MICROBIOLOGICAL CHARACTERIZATION OF RAW MATERIALS AND OBTAINED BIODEGRADABLE PACKAGING

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Abstract. The present study refers to the microbiological characterization of some biodegradable packaging made on the basis of polylactic acid and other additives with antimicrobial properties that confer a longer shelf life for fresh food products compared to their traditional packaging, i.e. with different compositions of polyethylene or polyethylene terephthalate. As additives for the final chemical composition of biodegradable packaging, several volatile oils and products resulting from the secondary valorization of agricultural waste from the manufacture of wine, respectively, were tested. The data obtained showed that the hydroalcoholic extract of the grape pomace does not contain compounds with antimicrobial activity and the results of the UV-VIS analyses indicated a significant composition of it in polyphenolic compounds. Also, the investigations demonstrated that a chemical composition of the newly obtained packaging supplemented with thyme essential oil increased the shelf life of freshly packaged food products in the traditional system up to 8 days from packaging.

Keywords: biodegradable packaging, polylactic acid, antimicrobial properties, grape pomace.

Rezumat. Caracterizarea microbiologică a unor materii prime și a ambalajelor biodegradabile obținute. Prezentul studiu se referă la caracterizarea microbiologică a unor ambalaje biodegradabile fabricate pe bază de acid polilactic și alți aditivi cu proprietăți antimicrobiene care să confere un termen de valabilitate mai mare pentru produse alimentare proaspete comparativ cu ambalarea acestora în sistem clasic, în ambalaje cu diferite compoziții de polietilenă sau polietilentereftalat. Au fost testate ca aditivi pentru compoziția chimică finală a ambalajului biodegradabil mai multe uleiuri volatile și produși rezultați din valorificarea secundară a deșeurilor agricole provenite de la fabricarea vinului, respectiv tescovina. Datele obținute au arătat că extractul hidroalcoolic al tescovinei nu conține compuși cu activitate antimicrobiană iar rezultatele analizelor UV-VIS au indicat o compoziție semnificativă a acestuia în compuși polifenolici. De asemenea, investigațiile au demonstrat că o compoziție chimică a ambalajului nou obținut suplimentată cu ulei esențial de cimbru a crescut termenul de valabilitate al produsului alimentar proaspăt ambalat în sistem clasic până la 8 zile de la ambalare.

Cuvinte cheie: ambalaj biodegradabil, acid polilactic, proprietăți antimicrobiene, tescovina.

INTRODUCTION

The packaging intended for the food industry is defined by different shapes, properties and compositions that ensure the preservation of the organoleptic characteristics of food in the short or longer term. Their chemical composition must ensure the quality and validity of the food both for short periods of time (fresh meat products, for example), and for longer, by freezing. Classified as perishable based on its physical, chemical, mechanical or biological degradation factors (JAFARZADEH et al., 2017), food requires packaging that prevents the action of these factors during the transport, distribution and marketing process. Thus, a packaging must meet a critical condition regarding the preservation of the organoleptic properties of food (PILEVAR et al., 2019). Recent research has demonstrated the usefulness of different nanomaterials such as metal nanoparticles or their oxides, nanoclays or graphene-type materials in obtaining mixed structures for the manufacture of modern packaging, without significant impact on the environment (JAFARZADEH et. al., 2020). Their mechanisms of action against microbial contamination of food may include cell membrane penetration (SINGH et al., 2014), modification of protein structure (DUIAZ et al., 2018), intercalation in the DNA structure, inhibition of enzymatic activities (LIRA et al., 2019) or oxidative stress (SINGH et al., 2014).

People's concerns for the preservation and transportation of food have their origins in the emergence and development of human society. The methods and means in this sense have gradually evolved, at first being based on drying, smoking, salting or fermenting food (KELSEY, 1989). The transport methods were marked by the appearance of braids made of burlap, herbs or vines (SACHAROW & GRIFFIN, 1970) also used in current human civilization. There are historical data that show that, in ancient Egypt, glass and ceramics were used for food storage around 3000 B.C. (SULLIVAN, 1976). Some archaeological evidence regarding the use of ceramic vessels for storing and transporting food as well as their microbiological characterization were also identified in the territory of Dacia (BĂTRÎNESCU et al., 2022). The development of society led to the discovery and manufacture of tin, used in the production of metal boxes for preserving food during 1200 A.D. (SACHAROW & GRIFFIN, 1970). The evolution of food storage and preservation packaging is marked in 1809 by the development of glass containers closed with cork and heated in a water bath (KELSEY, 1989), in 1810 by the appearance of metal cans and after 1840 by Pasteur's experiments. Later, the methods and means of packaging and preserving food were closely linked to industrial development, the appearance of rubber, adhesives, and polymers stable at wide temperature ranges (CUTTER, 2002). Thus, a series of materials such as bottles, plastic jars, and materials based on polyolefins, polyvinyl, vinyl chloride, polyethylene, nylon, etc. are found.

Currently, the flexibility of the food packaging process has led to the development of packaging procedures in controlled atmosphere, vacuum, modified atmosphere, edible films, active packaging (SACHAROW & GRIFFIN, 1970; BRODY, 1989; DAVIES, 1995). The latter are defined by preferential permeability, systems for capturing some gases released by canned foods, antimicrobial compounds embedded in the polymer matrix of the packaging and with controlled release or the ability to preserve the organoleptic properties of foods for a longer period (ROONEY, 1995; APPENDINI & HOTCHKISS, 2002; QUINTAVALLA & VICINI, 2002).

On the other hand, packaging and preservation using rigid materials such as glass, metal cans, plastic composites, materials that can be subjected to a subsequent sterilization process for longer shelf life (FARKAS, 1997) are also used (APPENDINI & HOTCHKISS, 2002). Current considerations regarding the microbiological characteristics of food packaging and packaging technologies assume combinations between different technologies (for example, the use of antimicrobial films for modified atmosphere packaging), the implementation of biosensors to determine bacterial toxins based on immunological properties, methods for quantifying microorganisms in different types of packaged foods (SILLIKER, 1980; CUTTER 2002). Some studies (COOKSEY, 2005) have demonstrated the effectiveness of nisin as an antimicrobial agent, the packaging covering systems being an effective carrier of it. The effectiveness of the agent decreases when it is incorporated into the polymer matrix, but increasing its concentration leads to increased effectiveness, reducing the content of *Lysteria monocytogenes*. Also, a series of other compounds such as chitosan and chlorine dioxide showed efficiency against the same bacterial strain and respectively *Salmonella typhirium* for packaged poultry meat but without significant effect regarding the natural microbial community (COOKSEY, 2005). Thus, as a result of their structure, antimicrobial agents act differently compared to pathogenic microorganisms and can be added to packaged foods or in the composition of the packaging, their choice being influenced by the characterization of the microorganisms. They perform both a bactericidal and bacteriostatic role and some of the most frequently used are antioxidant compounds, antimicrobials, products resulting from different biotechnologies, essential oils, polymers, enzymes, bacteriocins, archaeocins, natural extracts, etc. (MALHOTRA et al., 2015).

The present study refers to the evaluation of the antimicrobial activity of some raw materials (essential oils, polymers, products resulting from the recovery of agricultural waste from wine production) used to obtain new, biodegradable, environmentally friendly packaging, intended for the packaging of fresh meat products of poultry. On the other hand, the study aims to demonstrate the increase in the validity of the final packaged product by at least 20% compared to the classic system packaging procedure.

MATERIALS AND METHODS

Testing the antibacterial effect of essential oils. The tested essential oils were obtained from the following 15 plant extracts: oregano, mint, pine, sage, basil, clove, coriander, tea tree, lime, lemongrass, lemon, lavender, thyme, rosemary and cinnamon. To facilitate dilution in water, they were mixed with 5% DMSO and 0.5% Tween 80, after which they were mixed with sterile ultrapure water (v/v) to obtain oil concentrations of 75%, 50%, 25%, 10%, 5% and 1%. The bacterial strains on which the antibacterial effect was tested were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* ATCC 14028, *Pseudomonas aeruginosa* ATCC 15442, *Listeria monocytogenes* ATCC 1911. The testing was carried out by the disk-diffusimetric method (Kirby-Bauer) described in the CLSI standard M07 (***. 2018). The method involves the preparation of the bacterial inoculum from fresh cultures (of 24 h) at a turbidity equivalent to the McFarland standard 0.5, which corresponds to a bacterial density of $1\text{-}2\times10^8$ CFU/mL, the inoculation of Mueller-Hinton Agar medium (17.5g/L peptone, 1.5 g/L starch, 17.5 g/L agar) plates in cloth, aseptically spreading filter paper discs (4 mm diameter) on the surface of the inoculated culture media and 5 µL of essential oil (undiluted and diluted) on the surface of the paper discs. Afterwards, the plates are incubated at 35^0C for 18 h, after which the diameter of the zones of inhibition of bacterial growth is measured. The negative control was represented by the water, DMSO and Tween 80 mixture.

The potential of polylactic acid to inhibit the growth of microorganisms. The potential of polylactic acid (PLA) 2003D to inhibit the growth of microorganisms was tested against *Staphylococcus aureus* ATCC 25923 (Gram positive) and *Escherichia coli* ATCC 25922 (Gram negative) strains. The method assumes that in the initial stage the bacterial cultures are adjusted (in sterile physiological water) to a turbidity equivalent to the McFarland 0.5 standard. Afterwards, add 2 g of PLA 2003D (sterilized in advance by immersion for 10 min in 96% ethanol and repeated washings with sterile distilled water) and leave for 4 h at room temperature. After incubation, the cultures treated with PLA 2003D are diluted and seeded with a Digralsky spatula (volume 100 µL) on Mueller-Hinton Agar solid culture media. The cultures are incubated at 35^0C for 18 h, after which the colonies that have developed are counted and the results are compared with those of a control not treated with PLA 2003D.

Extraction of polyphenols from grape pomace. The studied samples, after lyophilization for 48 hours, were ground and subjected to the sieving operation. 3 grams of each sample were mixed with 30 ml ethanol 80% v/v and subjected to stirring at 160 rpm for 18 hours at temperatures of 28^0C, 37^0C and 50^0C (ĆUJIĆ et al., 2016; SHEWALE & RATHOD, 2018). Also, the samples were studied in static conditions, at 37^0C. After extraction, centrifugation was performed at 9000 rpm for 10 minutes. The supernatant was taken for the analysis of total polyphenol content.

Determination of the total content of polyphenols. The total polyphenol content of the four types of used materials (grape seeds, grape skins, grape pomace I and II) was determined by the Folin-Ciocalteu method (ALEXANDRE et al., 2019). Thus, 80 µL of Folin–Ciocalteu reagent 10% (v/v) were added to 20 µL of grape pomace extract (previously extracted in 80% ethanol), followed by 100 µL of sodium carbonate (7.5% (m/v)) and allowed to react in the dark at room temperature for 1 h. After incubation, absorbance was measured at 750 nm (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) in a 96-well microplate (Nunc™, Thermo Fisher Scientific Inc., Waltham, MA, US). Gallic acid (0.010–0.125 mg/mL, y = 5.991x + 0.126, R2 = 0.999) was used as the standard for the calibration curve and the results were expressed as equivalent milligrams of gallic acid per gram of dry extract.

The absorption spectra of the polyphenol samples (1-16) were recorded in the spectral range of 200-800 nm with the Jasco V-630 Spectrophotometer, using quartz cuvettes with an optical path of 1 cm. The samples were read against the 80% ethanol reference and were diluted 200 times with 80% ethanol.

Determining the antibacterial effect of some biodegradable packaging recipes. For this experiment, the samples were distributed in 50 mL falcons after being sterilized by exposure to UV radiation for 30-40 min on each side and 6 mL of nutrient broth culture medium (10 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract, 5 g/L NaCl, pH 7) were added. The culture medium was inoculated with 30 µL of the inoculum from the tested bacterial cultures (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 15442) having a turbidity equivalent to the McFarland standard 0.5, so that the inoculum density in each sample to be 5×10^5 CFU/mL. The falcons were incubated at 30^0C for 24 to 70 h with shaking at 140 rpm. After incubation, 100 µL of each sample (properly diluted in sterile physiological serum) was seeded on the surface of the nutrient agar solid culture medium (10 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract, 5 g/L NaCl, 18 g agar; pH 7.0) and incubation at 37^0C for 24 h. Subsequently, CFU/mL was quantified in the tested samples compared to the positive control (without recipe) and the percentage of growth reduction bacteria was calculated based on the formula: 100-[100 × (CFU sample/CFU control)].

Microbiological testing of poultry meat samples packed in biodegradable packaging. The determination of the total number of aerobic bacteria on the surface of the meat samples represented by poultry wings was carried out according to the methodology described in the international standard ISO 4833 (2013) with some modifications. In this sense, the sample preparation was carried out as follows: separate 1 g of meat (mainly skin) from several samples, distribute the samples in sterile vials in 10 mL of sterile physiological serum, homogenize by vigorous vortexing for 3 min, make serial decimal dilutions in sterile physiological serum. Sample inoculation: a volume of 50-100 µL of each appropriately diluted sample is inoculated with a Drigalski spatula onto the surface of the Plate Count Agar culture medium (5 g/L casein hydrolyzate, 2.5 g/L yeast extract, 1 g/L glucose and 18 g agar; pH 7.0) or nutrient agar (10 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract, 5 g/L NaCl, 18 g agar; pH 7.0). The Petri plates were incubated at 37^0C for 24 h, after which CFU were quantified and reported per 1 g of meat.

RESULTS

The antibacterial effect of essential oils. Oregano essential oil showed a strong inhibitory effect on the growth of the bacterial tested strains. Inhibition was maximal in the case of undiluted oil (100%), but relatively strong effects were also obtained at concentrations of 75-50% (in the case of *E. coli*) and 75-25% (in the case of *S. aureus*) (Table 1; Fig. 1). In the case of *S. enterica* inhibition was maximal with undiluted oil (100%), but relatively strong effects were also obtained at 75-25% concentrations. *P. aeruginosa* was less sensitive to oregano oil compared to the other bacterial species tested. Thyme essential oil showed a strong inhibitory effect and the concentrations at which it was effective ranged from 100% to 25%. The efficiency of this compound on *S. enterica* was very similar to that determined in the case of *E. coli* species. Thyme oil was also very effective in inhibiting the growth of *L. monocytogenes* (Table 2). *P. aeruginosa* species was less sensitive to thyme oil compared to the other bacterial species tested. Cinnamon essential oil showed a strong inhibitory effect and the concentrations at which it was effective were between 100% and 5%. At the 75% concentration, a stronger inhibitory effect was observed compared to the 100% concentration, probably due to better dispersion in the agar medium. Inhibitory effect on the growth of *S. enterica* species is very similar to that determined in the case of *E. coli* species (Table 2). On the other hand, this oil was also very effective in inhibiting the growth of *L. monocytogenes* and *P. aeruginosa* species (Table 2).

Peppermint essential oil specifically inhibited the growth of *S. aureus*, being effective at concentrations between 100% and 25%. In the case of the *E. coli* and *S. enterica* species, the inhibitory effect was weaker. Comparing this result with those determined for *E. coli* and *S. aureus* species, it can be stated that peppermint essential oil shows less efficiency on Gram negative species (*E. coli, S. enterica*) than on the tested Gram-positive species (*S. aureus*), due to structural differences in the bacterial cell wall.

Pine essential oil showed a weak inhibitory effect on the growth of *S. aureus* species (at 100% concentration) and was ineffective on *E. coli* and *S. enterica*. Sage essential oil showed inhibitory effect especially on *S. aureus* at concentrations between 100% and 25%. In the case of the *E. coli* and *S. enterica* species, the inhibitory effect was weaker. Basil essential oil particularly inhibited the growth of *E. coli*, with optimal concentrations ranging from 100% to 25%. The inhibitory effect was weaker on *S. aureus* and moderate on *S. enterica* species. A weak inhibitory effect was observed even

at low oil concentrations (5-10%). Clove essential oil was effective in inhibiting the growth of *E. coli* and *S. aureus* with advantage that it is active even at low concentrations (10%). Coriander essential oil inhibited the growth of the same strains but the inhibitory effect was stronger on *S. aureus* especially at 100% and 75% concentrations and weak on *S. enterica*. Tea tree essential oil specifically inhibited the growth of *E. coli* species (Table 1).

Table 1. The antibacterial effect of some essential oils. Values represent the diameter of growth inhibition zones (in mm).

Essential oils	***Escherichia coli* ATCC 25922**							***Staphylococcus aureus* ATCC 25923**						
	Essential oil concentrations (v/v)							**Essential oil concentrations (v/v)**						
	100%	75%	50%	25%	10%	5%	1%	100%	75%	50%	25%	10%	5%	1%
Oregano	30,5	28,5	27,5	7	6	6	6	42	25	24,5	23	5,7	5,7	0
Mint	9,5	7,5	6,7	6,2	6	0	0	16,5	14	9,7	6,5	0	0	0
Pine tree	0	0	0	0	0	0	0	8	0	0	0	0	0	0
Sage	8	6	6	5,5	0	0	0	14	11,5	9,5	5,7	0	0	0
Basil	12,2	11,2	11	8,7	7,2	6	4,7	8	6,5	5,5	0	0	0	0
Cloves	12	10,5	10	9,5	9	5,2	5,2	15	13	10,7	10,2	9	6,2	0
Coriander	14	10,9	9	8,2	7,2	5,7	0	19	16,5	10	7,5	0	0	0
Tea tree	12	13,5	10	7,5	5	5	0	9	7,5	6	0	0	0	0
Limes	14,5	8	7,5	5,5	0	0	0	18	15,5	11,7	6	0	0	0
Lemongrass	11	10	7,5	6,7	6	6	0	25,5	21,2	16,5	10	5,5	0	0
Lemon	9	8	7	0	0	0	0	12	9	2,5	0	0	0	0
Lavender	8,7	6,5	6	5	0	0	0	19	10	7,2	5,2	0	0	0
Thyme	29,5	27,5	20	9,5	6,7	6	5	38,5	28,5	27	10	6	0	0
Rosemary	10,5	9,5	6,5	5,5	0	0	0	17	10	8	5,5	0	0	0
Cinnamon	24	27,5	22,5	21,2	15	10,5	0	31,5	35,5	29	25,5	18,5	7	0

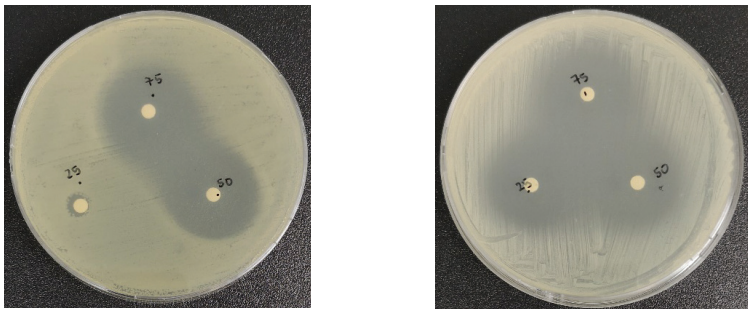

Figure 1 Growth inhibition of some tested bacterial strains by oregano essential oil.

Table 2. The antibacterial effect of some essential oils. Values represent the diameter of growth inhibition zones (in mm). Essential oils: A = Oregano; B = Thyme; C = Cinnamon.

	***Salmonella enterica* ATCC 14028**							***Listeria monocytogenes* ATCC 1911**							***Pseudomonas aeruginosa* ATCC 15442**						
	Essential oil concentrations (v/v)							Essential oil concentrations (v/v)							Essential oil concentrations (v/v)						
	100 %	75 %	50 %	25 %	10 %	5 %	1 %	100 %	75 %	50 %	25%	10 %	5 %	1 %	100 %	75 %	50 %	25 %	10 %	5 %	1 %
A	27	22	20,5	21,5	12	7,5	7,5	23,5	25	24	15	9,5	8,5	0	9	9	8,5	8	7	7	0
B	28	23,5	19	8	6	5	5	41	28	20,5	18,5	15	7	0	10,5	9	8	8	7,5	0	0
C	24,5	24,5	21,5	22,5	13,5	10,5	6	29	23	19	16,5	0	0	0	19	19	17	13	8	7,5	6

Relatively similar results were also presented in the specialized literature (SHAPIRO et al., 1994; HAMMER et al., 1999; CAMPANA et al., 2022), the minimum inhibitory and bactericidal concentrations being close to the data from the present study.

The potential of polylactic acid to inhibit the growth of microorganisms. The sample of polylactic acid 2003D analysed in the present study did not show the potential to inhibit the development of the Gram-positive strain

Staphylococcus aureus ATCC 25923 or the Gram-negative strain *Escherichia coli* ATCC 25922 (Table 3). Most likely, to obtain an antibacterial effect, treating PLA with substances can be known to exhibit the ability to inhibit bacterial growth. In this sense, the final mixture intended for the manufacture of biodegradable packaging containing the essential oils with antimicrobial effect can maintain its biological and eco-friendly activity. The results obtained in the case of polylactic acid known as bioplastic, being relatively easily degradable in the environment, are supported by data from specialized literature that show its combination with other compounds with antimicrobial activity such as carvacrol, enzymes and other natural compounds (SCAFFARO et al., 2018; 2020; TAWAKKAL et al. 2014) for obtaining biocomposites intended for the packaging industry.

Table 3. Results of evaluation of the antibacterial effect of PLA 2003D.
Values shown represent the number of colonies resulting from 100 µL of diluted 10^{-5} culture.

	Test 1	**Test 2**	**Average**	**Control**
Escherichia coli ATCC 25922	207	162	184	188
Staphylococcus aureus ATCC 25923	163	151	157	146

Extraction, quantification and spectral characterization of polyphenols as potential raw materials for packaging. Grape pomace I was obtained from one kg of black grapes. After squeezing the grapes and lyophilization, the result was 74.3 g, and after grinding and sieving, the result was 72.3 g. From this sample, 3 grams were used for the extraction of polyphenols by maceration with ethanol 80% v/v as described in the materials and methods. The data presented in table 4 show differences both between the types of pomace grapes investigated and the influence of temperature and stirring conditions regarding the total content of polyphenols that was extracted. In the case of pomace from grape seeds, the static conditions at 37^0C led to a slightly higher amount compared, for example, to the sample subjected to agitation at the same temperature. In the case of pomace from grape skins, the largest amount is observed under stirring conditions at 50^0C. Similarly, data were obtained for the sample of pomace grapes II. The sample of pomace grapes I stands out with higher amounts of polyphenols in all investigated conditions, the highest amount (3.14 mg/mL) being obtained under stirring conditions at 50^0C (Table 4).

Table 4. Determination of total polyphenol. Results are expressed in mg/mL. S1 – S16 = investigated samples.

	Grape seeds	**Grape skins**	**Grape pomace I**	**Grape pomace II**
28 °C, 160 rpm	1,88 (S3)	1,07 (S5)	2,69 (S9)	1,20 (S13)
37 °C, 160 rpm	2,04 (S2)	1,08 (S7)	2,83 (S10)	1,43 (S14)
50 °C, 160 rpm	1,93 (S4)	1,43 (S8)	3,14 (S12)	1,54 (S16)
37 °C, static	2,11 (S1)	1,22 (S6)	2,84 (S11)	1,24 (S15)

Although the data from the specialized literature (CAPONIO et al., 2022) support an antimicrobial activity of extracts from pomace grapes, the results obtained in this study were not supported in this sense, the tested bacterial cultures having the ability to develop in the presence of these extracts.

Spectral characterization. The spectral characterization of raw materials from agricultural waste from wine industry demonstrated that in the range 400-800 nm, the samples have a different spectral behaviour (Fig. 2A), forming 3 groups: a) Samples 1-4 (cores) show an absorption maximum of around 662 nm (Fig. 2A inset), but the intensity is much lower compared to samples 5-8 (skins) and 13-16 (grape pomace II); b) Samples 5-8 and 13-16 (grape pomace II) have a similar behaviour (Fig. 2Ab), respectively a maximum around 662 nm and a broad absorption band in the 480-560 nm range; c) Samples 9-12 (grape pomace I) present a broad absorption band around 550 nm with a higher intensity than in the case of samples 5-8 and 13-16. Also, at 662 nm samples 9-12 show a shoulder and not a well-defined maximum. In the UV range, all samples show an absorption maximum around 280 nm characteristic of all phenolic compounds (Fig. 2Aa) (ALEIXANDRE-TUDO et al., 2018). In the visible range, the broad absorption band from 480-580 nm that appears in the case of samples 5-8, 9-12 and 13-16 is generally attributed to anthocyanins (GIUSTI & WROLSTAD, 2001). Samples 1-4 coming from the kernels do not present this band, so anthocyanins are only present in the peel and pomace samples. The shoulder that appears around 320 nm for samples 5-16 and is missing in the case of samples 1-4 can be associated with hydroxycinnamic acids.

Regarding the influence of temperature, it is observed that, in the case of samples 13, 14, 16 (grape pomace II), the increase in temperatures induces a more intense absorption band (480-580 nm) characteristic of anthocyanins (Fig. 2Bb). Also, this characteristic band of anthocyanins is more intense in the case of sample 14, obtained under centrifugation conditions compared to sample 15 under static conditions (Fig. 2Ca). In the case of samples 9, 10, 12 (grape pomace I), the absorption band (480-580 nm) characteristic of anthocyanins is more intense in the case of sample 12 obtained at 5^0C (Fig. 2Ba). Also, the characteristic band of anthocyanins does not change in intensity in the case of samples 10 and 11 obtained under static conditions and shaking at 160 rpm (Fig. 2Cb). In the case of samples 5, 7 and 8, the absorption band (480-580 nm) characteristic of anthocyanins has the same intensity at 37^0C and 50^0C for samples 7 and 8 and is higher than in the case of sample 5 (28^0C) (Fig 2Da). Also, the characteristic band of anthocyanins is more intense in the case of sample 7, obtained under centrifugation conditions compared with sample 6 obtained under static conditions (Fig. 2Db).

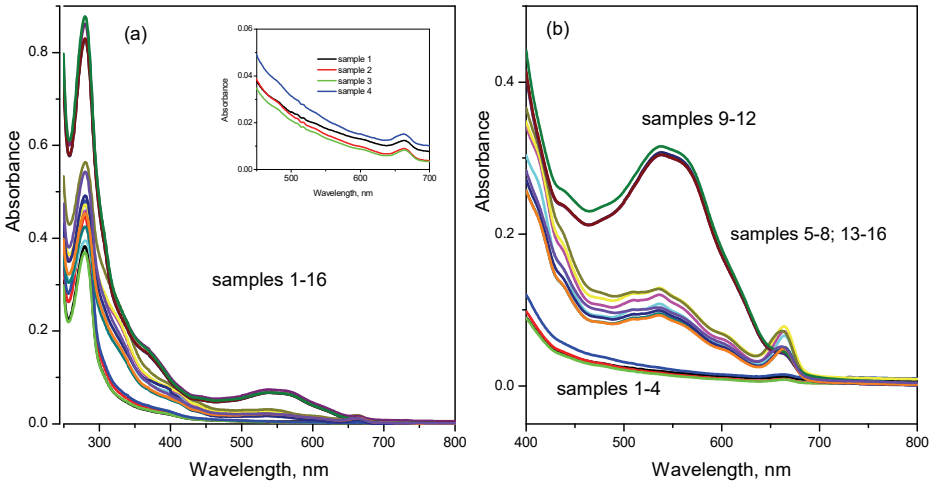

Figure 2A. Absorption spectra of samples 1-16 in the spectral range: (a) 250-800 nm and (b) 400-800 nm.

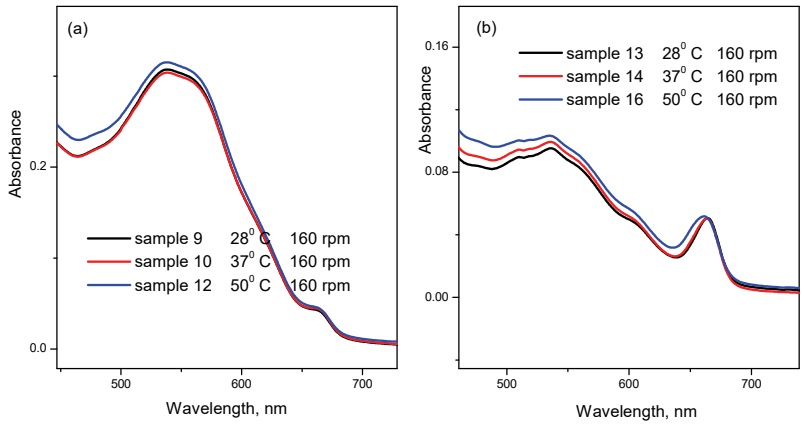

Figure 2B. Absorption spectra of samples: (a) 9, 10, 12 and (b) 13, 14, 16 at different temperatures.

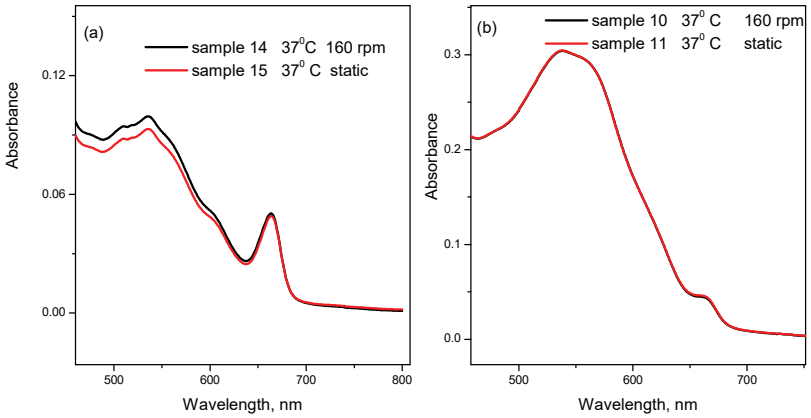

Figure 2C. Absorption spectra of samples (a) 14, 15 and (b) 10, 11 at 37^0 C, under static and centrifugation at 160 rpm.

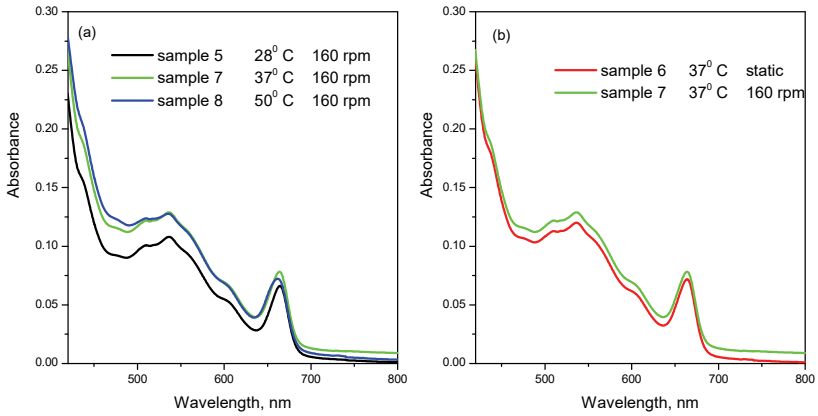

Figure 2D. Absorption spectra of samples: (a) 5, 7 and 8 at different temperatures and (b) 6 and 7 at 37^0 C, under static shaking at 160 rpm.

The antibacterial effect of some biodegradable packaging recipes. The results of testing the effect of recipes 361-366 on the growth of four bacterial strains of importance in the food industry showed that recipe 361 inhibited the growth of *E. coli, S. enterica* and *P. aeruginosa* strains by 25-45% compared to the control, but did not show antimicrobial activity on the *S. aureus* species. Formula 362 showed antimicrobial activity on the four tested strains with efficiency between 8.6% and 88%, the strongest effect being detected in the case of *S. aureus* (88% inhibition) and *E. coli* (80% inhibition) (Fig. 3; Table 5). Formula 363 showed the strongest antimicrobial effect among the five tested, acting with highly increased efficiency against *S. aureus* (99.9%), *E. coli* (99.9%) and *S. enterica* (99.9%). Formula 364 inhibited the growth of *S. aureus, E. coli* and *P. aeruginosa* with an efficiency ranging from 9.9% to 41%. However, it did not show antimicrobial activity on *S. enterica.* Formula 365 inhibited all four tested bacterial strains by 7-46% compared to the control. Formula 366 showed relatively moderate antimicrobial activity (13.9%) exclusively on *E. coli*. This recipe, however, stimulated the growth of the other bacterial strains by 15-45% compared to the control. Likewise, other recipes tested, respectively 335 - 342, showed the ability to inhibit the growth of the reference strain *S. aureus* ATCC 25923 in percentages of 45.3% to 73.1%.

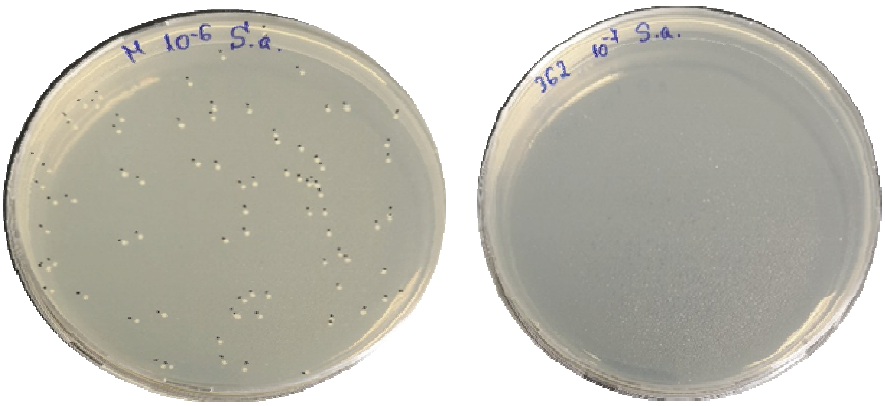

Figure 3. Left - Blank (10^{-6} dilution); Right – Recipe 362 (10^{-7} dilution); Bacterial strain: *S. aureus*.

Microbiological testing of poultry meat samples packed in biodegradable packaging. The total number of aerobic bacteria that grew from poultry meat samples stored in pans made of polylactic acid (PLA) or polyethylene terephthalate (PET) treated with antimicrobial and antioxidant compounds are shown in table 6. To determine the effectiveness of the used antimicrobial compounds and antioxidants, testing also included PLA and PET controls that were not treated with the respective compounds. The results obtained on the first day of testing are comparable to those in the specialized literature (IKEME et al., 1981; ENVER et al., 2021), with no differences observed between the treated and control casseroles because the bacteria on the surface of the meat did not enter the logarithmic phase of multiplication.

After 8 days of storage at 4^{0}C, meat from PLA casseroles exceeded 10^6 CFU/g, generally considered the maximum accepted limit for fresh poultry meat (MAHARJAN et al., 2019; ***. REGULATION (EC) NO. 2073/2005). However, casseroles treated with antimicrobial substances slowed down the multiplication of aerobic bacteria. For example, after 10 days of storage, the difference between PLA and control PLA was 10^8 CFU/g (Table 6; Fig. 4).

Table 5. The influence of recipes 361-366 on the growth of some bacterial strains of importance in the food industry.

Bacterial strains	Sample	C.F.U./mL	% Reduction of bacterial growth compared to the control
S. aureus	Blank (no receipt)	**8.5×10^{8}**	-
	Recipe 361	9.8×10^{8}	+15%
	Recipe 362	1.02×10^{8}	-88%
	Recipe 363	5.65×10^{4}	-99.9%
	Recipe 364	5×10^{8}	-41.1%
	Recipe 365	7.9×10^{8}	-7%
	Recipe 366	2.09×10^{9}	+45.8%
E. coli	Blank (no receipt)	**2.51×10^{9}**	-
	Recipe 361	1.78×10^{9}	-29%
	Recipe 362	5×10^{8}	-80%
	Recipe 363	7×10^{2}	-99.9%
	Recipe 364	1.685×10^{9}	-32.8%
	Recipe 365	2.26×10^{9}	-9.9%
	Recipe 366	2.16×10^{9}	-13.9%
S. enterica	Blank (no receipt)	**3.28×10^{9}**	-
	Recipe 361	2.46×10^{9}	-25%
	Recipe 362	2.99×10^{9}	-8.6%
	Recipe 363	6.73×10^{5}	-99.9%
	Recipe 364	3.34×10^{9}	+1.8%
	Recipe 365	2.79×10^{9}	-14.7%
	Recipe 366	4×10^{9}	+21.9%
P. aeruginosa	Blank (no receipt)	**3.23×10^{9}**	-
	Recipe 361	1.91×10^{9}	-40.86%
	Recipe 362	2.31×10^{9}	-28.48%
	Recipe 363	2.55×10^{9}	-21%
	Recipe 364	2.91×10^{9}	-9.9%
	Recipe 365	1.74×10^{9}	-46.1%
	Recipe 366	3.74×10^{9}	+15.9%

Table 6. Total number of aerobic bacteria (CFU/g) in meat samples.

Day	PLA	PLA control	PET	PET control
1	2.1×10^{3}	2×10^{3}	2.2×10^{3}	2.3×10^{3}
8	4.6×10^{6}	7×10^{6}	8.6×10^{4}	2.6×10^{5}
9	9.8×10^{6}	6.3×10^{7}	1.8×10^{6}	3.2×10^{7}
10	2×10^{7}	1.2×10^{8}	1×10^{7}	2.2×10^{7}

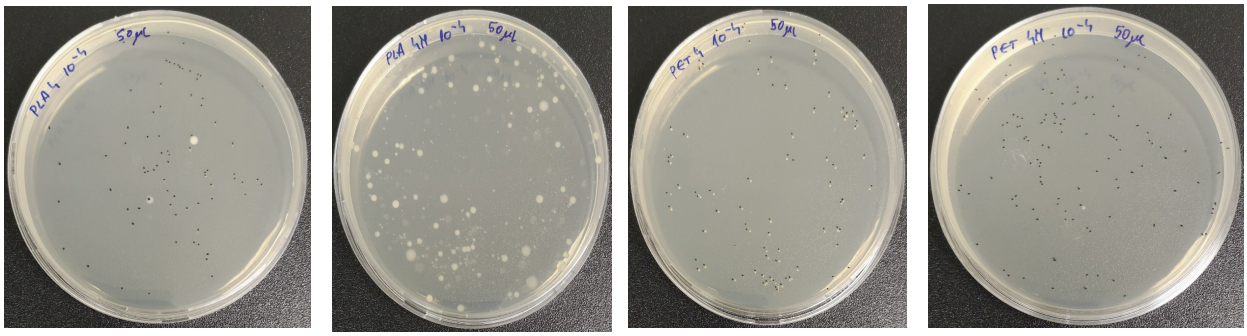

Figure 4. Bacterial colonies resulting from meat samples after 10 days of storage. A) PLA casserole, 10^{-4} dilution, 50 µL inoculum; B) blank PLA casserole, 10^{-4} dilution, 50 µL inoculum; C) PET dish, 10^{-4} dilution, 50 µL inoculum; D) control PET dish, 10^{-4} dilution, 50 µL inoculum.

On the other hand, meat stored in PET casseroles showed a microbial load acceptable for food consumption (10^{4} CFU/g) after 8 days of storage. In this case too, a significant difference of 1.7×10^{5} CFU/g was observed between

PET casseroles and control PET. After 9 days of storage, the meat could no longer be considered acceptable for consumption, but significant differences were maintained between the treated and control casseroles. Also, based on the experiments, it was found that the samples with a microbial load of more than 10^7 CFU/g have a yellowish appearance, become sticky and develop a smell that is specific to spoiled meat due to the development of bacterial biofilms.

From the obtained results, it can be seen that the tested PLA casseroles allow a shelf life of poultry meat of a maximum of 7 days, and the PET ones of 8-9 days, and the antimicrobial and antioxidant compounds in the composition of the casseroles slowed down the development of aerobic bacteria in the tested meat samples.

CONCLUSIONS

The obtained data revealed that fourteen of the fifteen essential oils showed the potential to inhibit the growth of tested bacterial species. Among these, oregano, thyme and cinnamon oils showed the strongest antibacterial effects on *E. coli, S. enterica, L. monocytogenes*, *P. aeruginosa* and *S. aureus* species. The cinnamon oil was effective even at low concentrations (5-10%) and inhibited particularly the growth of *P. aeruginosa*. Lemongrass oil was also effective in inhibiting the growth of *S. aureus*, but showed weaker action on *E. coli*. Pine oil showed the weakest antibacterial effect, acting exclusively on the *S. aureus* species at 100% concentration. Clove and lime oils showed moderate antibacterial effects on *S. enterica*, being particularly effective at 100-50% concentrations. Lemongrass and rosemary oils also showed moderate antibacterial activity, but were particularly effective at the 100% concentration. On the other hand, peppermint, sage, basil, coriander, tea tree and lavender oils showed relatively weak antibacterial activity against *S. enterica* and pine and lemon oils were not effective. Similarly, sea buckthorn oil did not show antibacterial activity on *S. aureus, E. coli, S. enterica, L. monocytogenes* or *P. aeruginosa*.

Phenolic compounds extracted from grapes and sea buckthorn showed no antibacterial activity. The 16 recipes tested for the antibacterial effect demonstrated that they are effective in different proportions, in most cases over 50% compared to the bacterial strains tested. The obtained data confirm that the tested PLA casseroles allow a shelf life of poultry meat of a maximum of 7 days, and the PET ones of 8-9 days, and the antimicrobial compounds in the composition of the casseroles slowed down the development of aerobic bacteria in the tested meat samples. On the other hand, the addition to the final recipes of some bioactive compounds, stable under the conditions of food packaging both in the classical and modern systems, with a controlled atmosphere for example, and which preserve their biological properties in this context, such as be extremozymes (KHAN & SATHYA, 2017) could lead to an increase in the shelf life of packaged products. Such enzymes are active and structurally stable in physical and -chemical conditions different from normal ones (GOMES & STEINER, 2004) and which can be encountered during the food packaging process through modern technologies.

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