\bullet+}$ radicals of BIOLIGNIN™ soluble fractions was rather close to that of antioxidant Trolox, which is often used as a standard (Table 4). In opposite, in the test with free radical $DPPH^{\bullet}$, the radical scavenging activity of all fractions under study was almost 10-fold lower than that for Trolox. The values of radical scavenging activity obtained using the both above mentioned test did not strongly differ for various fractions. Unlike this, in the test with reactive oxygen form, namely superoxide radical anion, the radical scavenging capacity of C fraction was threefold higher as compared with the value found for the A fraction (Table 4). In this test the radical scavenging capacity detected for the C fraction was not much lower than that for Trolox. Some discrepancy between the results of $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ tests, from one side, and $DPPH^{\bullet}$ test from other side, could be connected with the difference in mechanisms of the lignin radical scavenging activity in the various assays [20]. For the $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ tests scavenging activity occurs by electron transfer–proton transfer (ET–PT) mechanism, whereas for the $DPPH^{\bullet}$ test the combination of ET path with H atom transfer is considered. In the latter case, the reaction could be very slow and hindered by side reaction, in particular, for o-methoxyphenols [20].

The CH_2Cl_2 soluble fraction (A fraction) showed the lowest activity in the all tests applied that can be explained by the relatively high content of lipophilic admixtures in this fraction (Fig. 2). Besides, this fraction is characterized by the presence of the highest portion of lignin-derived compounds with α-carbonyl groups in the volatile products of pyrolysis (Table 3). The negative effect of conjugated carbonyl groups on antioxidant activity was noted earlier for monomeric lignin-related phenols [10].

CH_3OH/CH_2Cl_2 soluble fraction (C fraction) showed the highest radical scavenging activity, especially against superoxide-anion radicals, which can be compared with of flavanoids and other natural antioxidants. IC_{50} values of CH_3OH soluble fraction in tests with three different radicals also are relatively low.

Consideration of the results of radical scavenging tests together with the data of analytical pyrolysis opened an opportunity to precise some "structure–activity" correlations needed for understanding and tuning of antioxidant properties of lignins. It was found that the IC_{50} value in the test with the reactive oxygen species (superoxide radical anion) linearly decreased with the increasing condensation degree of lignin macromolecules and aromaticity (the Pearson correlation coefficients $r = 0.993$ at the critical coefficient value $r = 0.988$). Formation of phenylpropanoid aroxyl radicals is an essential step in the realization of antioxidant activity of lignin. The stability of the aroxyl radicals strongly depends on unpaired electron delocalization. The development of condensed lignin structure with increased aromaticity leads to the extension of electron delocalization thus decreasing dangerous prooxidant potential of polyphenols.

Table 4
The results of the tests on radical scavenging activity of BIOLIGNIN™ soluble fractions presented in terms of IC_{50} (the concentration of the tested sample required for a 50% inhibition of radical species).

Sample	IC_{50}, mg/L, in the tests with:		
	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	$O_2^{\bullet-}$
CH_2Cl_2 fraction	10.7 ± 0.3	50.0 ± 1.4	125.6 ± 5.5
CH_3OH fraction	7.1 ± 0.3	42.7 ± 0.8	60.6 ± 10.7
CH_3OH/CH_2Cl_2 fraction	9.1 ± 0.3	42.8 ± 1.0	43.9 ± 2.0
Trolox	4.0 ± 0.1	4.7 ± 0.1	17.7 ± 0.4

3.4. *The antioxidant effect of BIOLIGNIN™ soluble fractions on PU thermo-oxidative destruction*

The antioxidant activity of the BIOLIGNIN™ soluble fractions was tested by their influence on thermo-oxidative destruction of model PU films in air atmosphere. The PU films obtained were

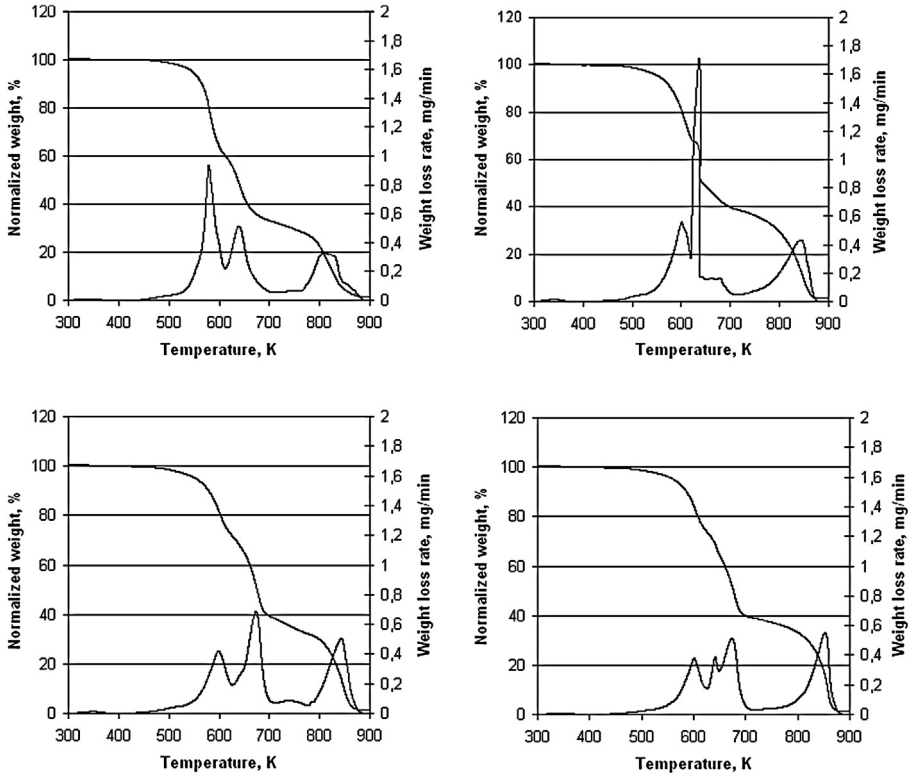

Fig. 2. Thermo-oxidative destruction results (TGA and DTG curves) for PU films: Control PU – lignin free PU film, (A) Fraction – PU film with addition of CH_2Cl_2 soluble fraction, (B) Fraction – PU film with addition of CH_3OH soluble fraction, (C) Fraction – PU film with addition of CH_3OH/CH_2Cl_2 soluble fraction.

Table 5
Effect of BIOLIGNIN™ soluble fractions on characteristics of thermo-oxidative destruction of PU films. A–F – CH_2Cl_2 soluble fraction; B–F – CH_3OH soluble fraction; C–F – CH_3OH/CH_2Cl_2 soluble fraction.

Sample	T_{start}, K	DTG T_{max}, K	Maximal weight loss rate, mg min^{-1}	$T_{50\%}$,[a] K		DTA exo-peaks T_{max}, K	
Lignin free PU	536 ± 2	577 ± 2	0.94 ± 0.05	633 ± 2	580 ± 2	639 ± 2	804 ± 3
PU with A-F	544 ± 2	616 ± 2	0.56 ± 0.02	638 ± 2	590 ± 2	639 ± 2	834 ± 3
PU with B-F	552 ± 2	624 ± 2	0.44 ± 0.03	675 ± 2	603 ± 2	675 ± 2	841 ± 3
PU with C-F	559 ± 2	675 ± 2	0.27 ± 0.02	675 ± 2	608 ± 2	675 ± 2	852 ± 3

[a] Temperature, when 50% weight loss of the sample is observed.

transparent, without solid inclusions and had the uniform thickness (~200 mkm).

The data of TGA (Fig. 2, Table 5) clearly testified the antioxidant effect of all three fractions, which is revealed in increasing temperatures of starting (T_{start}) and maximal development (T_{max}) of PU thermo-oxidative destruction as well as decreasing the process rate on the first stage of PU thermo-oxidative degradation. The data of DTA (Table 5) also confirmed the changes in thermo-oxidative behavior of model PU films: the exothermal maximum connected with oxidizing of PU destruction volatile products shifted to the high temperature region by 20-30 K (Table 5). It is considered in the literature [21] that for the polyether-based polyurethanes (used in the present study as model elastomers) this step involves the scission of the PU molecule into primary amine and proceeds by a radical chain process. The addition of antioxidants, in particular sterically hindered phenols, can stabilize PU against oxidation [22].

Lignins and lignocelluloses, mostly modified by oxialkylation, have long been a research subject in polyurethane chemistry as an aromatic polyol components [23,24]. It has been published that thermostability of PU synthesized using lignin-based polyols in enough quantities (10% and more) increases owing to significant changes in PU network structure, in particular an increase in PU cross-linked density up to 1.5–2.5 × 10^{-3} mol cm^{-3} [25]. However, in the present study, the BIOLIGNIN™ fractions were added in small amounts (5%) that did not lead to increase in the cross-linked density of PU elastomers: it was changed from (0.25 ± 0.02) × 10^{-3} mol cm^{-3} (lignin-free elastomer) to (0.26 ± 0.02) × 10^{-3} mol cm^{-3} (A fraction was added), to (0.35 ± 0.02) × 10^{-3} mol cm^{-3} (B fraction was added) and (0.6 ± 0.05) × 10^{-3} mol cm^{-3} (C fraction was added). Therefore, the noticeable retardation effect of lignin fractions on PU thermo-oxidative degradation observed cannot be connected with the cross-linking action of lignin.

In correlation with the results of the test on radical scavenging activity towards superoxide radical anion, CH_3OH and CH_3OH/CH_2Cl_2 soluble fractions decreased the PU thermo-oxidation rate stronger than that soluble in CH_2Cl_2. The action of lignin-based antioxidants manifested itself also in increasing the temperature of 50% mass loss by PU from 364 (control PU sample) up to 400 (PU with lignin fractions).

4. Conclusions

Fractionation of BIOLIGNIN™ with solvents of different polarity is a prospective tool for obtaining of more homogeneous lignin products, which reveal prominent antioxidant activity.

The results obtained clearly show that antioxidant activity of fractionated technical lignin is one more advantage for lignin application in PU production.

Application of Py-GC/MS opened an opportunity to found some novel "structure–activity" correlations needed for understanding and tuning of antioxidant properties of lignins.

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