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Development of Bioactive Polymeric Materials by Incorporation of Essential/Vegetal Oils into Biopolymer Matrices †

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Abstract: Microbial contamination represents an undesirable event in various domains. Bioactive natural compounds possess plenty of health benefits, including antimicrobial, antifungal and antioxidative activity; however, they are chemically unstable and susceptible to oxidative degradation. In this context encapsulation or immobilization methods play a key role in enhancing efficiency. Therefore, in this paper are presented some results regarding the development of antimicrobial polymeric materials using surface-modification and emulsion-stabilization approaches. Two polymeric substrates, one biodegradable, polylactic acid, and one non-biodegradable, polyethylene, functionalized by γ-irradiation have been modified with different active compounds in order to obtain bioactive food packaging materials. Surface immobilization of the bioactive layers was achieved by a wet-treatment involving carbodiimide chemistry. The bioactive agents, clove essential oil and argan oil, were incorporated into a biopolymer matrix (chitosan). The resulted materials were physico-chemical characterized in order to evaluate the molecular interactions between natural bioactive compounds and polymeric matrix, the stability of the immobilized surface layer, and barrier properties. Antimicrobial and antioxidant activities were also evaluated. Moreover, the surface functionalized polymeric substrates were tested as potential packaging materials for cheese preservation. The obtained materials have demonstrated improved barrier properties, good antioxidant and antimicrobial properties, and they prolong shelf-life of the tested food.

Keywords: poly(lactic acid); poly(ethylene); chitosan; gamma irradiation; bioactive oils; emulsions; surface coating; food packaging

1. Introduction

Microbial contamination represents an undesirable event in various domains, such as in biomedicine [1], food preservation [2], and cosmetics [3]. Microbial adhesion and growth on medical devices and implants can cause serious complications to human health, being source of severe nosocomial infections [4]. The continuing emergence, development and spread of pathogenic organisms that are resistant to antimicrobials constitute an increasing global concern [5]. The demand of new antimicrobial products is increasing due to the reduction in the effectiveness of the existing industrial products, which leads to continuous spread of infections. It is, however, extremely difficult

to develop a universal product that could regulate or stop the entire infectious occurrence [6]. In the field of food preservation, substantial research on the development of innovative food packaging materials has been carried out aiming to combat pathogens, to reduce spoilage and waste, to optimize process efficiency, to decrease the need for chemical preservatives, improving the functionality, and the nutritional and sensorial properties of food. Despite efforts and improvements in the food production industry, foodborne pathogens still cause a number of illness outbreaks yearly all over the world [7].

Plants remain a unique and underexploited resource of bioactive compounds, and existing ethnobotanical tradition can be used to guide future research efforts and narrow down the search to the most suitable biocomponents to be used in modern antimicrobial materials [8]. The application of bioactive compounds in native form is very limited in food and drug formulations due to their poor bioavailability, low solubility, and fast release from traditional carriers [9]. In order to use these substances in specific applications, protection and stabilization in formulations, as well as controlled release is mandatory.

In most circumstances, food-packaging materials are polymer-based. However, this has limitations, since plastics are semi-permeable to gases and can undesirably affect food and drink quality over relatively short time periods. Barrier properties of plastic materials can be improved by coatings [10,11] or through the inclusion of fillers (e.g., nanoparticles) within the polymer matrix [12]. Because of the inert nature of most commercially polymers they must be subjected to surface functionalization before the attachment of bioactive compounds [13]. In this context, high-energy radiation appears as useful and suitable techniques for functionalization of both synthetic and natural polymers since they do not require the use of toxic chemicals and no waste production involved. In the present study, we focused on the surface functionalization of two inert polymers (poly(lactic acid) and polyethylene) and aim to impart bioactive functionalities to the selected polymeric materials. In our research, chitosan (CS) was chosen as incorporating matrix for essential and vegetal oils and further as bioactive coating for the surface functionalized polymeric substrates. CS belongs to the class of natural polysaccharides, has film forming ability, oxygen barrier property, and antimicrobial activity (against various pathogenic and spoilage micro-organisms) [14,15]. However, the antimicrobial activity of chitosan depends on its molecular weight, the degree of deacetylation and of its chemical degradation [16], hence the incorporation of bioactive natural substances may lead to the improvement of its antimicrobial functions.

2. Experiments

Polymeric films, poly(lactic acid) (PLA) (2002D type, NatureWorks LLC, USA) and polyethylene (PE) (composed of two parts UV treated LDPE and one part HDPE, provided by SC LORACOM SRL, Romania) were γ-irradiated using an irradiation machine (GAMMATOR M-85) equipped with ^{137}Cs source. The irradiation doses for polyethylene were 20, 30 and 50 kGy, while PLA was exposed to 10, 20 and 30 kGy, at a dose rate of 0.4 kGy h^{-1}, in air, at room temperature. After γ-irradiation the polymeric films were removed from the treatment chamber and surface coated by immersion for 12 h at 4 °C into a 2 wt% chitosan solution (containing 5% acetic acid) or oil-loaded chitosan emulsions. Food grade vegetal oils (clove essential oil—CEO, and cold pressed argan oil—CAO) were incorporated in a ratio of 0.75 mL per gram of chitosan (Sigma Aldrich, Germany), and Tween 80 (Sigma Aldrich, Germany) (0.125 g/g chitosan) was added as an emulsifying agent. The vegetal oil-added coating solution was homogenized with an ultrasonic processor UP50H (Hielscher—Ultrasound Technology, Teltow, Germany) using a power of 50 W at 30 kHz.The films modified with chitosan or chitosan based emulsions were excessively rinsed first with 1% aqueous acetic acid solution and secondly with water, and further vacuum dried at 50 °C. For covalent bonding of chitosan coating onto PLA surface the hydroxyl groups of chitosan were previously activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) coupling agent. The samples thus prepared were kept in a desiccator until further analyses were performed.

The samples were characterized by FTIR spectroscopy at 4 cm^{-1} resolution with 64 scans (Bruker VERTEX 70 spectrometer) and in terms of barrier properties by using a manometric gas permeation

tester (Lyssy L100-5000, Systech Illinois, Johnsburg, Illinois, USA). Antioxidant activity was evaluated by DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay as described in previously reported paper [11]. **Antibacterial tests** were performed using specific bacteria usually tested for food products both of animal and non-animal origin, namely *Listeria spp*. and *Escherichia coli*, by colony counting method [10]. The **migration** of the active components from the oils loaded chitosan coatings was investigated by a total immersion migration test (EC, 1997) [17] using 50% aqueous ethanolic solution as a food simulant (D1) [18].

Food shelf-life testing: The PLA and PE-surface functionalized films with oils loaded chitosan coatings were tested as active materials for white cheese. Firstly, the white cheese was determined for total viable counts (TVC) at 30 °C (ISO 4833:2003; Plate Count Agar at 30 + 1 °C for 72 h in aerobic conditions). The number of those bacteria in fresh control sample was less than 10 CFU/g. Most often, the microbiological spoilage of white cheese during its storage is caused by yeast and moulds. Therefore, in this study we monitored the growth of those groups of microorganisms in white cheese stored in refrigerator in contact with active polymeric films.

3. Results

3.1. FTIR Spectroscopy

In Figure 1 are illustrated the FT-IR spectra of poly(lactic acid) (Figure 1a) and polyethylene (PE) (Figure 1b) based samples surface functionalized by chitosan and oils loaded-chitosan coatings.

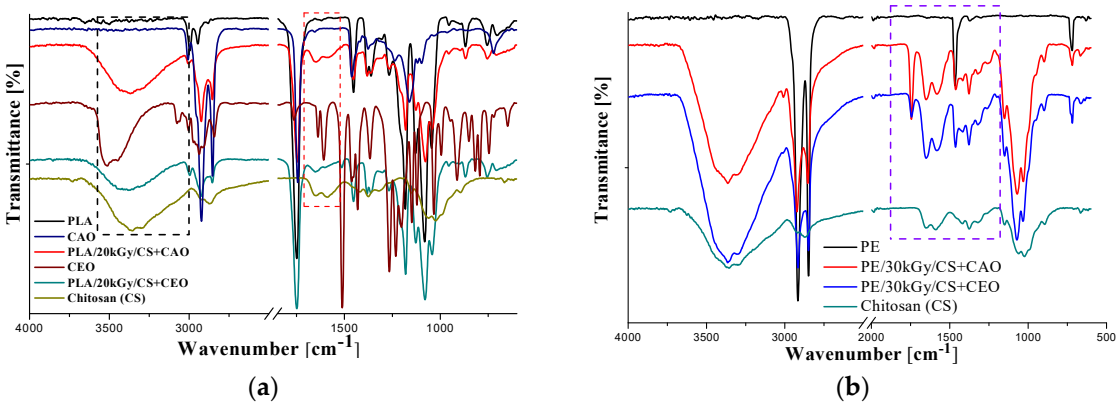

Figure 1. FTIR spectra of neat, γ-irradiated and surface functionalized with oil-chitosan loaded coatings of (**a**) PLA- and (**b**) PE-based films.

Significant differences are observed in FTIR spectra by oils incorporation into chitosan matrix. The vibration absorption band given by the free O-H bond valence occurs in the region 3590–3650 cm^{-1}, and the association through polymer hydrogen bonds leads to wide bands in the region 3200–3600 cm^{-1}. The vibration absorption band given by the free O-H bond valence (monomeric νOH) occurs in the region of 3590–3650 cm^{-1}, and the association through polymer hydrogen bonds leads to wide bands in the region 3200–3600 cm^{-1}. In the charged region 1400–400 cm^{-1} (fingerprint) of the IR spectrum of the absorption bands can be identified intense absorptions due to the deformation vibrations of the C-H bond and the valence vibrations of C-O single bonds of alcohols, ethers and esters. The major absorbance bands found in FTIR spectrum of clove oil (Figure 1a) are the following: the O-H stretch from phenol at 3511.3 cm^{-1}, C-H stretch of aromatic ring at 2937.6 cm^{-1}, C-C stretch (aromatic ring) at 1511.2 cm^{-1}, C-H substituted at 793.7 cm^{-1}, C-O stretch from ether group at 1265.3 cm^{-1}, and =C-H bend (alkene) at 1033 cm^{-1} [19]. In the FTIR spectra of gamma irradiated PLA and coated with CEO loaded-chitosan emulsion are observed both the characteristic bands of chitosan and CEO. The band found at 1511 cm^{-1} in the clove oil spectrum assigned to C-C stretch from aromatic ring is evidenced also in the FTIR spectra of PLA/20kGy/CS+CEO sample.

For argan oil (CAO) the IR spectrum (Figure 1a) reveals that: in the interval of 3100–2800 cm^{-1} the major absorption bands are located in the vicinity of frequencies 3006.9, 2923, 2853 cm^{-1}. The band

at 3006 cm^{-1} is specific to the methyl-oleate and the other absorptions are characteristic to the symmetrical and asymmetrical vibrations ν(C-H) of the CH_2 and CH_3 aliphatic groups from the alkyl rest of the triglycerides, which are found in large quantities in vegetal oils. The absorption at 1743 cm^{-1} is assigned to ν(C = O) from RC = OOR compounds, feature to the oils with a high content in saturated fatty acids and short carbohydrate chain. The spectral band near to 1654 cm^{-1} corresponds to the double C = C link and may be correlated with the content of polyunsaturated fatty acids in the oil. At 1461 cm^{-1} is noticed a band associated to the vibrations of deformation δ(CH). Other two bands are observed at 1376 and 1237 cm^{-1}, the first band corresponds to the deformation vibration in the phase of methylene group, while the second band corresponds to the deformation vibration in the plan of the group =CH, from the double links cis unconjugated [20]. By CAO loading CS emulsion a new band appears in the FTIR spectra of PE/30kGy/CS+CAO at 1744.5 cm^{-1} (when compared with γ-irradiated PE coated only with chitosan) that is assigned to ν(C = O) from RC = OOR functional group. In the case of PLA substrate surface modified with CAO-loaded chitosan coating some characteristic bands of PLA and chitosan overlaps the argan oil vibration bands. The FTIR spectrum of PLA/20kGy/CS+CAO sample reveals some characteristic bands (or shoulder peak) for argan oil namely at 1462 and 1377 cm^{-1} attributed to vibrations of deformation in-plane and in-phase of methylene group.

3.2. Barrier Properties, Antioxidant/Antimicrobial Activities, and Migration in Food Simulant

The barrier properties of PLA and PE surface functionalized samples were estimated by measuring the gas transmission rate of oxygen (OTR) at 23 °C in relative dry state. Surface immobilization of chitosan and oil loaded chitosan emulsions onto γ-irradiated poly(lactic acid) and polyethylene substrates lead to a significant decrease of OTR (Table 1). The antioxidant activity of the polymeric substrates functionalized by oil loaded chitosan coatings was determined by measuring the capacity of each sample to scavenge the DPPH free radicals. Gamma irradiated PLA and PE modified only with chitosan presents only a slight antioxidant activity, while the substrates functionalized with vegetal oils loaded chitosan emulsions exhibit enhanced radical scavenging activity (RSA) (Table 1). The samples functionalized with CEO loaded chitosan manifest higher RSA compared to CAO loaded coatings, as revealed by the data listed in Table 1. The migration profiles of CEO in food simulant D1 shows a burst release of ~30% in the first hours, followed by a gradual but fast release in the first 4–5 days and then a slower release up to 2 weeks. The calculated kinetic parameters indicate a migration behavior with some tendency towards Fickian diffusion.

Table 1. Oxygen permeability, antioxidant activity and bacterial inhibition (for a Gram-negative bacteria, *E. coli*, and a Gram-positive bacteria, *Listeria monocytogenes*) for PLA and PE functionalized surfaces with oils-loaded chitosan coatings.

Samples	Oxygen Permeability ($mL/m^2.day$)	Antioxidant Activity (%)	Bacterial Inhibition (%)	
			Escherichia coli	*Listeria monocytogenes*
PLA	476	0	32	20
PLA/20kGy/CS	162	30	84	96
PLA/20kGy/CS+CAO	85	81	85	70
PLA/20kGy/CS+CEO	51	100	99	67
PE	3843	0	0	0
PE/20kGy/CS	2531	24	69	72
PE/30kGy/CS+CAO	1685	76	83	96
PE/30kGy/CS+CEO	1325	91	92	98

3.3. Testing the Polymeric Substrates as Active-Food Packaging for Fresh White Cheese

The population dynamics of spoilage-related microorganisms (Total Viable Counts—TVC) of white cheese is described in Figure 2. It can be noticed that the TVC of white cheese stored in refrigerator in contact with traditionally packaging material (control) were significantly higher than in PLA and PE substrates surface modified with chitosan and bioactive oils loaded CS coating.

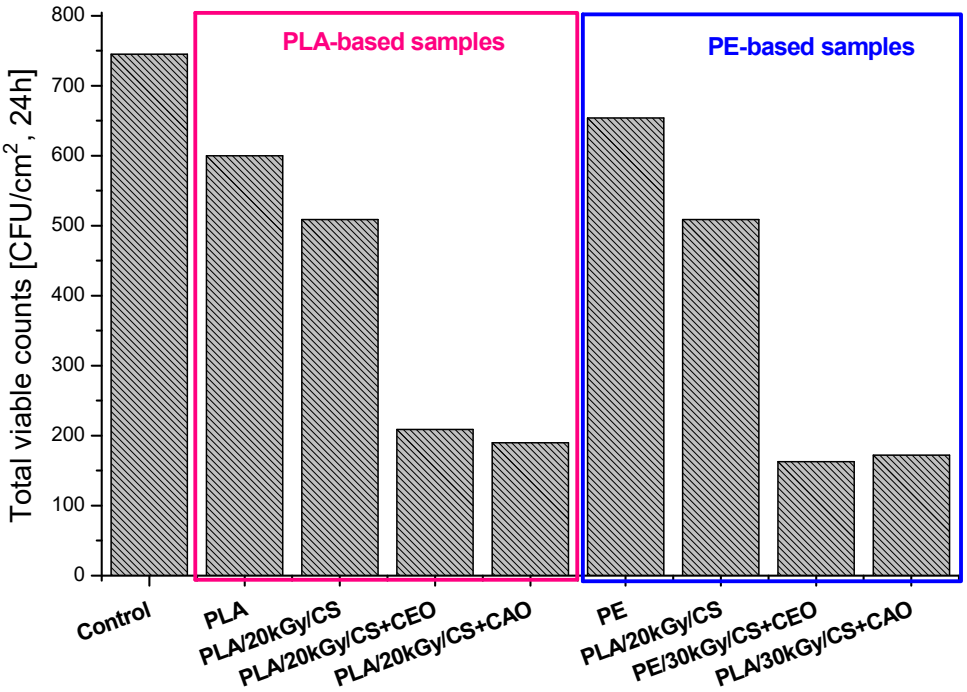

Figure 2. Variation in time of Total Viable Counts for white cheese packed in poly(lactic acid) and polyethylene modified substrates with chitosan and oils loaded chitosan emulsions.

4. Discussion

FTIR spectroscopy analysis of the samples had revealed that γ-irradiation of both polymeric substrates create reactive sites onto the surface that facilitates the attachment of biopolymeric formulations (chitosan or oil-loaded CS emulsions). Oils loaded chitosan coatings are characterized mainly by strong hydrogen bond interactions (highlighted by the O-H band shifts to lower frequency). The FTIR spectroscopy have demonstrated both the clove and argan oil incorporation into chitosan matrix and the surface immobilization of the chitosan-oil mixture onto γ-irradiated PE and PLA substrates.

In dry conditions, the coated films were significantly less gas (oxygen) permeable than PLA and PE substrates. However, we must take into account the hygroscopic nature of chitosan, meaning that a rise of the relative humidity is possible to determine a decrease of its barrier properties. Moreover, the oxygen barrier properties are influenced by the thickness of the analysed substrate, and this is one of the reasons that the OTR values for PLA-coated samples are lower than for PE-based samples (PLA films are 5 times thicker).

The antioxidant activity of the essential and vegetal oils is mainly dictated by its complex composition. In the case of CEO the antioxidant activity is mainly attributed to eugenol, which is the major compound, and for CAO the antioxidant feature is dictated probably by the content in caffeic acid, vanillic acid, and ferulic acid. The migration of CEO active compounds into D1 food simulant was characterized by a prolonged and sustained release. This behaviour was determined by the incorporation of CEO in CS and subsequently the physico-chemical interactions at the oil-biopolymer interphase, which delayed the migration of active compounds. The migration behaviour of CAO could not be determined using D1 as food simulant, hence additional studies are planned further.

The antibacterial tests shows that the oil loading into chitosan coating leads to a slightly decrease of CS antibacterial activity towards Listeria monocytogenes, which can be attributed to the interactions that take place between active components of oils and biopolymer (some functional groups of chitosan are hindered). Significantly lower values for TVC of spoilage-related microorganisms found in white cheese stored in contact with PE and PLA functionalized materials with oil-loaded CS coatings indicates a delay in food spoilage.

5. Conclusions

Surface functionalization of PLA and PE by gamma irradiation and active oil loaded CS coatings have improved their gas barrier properties, and impart to them antioxidant and antimicrobial properties. The incorporation of active vegetal oils into chitosan coatings leads to a significant decrease of spoilage-related microorganisms of cheese when compared with the native polymeric substrates, PLA and PE, and to a sustained release of active compounds. Both clove essential oil and cold pressed argan oil proved to be valuable antimicrobial agents for delaying the spoilage of white cheese.

Author Contributions: E.S. and C.V. conceived and designed the experiments; E.S., R.P.D., M.A.B. performed the experiments; E.S., R.P.D., and M.A.B. analyzed the data; A.E. contributed with analysis tools; E.S. wrote the paper.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PLA	poly(lactic acid)
PE	polyethylene
CEO	Clove essential oil
CAO	Cold pressed argan oil
CS	Chitosan
TVC	Total viable counts

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