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ORIGINAL ARTICLE

Orange essential oil as antimicrobial additives in poly(vinyl chloride) films

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Abstract

In this work were developed and evaluated films of poly(vinyl chloride)-PVC additivated with orange essential oil – OEO. These films were evaluated with FT-IR spectroscopy; mechanical tests; migration OEO in simulants; and determination of stability after sterilization by gamma radiation at a dose of 25 kGy. The OEO was assessed with GC-MS and analysis of antimicrobial activity. The films were prepared by the casting solution technique. The essential oil concentrations in PVC were 2%, 10% and 30% (w/w). The results showed that the OEO was incorporated into the polymer matrix and that this oil had antimicrobial activity against the bacteria E. coli and S. aureus. The migration of OEO in the films occurred with all simulants. The incorporation of OEO in the films also made them more flexible. It was also found that additive with 30% w/w OEO provides a protective effect for the polymer after sterilization by gamma radiation.

Keywords: *antimicrobial activity, mechanical tests, migration, orange essential oil, poly(vinyl chloride).*

1. Introduction

Food packaging has improved over the years in order to match the demands of modern society[1]. The search for alternative in packaging systems has been carried out to preserve the quality of food and prolong its commercial validity[2]. Among these systems is antimicrobial packaging, acting by the slow migration of active agents incorporated into the polymeric matrix to the surface of the food[3,4].

One possibility for the formulation of antimicrobial packaging is to use an additive with essential oils (EOs). EOs are liquid mixtures of volatile compounds extracted from leaves, flowers, stems, roots, seeds or fruit peel that have attracted interest of the food industry for their antimicrobial nature[5-8]. This antimicrobial action is due to the presence of components that have the ability to alter the permeability of the outer membrane of micro-organisms and/or inhibit important enzymes for their growth and survival[9].

An alternative to this kind of antimicrobial packaging would be the combination of poly(vinyl chloride) (PVC) with essential oil. PVC is one of the most consumed thermoplastics in the world, with good cost-benefit and the ability to incorporate diverse types of additives, besides being recyclable, non-toxic and inert[10-13]. PVC is a rigid polymer. This rigidity is attributed the forces of Van der Waals dipole-dipole caused by the hydrogen and chlorine attached to the same carbon atom[12]. The additives incorporated in the PVC can change their characteristics, such as the decrease in the rigidity or transparency; to promote greater resistance to weathering conditions; to promote antimicrobial action; and combined changes[13-15].

A potential antimicrobial agent is the orange essential oil (OEO). This oil has d-limonene as its main antimicrobial agent. This is a monocyclic monoterpene extracted from citrus peel, easily absorbed into the polymer matrix; and has intense antimicrobial activity, making it attractive for the food packaging industry[14]. Furthermore, the extraction of essential oil orange can be considered a sustainable raw material, as the shell of the citrus fruit is considered a loss to the industry of fruit juice[16,17]. In the literature reporting PVC added with substances that have the function of stabilizers, plasticizers or viscosity increasing agent, such inseed oil and gum rosin[18]. Also, works are reported of limonene added to other polymers, such as PLA[14,19,20], blends of PHB/PLA[21,22], starch-sodium caseinate blend films[23] and chitosan films[14,24,25]. In literature also are reported workes that used other essential oils with polymers, such as gelatin films with citrus oils[26], chitosan films with cinnamon oil[27]; films from soy protein with cinnamon oil[28], k-carrageenan film with savory oil[29], chitosan films with basil[30], films from whey protein with oregano oil[31] and chitosan films with Zataria oil multiflora[32].

This study were developed and evaluated PVC films additivated with orange essential oil aiming towards the application to antimicrobial packaging for the food industry. The additive with this EO permits a greater interaction of the packaging with the food an important differential compared to conventional packaging.

2. Materials and Methods

2.1 Materials

The orange essential oil (OEO) with specific density of 0.8420 g/mL was donated by AGROTERRENAS Company (São Paulo - BR) and the polymer was donated by TELETRON (Pernambuco - BR). The solvent tetrahydrofuran (THF) used from Sigma Aldrich.Text paragraph within a first subsection.

2.2 Characterization of the OEO by GC-MS

Characterization of the essential oil was performed with a gas chromatography mass spectrometry (GC-MS) system from Thermo Scientific. The chromatograph was a Trace 1300 model. The mass spectrometer was the ISQ Single Quadrupole system. The temperature parameters were used were: GC oven ramp 60 °C for 3 min (10 °C/min to 300 °C) and 300 °C for 15 min; injector temperature 270 °C; MS temperature of the transfer line 280 °C; and MS source temperature of 250 °C ions.

2.3 Production of polymer films

The films were produced by the solution casting method with 1.5g of PVC and 50 mL of THF[33]. The PVC films were prepared by additivating with orange essential oil in different amounts (0, 2, 10 and 30% w/w). The orange essential oil was added to the polymer according to the methodology adopted by Morelli et al.[34]. The glass Petri dishes used in the solvent evaporation step had the following dimensions: 15.0 × 2.0 cm. The PVC films and PVC additivated with OEL had an average thickness of (0.083 + 0.015) mm.

2.4 Mechanical properties

Mechanical tests were carried out in a universal tensile testing instrument, DL-500MF brand model EMIC, in accordance with the ASTM D882-12 standard[35]. Assays were conducted at room temperature without humidity control. Assays were performed under the following conditions: load cell of 500 N; jaw speed of 100 mm/min; initial distance between the jaws 40 mm; and dimension of the specimen (20 × 50) mm. For each film composition, there were 9 replicates.

2.5 Mid-infrared spectra acquisition (FTIR)

Mid-infrared (MIR) spectra of the films were acquired in a Tensor 27 spectrometer (Bruker) with an Attenuated Total Reflectance-ATR accessory. The spectra of the films were recorded under the following conditions: mid-infrared region 4000-400 cm^{-1}, resolution of 4 cm^{-1} and 16 scans.

2.6 Migration test

To follow the migration of orange essential oil, we used mid-infrared spectroscopy, using the attenuated total reflection technique (ATR)[4]. The conditions chosen were: spectral range 1670 to 1616 cm^{-1}; resolution of 4 cm^{-1}; and 16 scans. Samples of scale films (30x10) mm were used in the migration tests. To perform the test, the samples were immersed in food simulants: distilled water, olive oil and 10% ethanol. Each film sample was immersed in 6 mL of simulant, sealed and placed in a hot air oven at 40 °C. The migration periods were monitored at 0, 36, 84 and 162 hours.

2.7 Antimicrobial activity of OEL

The activity of the orange essential oil was investigated by disk diffusion assay with medium Plate Count Agar (PCA)[36]. Filter paper disks of 2 cm diameter were utilized, having been sterilized by UV irradiation for 10 min (each side for 5 min). Aliquots of 0.5 mL of *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739) in the order of 10^7 CFU/mL, were quantified by turbidity on the Mcfarland comparison scale. They were inoculated into the PCA by the pour plate method. After solidification of the PCA, these were placed on discs immersed with orange essential oil, in the center of the petri dish. The plates were incubated at 35 °C for 48h.

2.8 Radiolytic sterilization of films

The films were exposed to gamma radiation with a Gammacell (GC)-220 Cobalt-60 irradiator at a dose of 25 kGy. This dose is also used to sterilize the food packaging[37].

2.9 Statistical analysis

All data were analyzed by One-way analysis of variance (ANOVA) using Duncan's test for comparison between the means ($p < 0.05$). The statistical analyses were performed with STATISTICA 7.0 software.

3. Results and Discussion

3.1 GC-MS of orange essential oil

The GC-MS analysis identified over 150 constituents present in the OEO. Figure 1 highlights main constituents, representing 89.78% of the oil composition.

Figure 1 shows the major components of the OEO were p-Mentha-1(7),3-dieno **(1)**, D-Limoneno **(2)**, Linalol **(3)**, Decanal **(4)**, n-Hexadecanoic acid **(5)** and cis-13-Octadecenoic acid **(6)**. These compounds are classified as terpenes, alcohol, aldehyde and carboxylic acids and their molecular structures are summarized in Table 1, with their respective retention times (RT) and peak areas.

The most known for their antimicrobial compounds are the phenols, terpenes and aldehydes. These act by altering the concentration of fatty acids in the microbial cell membrane, causing damage to its structure[38].

D-limonene was expected as the major constituent as described in the literature on Citrus oils[39]. Other authors who studied Citrus oils quantified 84.7% in grapefruit oil, 94.51% in orange oil and 60.0% in lemon oil[40-42].

3.2 Mid-infrared spectra (FTIR) of PVC/OEO films

Figure 2 shows the FTIR spectra obtained in the mid-infrared region of orange essential oil, pure PVC film and PVC films additivated with 2, 10 and 30% w/w of orange essential oil. In this figure the main bands have been identified in accordance with the literature, found in pure PVC film which are 2911, 1249, 957, 837 and 616 cm^{-1} related to the CH stretching, CH rocking, trans CH wagging C-Cl stretching and cis CH wagging, respectively[43]. In PVC

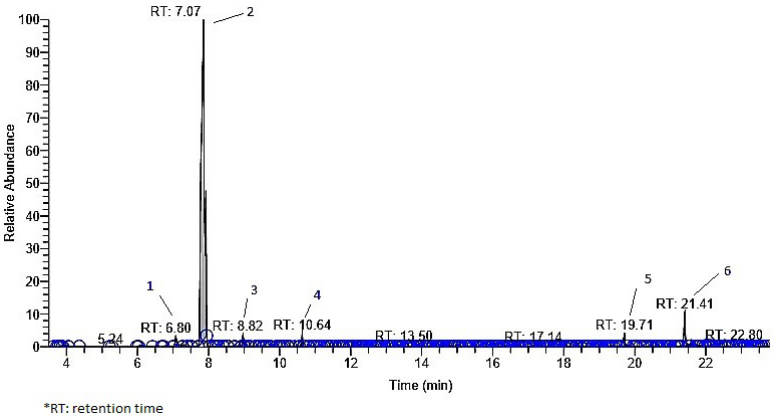

Figure 1. GC-MS chromatogram of orange essential oil (OEO).

Table 1. Major components orange essential oil determined by GC-MS.

Number	Component	Retention time (RT) (min)	Peak area (%)
1	p-Mentha-1(7), 3-diene	6.80	1.98
2	Limonene	7.07	79.97
3	Linalol	8.82	1.11
4	Decanal	10.64	0.93
5	n-Hexadecanoic acid	19.71	1.84
6	cis-13-Octadecenoic acid	21.41	3.95

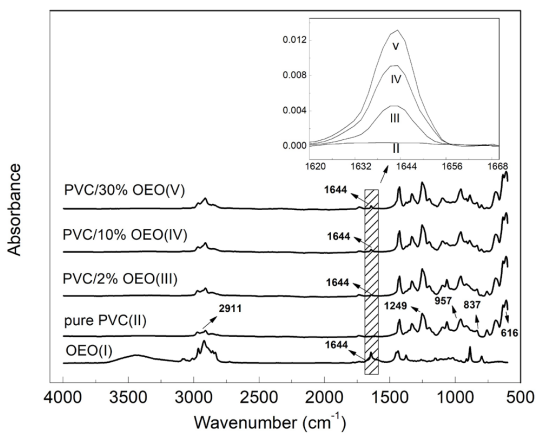

Figure 2. FTIR spectra of the orange essential oil (OEO), pure poly(vinyl chloride) film (PVC) and poly(vinyl chloride) films additivated with 2, 10 and 30% of w/w of orange essential oil (PVC/2%OEO, PVC/10%OEO and PVC/30%OEO, respectively).

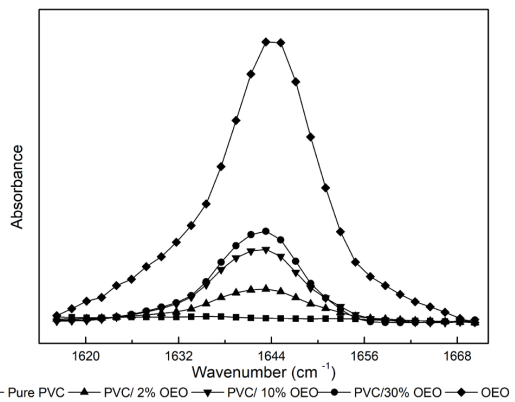

Figure 3. FTIR spectra of the orange essential oil (OEO), pure poly(vinyl chloride) film (PVC) and poly(vinyl chloride) films additivated with 2, 10 and 30% of w/w of orange essential oil (PVC/2%OEO, PVC/10%OEO and PVC/30%OEO, respectively) in the region of 1616-1670 cm^{-1}.

films additivated with orange essential oil, a 1644 cm^{-1} peak band is observed. This band gives evidence of the presence of orange essential oil which is identified as the stretch of the C = C bond. This band is present in orange essential oil, but it is not present in the PVC FTIR spectrum, so this band can be used for purposes of evaluating oil migration in a food simulant environment. In Figure 3 there is a 1670-1616 cm^{-1} region in the spectra of films evaluated. As can be seen in Figure 3, the increased peak of the band is due to the increase in the percentage of oil.

3.3 Migration test of the orange essential oil

For migration analysis, samples of PVC films were evaluated. These PVC films were additivated with 2%, 10% and 30% w/w of OEO exposed to the following chemical agents: 10% ethanol, olive oil and water. These media simulate alcoholic foods (ethanol), greasy food (olive oil) and aqueous non-acid foods (pH > 4.5) (water), as established by Resolution Nº. 32 of the Common Market Group, MERCOSUR (2010)[44].The acquisition of the spectra was carried out in periods of 0, 36, 84 and 162 hours. Figure 4

shows the spectra of PVC samples additivated with 2% w/w of OEO. The essential oil migration to the film surface can be observed by the decrease in peak at the 1644 cm^{-1} band.

Figure 4 shows a decrease in the intensity of the peak at periods of 36, 84 and 162 hours. This migration is justified by the diffusion mechanism that is strongly influenced by interactions occurring between the media and the packaging material[45].

Figure 5 illustrates the spectra of PVC samples with 30% w/w of OEO. Figure 5a shows that OEO migration in the ethanol simulant occurs gradually over the period. Figure 5b shows migration in the simulant olive oil with higher speed, being completed in the first 36 h. Figure 5c shows OEO migration at a slower speed, as can be verified by the intensity of the peak at 1644 cm^{-1}.

The migration of orange essential oil in the simulant olive oil occurred with higher speed due to the affinity and solubility between them. The diffusion of the active agent

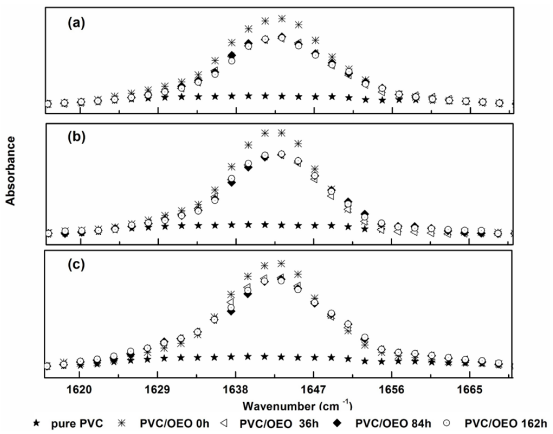

Figure 4. Migration in pure poly(vinyl chloride) film (PVC) and poly(vinyl chloride) film additivated with 2% of w/w of orange essential oil (PVC/OEO) in the simulants: (a) ethanol; (b) olive; and (c) water by Infrared.

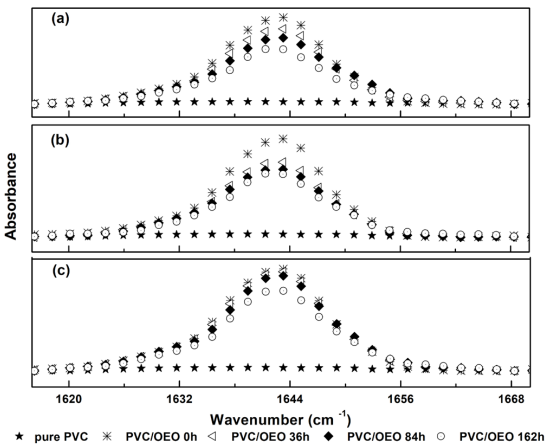

Figure 5. Migration in pure poly(vinyl chloride) film (PVC) and poly(vinyl chloride) film additivated with 30% of w/w of orange essential oil (PVC/OEO) in the simulants: (a) ethanol; (b) olive; and (c) water by Infrared.

and its solubility of the polymer is extremely important to define the basic conditions for their use. The diffusion behavior of chemicals incorporated in the polymers is a very complex process and depends on several parameters, such as the concentration of substances in the packing, nature of the food, temperature and the period of time during which the contact lasts[46]. In the literature, there are studies that have evaluated the migration of limonene in other polymers. Authors evaluated the diffusion of limonene in low-density polyethylene film[47].They found that limonene diffusion velocity in the polymer was low due to the morphological differences in the polymer.

3.4 Antimicrobial activity of OEL

Figures 6a and 6b illustrate the antimicrobial test through the zone of inhibition for *S. aureus* (Gram positive) and *E. coli* (Gram-negative). It can be seen that these figures the zone of inhibition showed antimicrobial activity for the bacteria tested in the oil. Diameters of the inhibition halos shown in Figures 6a and 6b were 21,6 mm and 38,5 mm, respectively. The antimicrobial activity of orange essential oil has also been observed by other authors[48,49].

3.5 Mechanical properties

Table 2 shows the results of tensile tests for mechanical properties, using Young's modulus, percentage elongation at break and tensile strength of PVC films, PVC/2%OEO, PVC/10%OEO and PVC/30%OEO. The mean values of the mechanical properties obtained through the mechanical tests were compared statistically with Duncan's test at a significance level of 5% ($p < 0.05$). We verified that there was a reduction of the values of Young's modulus for the additive with 30% w/w of OEO. For the percentage elongation at break, the values presented no statistical differences for the level of significance of 5%. For maximum stress, significant changes were observed from the additive with 10% w/w of OEO.

Table 3 shows the results of tensile tests for Young's modulus, percentage elongation at break and tensile strength of PVC films, PVC/2% OEO, PVC/10% OEO and PVC/30% OEO after exposure to gamma radiation. The mean values of the mechanical properties were compared statistically by Duncan's test at a significance level of 5% ($p < 0.05$). This verified that there were no significant changes in the values of Young's modulus. For the percentage elongation at break, there was a decrease in value of this property with the additive at 30% w/w of OEO.

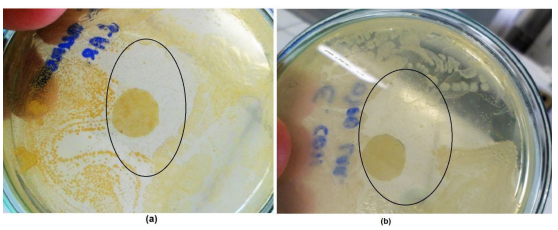

Figure 6. Antimicrobial test through the zone of inhibition: (a) *S. aureus* (Gram positive); (b) *E. coli* (Gram-negative).

Table 2. Average values obtained for the mechanical properties tensile strength, percentage elongation at break and Young's modulus in pure poly(vinyl chloride) film (PVC) and poly(vinyl chloride) films additivated with 2, 10 and 30% of w/w of orange essential oil (PVC/2%OEO, PVC/10%OEO and PVC/30%OEO, respectively).

Samples	Young's Modulus (MPa)*	Elongation-at-break (%)*	Maximum Tensile(MPa)*
PVC/2%OEO	1224.00 ± 28.83[a]	5.628 ± 0.197[a]	42.150 ± 1.451[a]
PVC/10%OEO	1153.00 ± 143.28[a]	6.056 ± 0.030[a]	31.505 ± 1.278[b]
PVC/30%OEO	755.93 ± 37.46[b]	6.018 ± 0.726[a]	33.410 ± 2.272[b]
PVC	1275.00 ± 77.79[a]	5.901 ± 0.344[a]	43.580 ± 0.850[a]

*Values are presented as means ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

Table 3. Average values obtained for the mechanical properties tensile strength, percentage elongation at break and Young's modulus of the irradiated samples in pure poly(vinyl chloride) film (PVC) and poly(vinyl chloride) films additivated with 2, 10 and 30% of w/w of orange essential oil (PVC/2%OEO, PVC/10%OEO and PVC/30%OEO, respectively).

Samples	Young's Modulus (MPa)*	Elongation-at-break (%)*	Maximum Tensile (MPa)*
PVC/2%OEO	0.083 ± 0.006[a]	1059.77 ± 112.18[a]	6.232 ± 0.090[a]
PVC/10%OEO	0.076 ± 0.025[a]	941.65 ± 89.76[a]	6.062 ± 0.192[a]
PVC/30%OEO	0.089 ± 0.029[a]	738.17 ± 10.01[b]	5.770 ± 0.575[ab]
PVC	0.076 ± 0.011[a]	947.72 ± 44.19[a]	5.476 ± 0.169[b]

*Values are presented as means ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

Comparing the mean values for each property before and after gamma radiation, we observed that there was a reduction in all parameters for each PVC film with oil additives. Similar result was observed in the work done by Landgraf[37].The author affirms that although sterilization by gamma radiation at 25 kGy dose inactivates the antimicrobial agent, the highly reactive species generated in the irradiation process can have undesirable effects on packaging materials, degrading the polymer may lower its resistance, change the color and transparency.

A comparison of the reduction obtained before and after the sterilization process showed that the Young's modulus of the control film decreased by 25.67%, while for the films with 30% OEO this reduction was only 2.35%. For elongation, these reductions were 7.20% and 4.12%, respectively for the film control and 30% OEO. For maximum stress, there was a reduction of 1.23% for the films with 30% OEO and an increase of 11.17% for the control film. These results indicate that irradiation affects in the PVC film is more intense in the PVC film without OEO, while in the presence of 30% w/w of OEO these changes were minimal.Accordingto Uzeli (2013), packaging properties should be maintained after sterilization[50]. Thus, PVC films with 30% orange oil meets this requirement.

Table 3 also shows that the flexibility in PVC film with oil increased after sterilization by gamma radiation. This is important for packaging, since flexibility is a desirable property for this polymer.

4. Conclusions

OEO presented antimicrobial activity to *E. coli* and *S. aureus*, two microorganism pathogens of great relevance to food area. Through the results of the migration test, it was found that the OEO migration speed for each food simulant is related to the amount of additive used in the active film. The higher the percentage of additives, the most essential oil migration speed to the surface of the film. The mechanical properties demonstrated that in the presence of OEO, the PVC films were more flexible, even after being irradiated with gamma radiation. The results from the mechanical and migration properties showed that orange essential oil is promising for use in antimicrobial packages, because the essential oil is an antimicrobial agent that migrates to the surface of the film in food simulants and also contributes to improve flexibility of the film.

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