Evaluation of the Rosemary Extract Effect on the Properties of the Poly Lactic Acid-Based Materials

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Abstract

Nature offers countless solutions to obtain appropriate bioactive agents with higher activity than synthesis ones. New multifunctional materials containing additives derived from natural resources, such as powdered rosemary ethanolic extract, were obtained by melt mixing. Composition of the rosemary ethanolic extract was detailed studied and the new-obtained PLA-based materials were investigated in respect with structural and physico-chemical properties. The results of antioxidant and antimicrobial activity, permeability to gases and migration kinetics indicated that PLA/R-based films show promising properties for application as bioactive food packaging materials.

Experimental

- **Polylactic acid (PLA)** from NatureWorks LLC (trade name: PLA 2002D) with av. Mw (GPC) of 4475 kDa.
- **Poly(ethylene glycol) (PEG)** BioUltra 4000 (Sigma-Aldrich) as plasticizer.
- **Powdered rosemary ethanolic extract (R)** was obtained by solvent extraction method from the rosemary leaves in a Soxhlet unit, followed by precipitation and its ***composition*** was detailed studied:

- The ***Total Phenolic Content*** was determined using Folin-Ciocalteu's reagent (FC) method, by reading the absorbance at 740 nm with a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), using gallic acid as standard. The resulted total phenolic content was of 112.5 mg GAE/g dw.
- The ***Total Flavonoids Content*** was determined by the aluminum chloride colorimetric assay with quercetin as standard, measuring the absorbance at 510 nm. A total flavonoids content of 261.5 (mg QE/g dw) was found.

- **Processing:** The compounding was performed for 10 min, at 175 C, and 60 rpm, by means of a Brabender mixer (30EHT, Duisburg, Germany).

A Carver press (Wabash MPI, IN, USA) and special parameters for compression molding (175 ºC, or 165 ºC for PLA/R blends, pre-pressing for 3 min at 50 atm and a pressing for 2 min at 150 atm) were used to obtain specimens for different analyses, with different compositions:

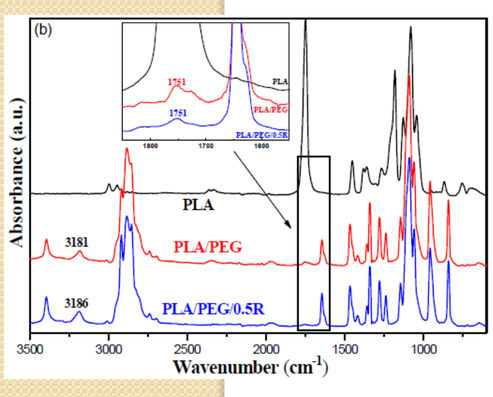
No.	Sample	PLA (wt %)	Powdered Rosemary Ethanolic Extract (R) (wt %)	PEG (wt %)
1	PLA	100	-	-
2	PLA/0.25R	99.75	0.25	-
3	PLA/0.5R	99.5	0.5	-
4	PLA/0.75R	99.25	0.75	-
5	PLA/PEG	80	-	20
6	PLA/PEG/0.5R	79.5	0.5	20

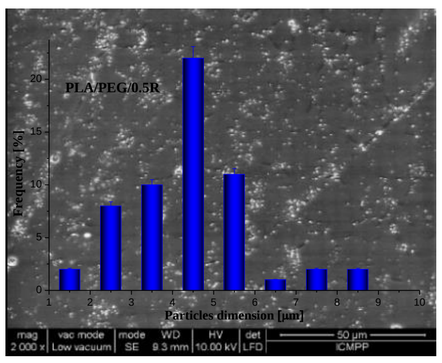
- Incorporation of R doesn't change the melt processing characteristics of PLA and its blends;
- Plasticizer PEG presence determines a sharp change in the melt mixing behavior: the melt flow was improved, the materials being easily processed;
- No oxidation occurred during processing

Sample	TQ_{max1} (Nm)	TQ_{1min} (Nm)	TQ_{max2} (Nm)	TQ_{5min} (Nm)	TQ_{final} (Nm)
PLA	66.4	17.2	-	13.3	10.2
PLA/0.25R	73.8	15.7	-	11.2	10.2
PLA/0.5R	72.0	17	-	11.4	10.5
PLA/0.75R	74.6	17.6	-	11.1	10.1
PLA/PEG	12.9	0.9	-	7.3	6.5
PLA/PEG/0.5R	10.1	2.8	-	7.3	5.7

TQ_{max1}—maximum torque; TQ_{1min}—torque after one minute of mixing; TQ_{max2}—maximum torque after 1.5 min of mixing; TQ_{5min}—torque after 5 min of mixing (half processing time); TQ_{final}—torque at the end of mixing.

Structure and morphology





- The COOH, C–O, phenolic –OH groups of the R components show corresponding absorption bands in the region 1750–1600 cm^{-1} .
- The R sample exhibits a sharp band at 1692 cm^{-1} corresponding to C–O vibrations, also present in the samples loaded with rosemary extract.
- Bands' shift indicates some interactions between components for the systems containing plasticized PLA due to a better distribution.
- homogeneous distribution of the rosemary powder in the PEG plasticized PLA samples without agglomeration;
- average dimension of the particles of 4.1 µm

Mechanical properties

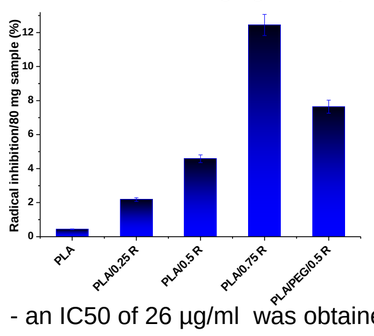
- The Young's modulus and tensile strength increase after incorporation of R into PLA and decrease in plasticized PLA
- The elongation at break increased and is two times higher for plasticized systems containing R

Rheology

- Decrease of all studied rheological parameters with incorporation of the powdered R extract in the PLA films
- A predominantly viscous behavior (G″>G′)

Antioxidant and antibacterial activity

ABTS•+ radical scavenging activity assay



- an IC50 of 26 µg/ml was obtained

Sample	ATCC *Bacillus cereus* 14579		ATCC *Salmonella typhymurium* 14028		ATCC *Escherichia coli* 25922	
	Inhibition %/24 h	Inhibition %/48 h	Inhibition %/24 h	Inhibition %/48 h	Inhibition %/24 h	Inhibition %/48 h
PLA	5	59	32	61	53	71
PLA/0.25 R	59	100	52	87	61	86
PLA/0.5 R	91	100	52	84	71	100
PLA/0.75 R	100	100	55	87	94	100
PLA/PEG	45	91	29	77	69	94
PLA/PEG/0.5R	86	100	48	100	76	100

- The antimicrobial activity is improving with an increasing concentration of the R extract, reaching values of about 100% for a concentration of 0.75% R

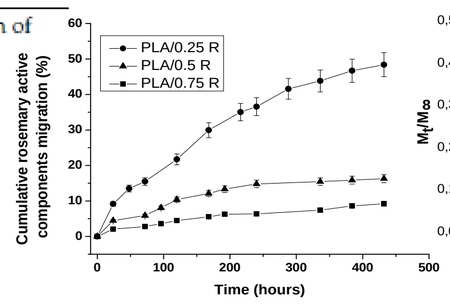
Gas Permeability

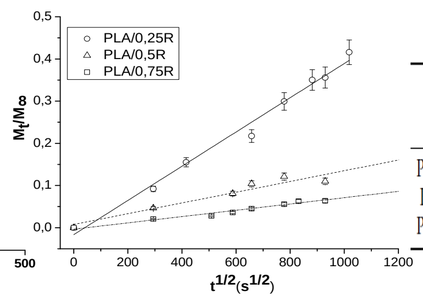
Sample	Thickness (mm)	CO_2 (mL/m²/day)	O_2 (mL/m²/day)
PLA	0.151	873	1308
PLA/0.25R	0.120	588	487
PLA/0.5R	0.122	535	273
PLA/0.75R	0.130	412	201
PLA/PEG	0.128	524	455
PLA/PEG/0.5R	0.126	489	278
Food freezing bag	0.020	64,601	50,266
Food packaging foil (LDPE/PP)	0.009	128,374	35,629

- An improvement of the gas permeability of the PLA-based films of approximately the same thickness was observed, depending on the R amount in films composition
- The oxygen barrier properties are better than those of CO_2.

Overall migration study

- At 40 °C for a minimum of 10 days using a 50% aqueous ethanol solution as a modified D1 food simulant;
- The active components' concentrations were calculated based on the calibration curve previously determined at 285 nm for the main components of rosemary extract;





$$M_t/M_\infty = k \cdot t^n$$

$$M_t/M_\infty = k_H \cdot t^{1/2}$$

Sample	Korsmeyer-Peppas Model			Higuchi Model		
	n	R^2	$K \times 10^{-3}\ (h)^{-n}$	R^2	$k_H \times 10^{-3}\ (h)^{-n}$	R^2
PLA/0.25R	0.61	0.99	14.1	0.989	27.57	0.98
PLA/0.5R	0.42	0.98	12.82	0.988	8.28	0.98
PLA/0.75R	0.54	0.985	3.52	0.993	4.48	0.99

- The migration behavior was quantitatively influenced by the R amount incorporated in the film samples: an increase of the R content led to a slower release of the active components;
- *n* values indicate a behavior closer to Fickian diffusion and the linearity of the plots $M_t/M_\infty < 0.6$ with respect to t½ demonstrates that the data are well described by the Higuchi model
- The trend of k_H values: $k_{PLA/0.25R} > k_{PLA/0.5R} > k_{PLA/0.75R}$
- Diffusion is indicated as the preferred mechanism for migration

Conclusions

- Incorporation of powdered rosemary ethanolic extract (R) into poly(lactic acid) (PLA) improved elongation at break, rheological properties, antibacterial and antioxidant activities.
- The good accordance between results of chemiluminescence method and radical scavenging activity determination by chemical method evidenced the increased thermo-oxidative stability of the PLA biocomposites in respect with neat PLA, R acting as an efficient antioxidant.
- A diffusion-controlled mechanism characterizes the migration process.
- The migration rate of the rosemary ethanolic extract components from PLA/R films directly depends on their permeability and degree of crystallization: for films of approximately the same thickness, the gas permeability decreased by two or six times, indicating difficult diffusion of components through the materials. Also, the interaction between the PLA/R blends components can slow the migration into the food simulant.
- The results obtained indicated that PLA/R-based films showed suitable properties for application as food packaging materials.

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