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Bioactive Paper Packaging for Extended Food Shelf Life

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Abstract: Food safety and quality are major problems for food producers and industry, governments, and consumers. Conventional plastic-based food packaging is difficult to dispose of and recycle due to its provenience from fossil resources and resistance to biodegradation. Therefore, currently, the trend is to develop new eco-friendly food packaging that can replace these materials. The limitations of conventional packaging can be solved by developing new active materials with antimicrobial and antioxidant properties, based on cellulose, a natural biodegradable organic compound derived from renewable resources. In this study, new materials with antioxidant and antibacterial activity were obtained by combining a "green" functionalization approach (enzymatic activation) and surface modification using bioactive agents (essential clove oil and cold-pressed grape seed oil). Kraft paper was firstly activated with cellulase, followed by impregnation with the above-mentioned oil solutions, and then its properties were evaluated. The increased values of the O/C ratio for modified Kraft paper indicate an increased polarity due to the presence of phenolic groups. This resulted in an improved hydrophobicity, with the water contact angle increasing from 97° to over 110°. Following different interactions with the functional groups of vegetable oils, the modified Kraft paper exhibited distinct antioxidant and antibacterial properties. However, modified paper with clove essential oil showed higher antioxidant activity (due to the higher content of phenolic compounds), while modified paper with cold-pressed grape seed oil had better antimicrobial activity against *Escherichia coli* (−), *Salmonella enteritidis* (−), and *Listeria monocytogenes* (+) bacterial strains, and was more effective at reducing bacterial growth on fresh beef and fresh curd. The newly obtained bioactive paper provides an effective packaging material that can help control foodborne pathogens in food, thus extending its shelf life and safety.

Keywords: active food packaging; Kraft paper; plant oils; food safety; shelf life

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1. Introduction

Food safety and its quality is a major concern for different stakeholders, governments, and consumers, especially since microbial growth can damage the overall quality and safety of a product. As a result, modification of the aroma, color, or texture of the food products can take place. Therefore, the food processing sector has encountered major challenges in providing safe and good-quality products [1].

Traditional food packaging materials have been produced using petroleum-derived polymers due to their attractive mechanical properties and cost-effectiveness [2]; however, due to their non-recyclability and non-biodegradability, these plastics have caused large ecological problems. Moreover, the functions of traditional packaging are passive and insufficient to provide and maintain the good quality of the food products, especially for fresh agricultural, dietary, and meat products [1].

In this context, active packaging, aside from its main role of protection, influences the storage of the packaged product [2]. This type of packaging refers to a system that provides dynamic protection to different food products, thus achieving quality control through food–packaging interactions [1]. These systems can absorb, extract, control, emit, or assess the foodstuff's state from inside or outside (as needed). Aside from the main packaging

materials, other components, such as moisture absorbers, antioxidant and antimicrobial compounds from natural or artificial sources, gas scavengers, or emitters, can be added into the matrix in order to extend food shelf life or to prevent food spoilage or alterations [1,3,4].

To reduce the growth and spread of pathogenic microorganisms in food products, different antimicrobial package materials may be developed. These materials present the ability to inhibit or kill pathogenic microorganisms and therefore extend the shelf life of perishable products, enhance the safety of packaged products, and add benefits to the consumer's health [5,6]. Antimicrobial packaging includes systems such as distributed bioactive agents in the packaging material, packaging material surfaces coated with bioactive agents, and film-forming antimicrobial macromolecules or edible matrices.

Kraft paper is a natural and biodegradable product, 100% sustainable, and one of the most popular packaging materials in the world. It is produced from pulp from all types of wood and is also a lightweight packaging material that helps maintain a low shipping cost [7]. In order to produce active packaging paper, it is necessary to covalently immobilize the bioactive compounds onto a functionalized surface. Thus, the first step is to design or select the properties matching the needs of the end use. The second step is the optimization of the surface functionalization techniques in order to introduce the necessary type and quantity of reactive functional groups. This step is important to improve bioactivity by reducing steric constraints and shielding the compound from hydrophobic surface-induced denaturation, and can be achieved by binding a bioactive compound to a solid substrate via a spacer molecule. The third and final step is to covalently attach a bioactive compound to the functionalized surface [8].

Cellulase is an enzyme which acts on cellulose and creates active centers such as aldehyde groups, which can further react with amino and phenolic groups in relatively mild conditions. Phenolic compounds can react with the enzymes to different extents and thus can be grafted on the cellulosic surface, developing covalently bound antioxidant materials [9]. Covalent bonding onto a polymeric substrate of some bioactive components will result in the morphological and structural changes of the substrate, leading to new materials with specific properties obtained through environmentally friendly methods for food packaging applications.

Bioactive compounds are generally suitable for use in active packaging and thus can provide longer shelf life and preserve food products. Among them, the essential oils or cold-pressed oils obtained from different parts of aromatic plants or seeds have the ability to stop the growth of different pathogens. These oils enhance the antibacterial properties of the packaging materials due to the presence of a high amount of bioactive compounds in their structure [3,10]. As the main components of the oils, phenolic acids are natural plant hydrophilic antioxidants obtained as vegetal oils by extraction, cold pressing, etc. Their antioxidant activity is related to the ability of these compounds to extinguish the free radicals, terminate the chain reactions in lipid oxidation, and chelate metal ions. The hydroxyl groups from their structure are associated with their antioxidant activity. Several types of essential oils (such as cinnamon, palmarosa, lemongrass, oregano, thyme, or lavender oils [3,11–17]) have been reported as being used as antimicrobial agents to preserve food products. In our previous works, we observed that clove essential oil presented high antimicrobial activity against different Gram-positive and Gram-negative bacteria (e.g., *Staphylococcus*, *Streptococcus*, *Listeria*, *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*) and fungi (e.g., *Aspergillus, Penicillium*) [11,12]. The oxygen radical absorption capacity tests indicated the strong antioxidant action of the clove essential oil, which was 3 to 10 times higher than that of other essential oils [13]. Grape seed oil contains a large amount of phenolic compounds, fatty acids, and vitamins, and presents important properties for health, such as anti-inflammatory, cardio-protective, antimicrobial, and anticancer properties, and can interact with cellular and molecular pathways [18,19].

The goal of this study was to design and develop new bioactive packaging materials with antimicrobial and antioxidant activities, based on natural biodegradable compounds,

using enzyme treatment. This can help control foodborne pathogens in food, extending shelf life and safety.

2. Materials and Methods

The surface activation of the Kraft paper was achieved with cellulase. Further, the activated paper was coated with two vegetable oil solutions: one essential (clove oil) and the other one obtained through the cold-pressing process (grape seed oil).

2.1. Materials

Bleached Kraft paper (BP) available commercially and used for packaging, with 0.6 g/cm^3 density and a 100-micron thickness, was purchased from Adi Center SRL, Iasi, Romania.

Cellulase from *Trichoderma reesei* (83 FPU/mL) was purchased from Sigma-Aldrich and was used as received for the activation of the paper.

Essential clove oil (ECO), purchased from Fares (Orastie, Romania), and cold-pressed grape seeds oil (CGO), purchased from S.C. Herbavit S.R.L (Iasi, Romania), were chosen for the Kraft paper functionalization. These two oils were selected due to their high content of antioxidant compounds, as shown in previous works [20,21]. The clove essential oil is mostly composed of eugenol (85.7%) and eugenol acetate (7.9%), while grape seed oil is mostly composed of about 86% unsaturated fats, of which about 70% is linoleic acid and about 16% is oleic acid [20,21].

2.2. Kraft Cellulose Paper Functionalization

The enzyme treatment of the bleached Kraft paper was carried out with 2 g/L cellulase at 50 °C for 1 h and pH5 (0.05 M acetate buffer). The ratio of the paper to enzyme solution was 1:50 (*w*/*w*). After incubation, the deactivation of the enzyme was achieved by washing the samples in distilled water at 90 °C for 5 min. After that, the papers were double-washed in hot water, double-washed in cold water, and then finally dried. Further, the paper was immersed in a methanol solution (10 wt%) of essential clove oil or chloroform solution (10 wt%) of pressed grape seed oil for 4 h at room temperature (~25 °C) with mechanical stirring (~150 rpm). After impregnation, the samples were dried at 60 °C and washed with methanol or chloroform in order to eliminate the unreacted compounds from vegetable oils which might have been only physically adsorbed onto the paper surface. The final materials were dried and prepared for analysis.

2.3. Investigation Methods

2.3.1. ATR-FTIR Spectroscopy

The spectra were recorded in the 4000–600 cm^{-1} region, at 4 cm^{-1} resolution, with 64 scans, by means of a VERTEX 70 spectrometer (Bruker Optics, Ettlingen, Germany) equipped with a ZnSe crystal. The evaluations were made on the average spectrum obtained from three recordings for each sample. The processing of the spectra was conducted using the OPUS 7.5 program.

2.3.2. SEM/EDAX Analysis

SEM/EDAX analysis was performed with a scanning electron microscope SEM/ESEM–EDAX QUANTA 200 (FEI Company, Portland, OR, USA) at 1000× magnification. No treatment was applied to the samples for this analysis. For EDAX results, the average of 3 measurements for different zones of the same sample were used.

2.3.3. Contact Angle Measurements

Static contact angle measurements performed on a CAM-200 goniometer (KSV Instruments Ltd., Helsinki, Finland) were used to determine the hydrophilic/hydrophobic character of the paper's surface. The water contact angle was determined by the sessile drop method, at room temperature (~25 °C) and controlled humidity, in the first 3 s after

placing 2 µL drops of water on the sample's surface. The presented values represent the average of at least 10 measurements performed on one sample.

2.3.4. DPPH Radical Scavenging Assay

The radical scavenging activity of Kraft cellulose paper was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable free radical with a violet color that, under the action of proton-donating compounds, is reduced to a light yellow color. These color changes can be monitored at 517 nm. Briefly, 0.05 g of each sample was placed into 100 mL of methanol (for essential clove oil) or chloroform (for cold-pressed grape seed oil), and shaken overnight. Further, volumes ranging from 0.1 to 2.5 mL of the obtained solution were mixed with 2 mL of 0.05 mM DPPH in methanol and placed in the dark for a period of 30 min in sealed recipients, before recording the UV absorbance spectra.

The radical scavenging activity was calculated according to the following equation [22]:

$$\%RSA = 100 \times \left(1 - \frac{A_{sample}}{A_{control}}\right) \quad (1)$$

where A_{sample} represents the absorbance of the sample solution and $A_{control}$ represents the absorbance of the DPPH solution with the addition of the unmodified sample.

The IC50 value is defined as the concentration at which the inhibition of DPPH free radical activity is 50%. The IC50 was calculated using linear regression analysis of the individual solutions prepared as described above. All assays were performed in duplicate.

2.3.5. *In Vitro* Antibacterial Activity

Antibacterial tests were performed using the colony-counting method based on the standard procedure on 3 different Type Culture Collection bacterial strains (ATCC, Rockville, MD, USA), namely SR ISO 16649-2/2007 for *Escherichia coli*-ATCC 25,922 (Gram-negative) [23], SR EN ISO 6579/2003/AC/2004/AC/2006, amendment 1:2007 for *Salmonella enteritidis*-ATCC 14,028 (Gram-negative) [24], and SR EN ISO 11290-1:2000/A1:2005 for *Listeria monocytogenes*-ATCC 7644 (Gram-positive) [25].

The procedure involves sterilization of the materials (for 20 min in an autoclave at 110 °C and 0.5 bars), followed by contamination by seeding 0.1 mL (10^2–10^3 CFU) of each microbial suspension using sterile swabs on the sample surface, inoculation and incubation for 24 h and 48 h at 37 °C, followed by colony counting. In the case of *E. coli*, identification was achieved by staining with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. *Listeria monocytogenes* was identified using the β-hemolysis test, while *Salmonella* was counted by the specific count method with xylose Lysine Deoxycholate agar (XLD agar).

2.3.6. Microbiological Analysis on Fresh Curd and Fresh Beef

The modified Kraft paper specimens, with proven antimicrobial properties, were tested in aseptic laboratory conditions on fresh curd and fresh beef, both having a short shelf life.

Briefly, the modified Kraft paper was cut into 4 × 4 cm^2 pieces and placed in sterile Petri dishes. Next, food samples aseptically cut to 1 × 1 × 1 cm^3 were added onto the center of the paper. After that, the Petri dishes were sealed and refrigerated at 7 °C for 24 and 48 h. At each mentioned interval, the samples were brought to room temperature (~25 °C) for analysis. The bottom surfaces of the food samples were checked using pH indicator paper. The surface of the Kraft paper in contact with food samples was wiped off with a sterile swab that was then immersed in a test tube with 10 mL of physiological serum. Next, 1 mL from these suspensions was seeded in 2 Petri dishes containing plate count agar (PCA). After solidification, the plates were kept at 30 °C for 72 h according to the SR ISO 4833/2014 standard [26]. The microbiological examinations and the interpretation of the results were performed according to the SR EN ISO 7218/2014 standard [27].

3. Results and Discussions

3.1. ATR-FTIR Spectra Results

Infrared spectroscopy was used in order to illustrate the structure of the studied materials, as well as the intermolecular interactions between the bleached Kraft paper and the plant oils. The spectra of the bleached Kraft paper (BP), plant oils (ECO and CGO), and paper treated with them (BP/ECO and BP/CGO) are shown in Figure 1. They were divided into two main regions, namely 3700–2700 cm^{-1}, which is usually assigned to different stretching vibrations of OH groups and hydrogen bonds, as well as to methyl and methylene groups, and the fingerprint region (1800–600 cm^{-1}), assigned to specific groups constituent in the studied materials.

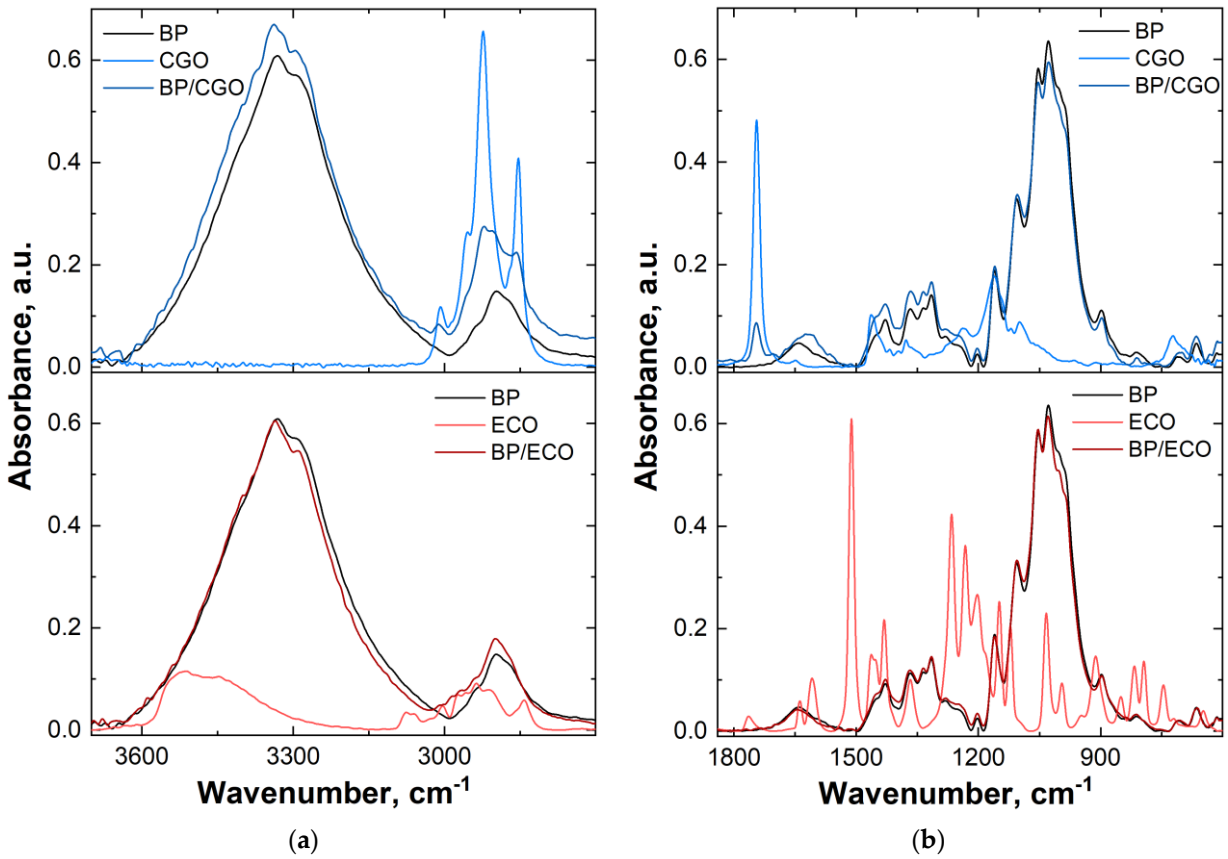

Figure 1. Infrared spectra of control and plant-oil-modified Kraft paper in the 3700–2700 cm^{-1} region (**a**) and 1800–600 cm^{-1} region (**b**).

In Figure 1a, the BP spectra present characteristic bands of cellulose, with a large band at 3330 cm^{-1} assigned to different inter- and intramolecular H-bonds and –OH stretching vibrations, and 2898 cm^{-1} assigned to the symmetric and asymmetric stretching vibration of methyl and methylene groups [28,29]. The ECO spectrum shows a large small band at about 3516 cm^{-1} assigned to OH stretching vibrations, and at 3005, 2966, 2938, 2914, and 2844 cm^{-1} assigned to -CH stretching vibrations from the methyl and methylene groups [30,31]. The BP/ECO spectra (treated paper with clove essential oil) do not present significant changes in this region: the band from 3330 cm^{-1} from BP is shifted to 3337 cm^{-1} in BP/ECO, and small differences can also be observed for the -CH stretching vibration region.

The CGO spectrum shows important bands at 3007, 2953, 2926, and 2854 cm^{-1}. These are assigned to =C-H (cis and trans) and -CH symmetric and asymmetric stretching vibrations from the methyl and methylene groups [31]. In this case, the BP/CGO spectrum

shows higher modifications in the 3030–2750 cm^{-1} region, due to the presence of the CGO on the surface of the paper.

In the fingerprint region (Figure 1b), all spectra present a higher number of bands. As mentioned above, the spectra of the bleached Kraft paper present specific bands of cellulose, namely at 1640 cm^{-1}, assigned to the stretching vibration of the absorbed O-H and conjugated C-O; at 1448 and 1428 cm^{-1}, assigned to the C-H deformation and O-H in-plane bending; at 1366 cm^{-1}, assigned to CH deformation and CH_3 symmetric deformation; at 1337 cm^{-1}, assigned to the CH_2 rocking vibration; at 1317, 1278, and 1254 cm^{-1}, assigned to the C-H bending mode; at 1161 cm^{-1}, assigned to the C-O-C stretching vibration mode of the pyranose ring; at 1200 and 1106 cm^{-1}, assigned to the C-O stretching vibration; at 1051 cm^{-1}, assigned to the C-O stretching vibration mainly from C(3)-O(3)H; at 1028 cm^{-1}, assigned to the C-O and C-C stretching ring; and at 897 cm^{-1}, assigned to CH deformation vibrations [28,29].

The ECO spectra, with the main component of eugenol, present several important bands: at 1637 and 1512 cm^{-1}, assigned to the stretching vibration of the C=C of vinyl groups; at 1461 and 1432 cm^{-1}, assigned to the phenyl ring's C–C extending vibrations; at 1366, 1266, and 1234 cm^{-1}, assigned to the stretching vibration of the C–O bond from phenolic hydroxyl; at 1202 and 1150 cm^{-1}, assigned to the bending vibration of C–O bonds; at 1119 and 1033 cm^{-1}, assigned to the stretching vibration of the C–O–C aromatic ether groups; at 997 and 911 cm^{-1}, assigned to O–H bending; and at 850 and 795 cm^{-1}, assigned to the C–H bending of the aromatic ring [30,32].

The spectrum of BP/ECO presents mainly the bands of cellulose. However, the presence of ECO can be observed by the presence of a small band at 1516 cm^{-1} (seen only in ECO at 1512 cm^{-1}) and a slight modification of the band shape in the 1430–1100 cm^{-1} region. In addition to these, no new bands were found in the BP/ECO sample spectrum with the addition of the ECO. Generally, the absorption bands of the cellulose are strong, and because the bands from the ECO overlap with those of cellulose, it is difficult to distinguish their presence in the spectrum.

The spectrum of CGO consists of characteristic triglyceride absorption bands; thus, here we can observe sharp bands at 1746 cm^{-1}, assigned to the C=O stretching vibration from the ester, aldehyde, ketone, and anhydride groups; at 1462, 1377, and 1160 cm^{-1}, assigned to the stretching vibration and deformation in -CH from the methyl groups; at 1236 cm^{-1}, assigned to the stretching vibrations of the C–C(=O)–O groups; and at 1098 cm^{-1}, assigned to the stretching vibration of the O–C–O groups associated with primary and secondary alcohols. The small bands at 960 and 911 cm^{-1} are assigned to the stretching vibrations of the cis-substituted olefinic groups and also to the vinyl groups, while the band from 721 cm^{-1} is assigned to the deformation and rocking vibrations associated with the –HC=CH– groups (cis-conformation, out of plane) [31,33].

The treated bleached Kraft paper with cold-pressed grape seed oil (BP/CGO) spectra present some differences compared to BP, namely a new band at 1744 cm^{-1} assigned to the C=O stretching vibration from the groups from CGO; the band from 1640 cm^{-1} (in BP spectrum) is shifted to 1616 cm^{-1} (in BP/CGO spectrum); the band from 1448 cm^{-1} (in BP spectrum) is increased in intensity and width and is slightly shifted to 1450 cm^{-1}; and the band from 1254 cm^{-1} is slightly shifted to 1249 cm^{-1}.

Following these, we can conclude that the bleached Kraft paper was modified with the plant oils; in both cases, the evidence of their presence on the surface of the paper could be observed.

3.2. SEM/EDX Results

Imaging of the surface morphology of studied materials was analyzed using SEM, as shown in Figure 2.

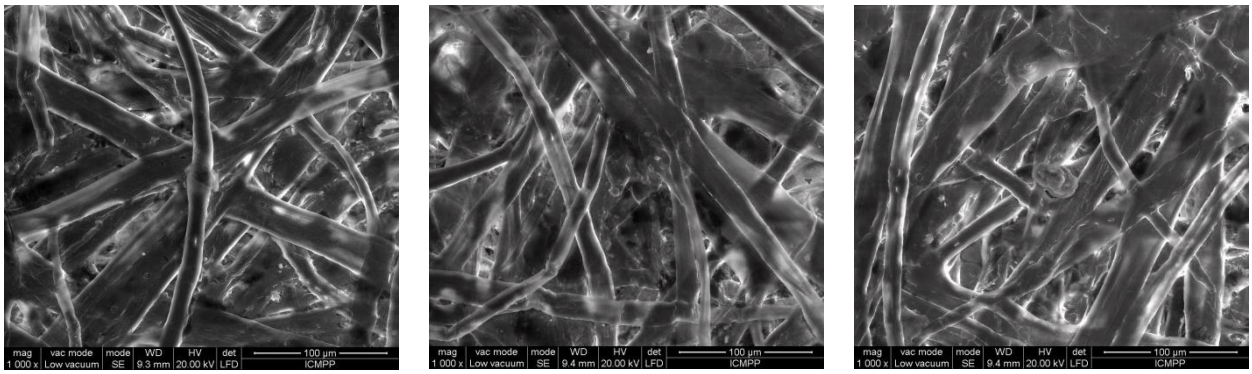

Figure 2. SEM images of the control and plant-oil-modified bleached Kraft paper.

SEM images of the unmodified (control) paper presented a dense fiber matrix with low porosity. Plant-oil-modified papers presented a surface with an increased homogeneity due to oil entering between the pores. Therefore, liquid penetration (like water) was expected to be lower in the case of plant-oil-modified materials.

Elemental composition analysis of the surfaces also provides information regarding the modification. Table 1 presents the average results of the EDAX measurements for the analyzed materials.

Table 1. EDAX data for control and plant-oil-modified bleached Kraft paper.

Sample	Element						
	C		O		N		O/C
	Wt%	At%	Wt%	At%	Wt%	At%	At%
BP	57.12	63.71	41.78	35.72	0.50	0.48	0.560
BP/ECO	54.82	62.15	43.97	36.97	0.98	0.85	0.594
BP/CGO	55.12	62.30	43.12	36.55	1.44	1.10	0.586

As expected, the main elements in the paper composition were carbon and oxygen.

After modification, increases in oxygen content and in the O/C atomic ratio were observed. Nitrogen content was also higher after modification, suggesting that nitrogen from the air also took part in the formation of the reactive centers on the paper surface. Both vegetable oils have carbon and oxygen in their molecules, but the content of carbon atoms is higher in cold-pressed oil due to the presence of long aliphatic chains in the fatty acids.

The O/C ratio increased after enzymatic activation and modification of the paper, suggesting increased polarity as a result of the oxygen-containing groups' presence, following the fixation of tested oils onto the activated paper.

3.3. Water Contact Angle (WCA)

WCA measurements were used to establish whether or not the paper surface was affected after modification with vegetable oils. A water contact angle above 90° describes a hydrophobic surface, while a water contact angle below 90° characterizes a hydrophilic surface. Complete wettability is considered when the contact angle is 0°. Therefore, measuring the values of a contact angle of a material is very important for practical applications in the food industry [34,35].

Figure 3 presents the water contact angles and photographs of water droplets deposited onto surfaces for the control and plant-oil-modified bleached Kraft papers.

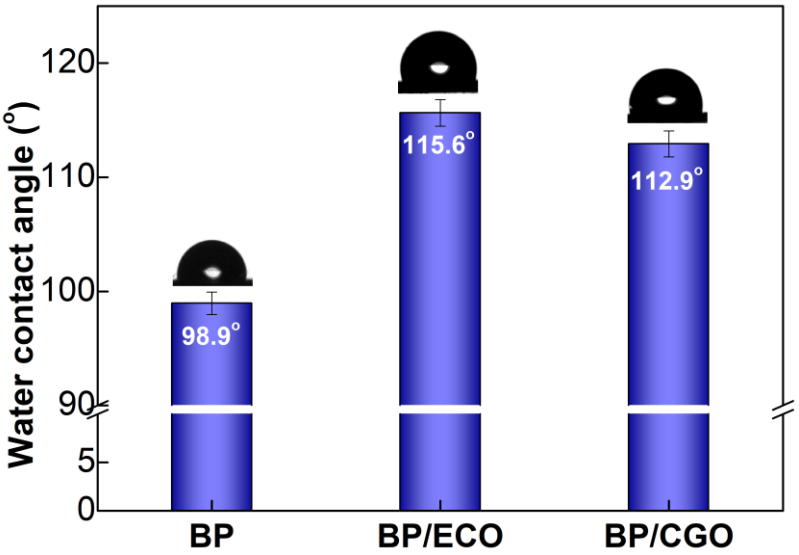

Figure 3. Water contact angles of control and plant-oil-modified bleached Kraft papers.

Control (unmodified) Kraft paper presented a water contact angle of 98.9°, exhibiting low hydrophobicity. Surface modification by vegetable oils strongly improved the hydrophobicity of the paper, the water contact angle increasing over 115° in the case of BP/ECO and about 113° in the case of BP/CGO. This could be related to the interactions that occur between the polar functional groups of the main compounds from the vegetable oils (e.g., carboxyl) and the enzyme-activated paper, thus resulting in fewer groups being available at the top surface to interact with water.

The water contact angle value for BP/ECO was slightly higher compared with BP/CGO paper. This may be the result of the stronger interactions that occurred between clove oil's main component (eugenol) and cellulose from the Kraft paper. Eugenol, being a smaller chain molecule than linoleic acid (the main component in CGO), can interact intermolecularly with cellulose, resulting in more hydrophobic functional groups being available on the surface of the paper.

3.4. DPPH Radical Scavenging Assay

DPPH tests were performed to assess if the vegetable oils impart their antioxidant efficiency to the cellulosic substrate on which they are immobilized. The IC50 value is assigned to the concentration required to yield 50% inhibition in the tested sample (Table 2).

Table 2. Half maximal Inhibitory Concentration (IC50) for control and plant-oil-modified bleached Kraft papers.

Sample	BP	BP/ECO	BP/CGO
IC50, mg/mL	---	0.101	41.43

The antioxidant capacity is conditioned by the efficiency of the individual vegetable oil to modify the cellulosic substrate and provide a stable product. The antioxidant activity is related to the existing hydroxyl groups in their structure. As indicated by the very low IC50 concentration, the BP/ECO-modified paper has stronger antioxidant activity, mostly owing to the major components and contributors to total antioxidant activity, which are eugenol and eugenol acetate [21,36].

The antioxidant activity of grape seed oil is associated with its main components, which are gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins, and could be the result of the synergistic combination of these phenolic compounds [18,37,38]. Nonetheless, phenolic compounds are minor constituents in contrast to fatty acids, which

comprise up to 85%–90% of the cold-pressed grape seed oil [18], this being the reason for the reduced antioxidant activity compared to essential clove oil. Similar results were obtained in our previous work, where cellulosic substrates were modified with phenolic compounds or vegetable oils using cold plasma or gamma irradiation as a paper activation method [12,19].

3.5. In Vitro Antibacterial Activity

In vitro antibacterial evaluation was conducted on three different bacteria that are important from a food safety point of view due to being main food contaminants, namely Gram-negative *Escherichia coli* and *Salmonella enteritidis*, and Gram-positive *Listeria monocytogenes* (Figure 4).

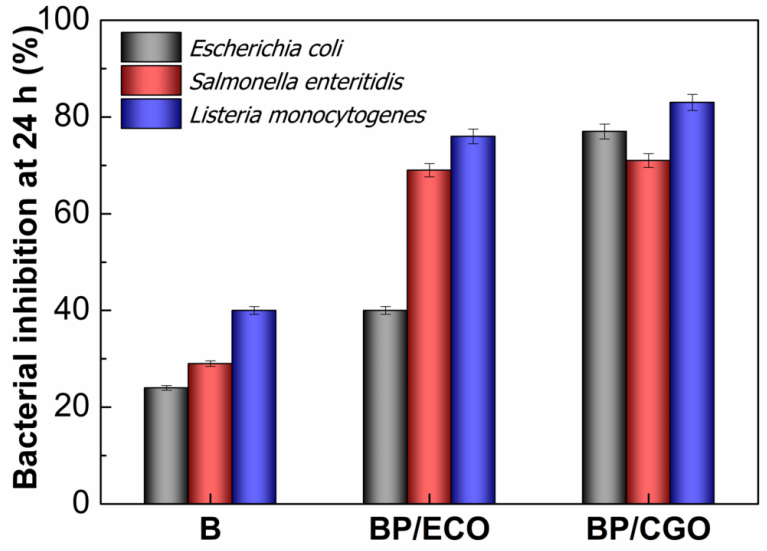

Figure 4. Percent bacterial inhibition for control and plant-oil-modified Kraft papers.

Observing the results from Figure 4, we can determine that by modifying Kraft paper with vegetable oils, improved bacterial inhibition was observed for both Gram-negative and Gram-positive bacteria at 24 h. The efficiency increased in the order of *E. coli* < *L. monocytogenes* < *S. enteritidis* in the case of BP/ECO (essential clove oil) and in the order of *L. monocytogenes* < *S. enteritidis* < *E. coli* in the case of BP/CGO (cold-pressed grape seed oil). Also, cold-pressed grape seed oil showed greater bacterial inhibition compared to essential clove oil for all bacterial strains tested.

Overall, the antimicrobial activity displayed by phenolic compounds in plant oils induced oxidative damage to bacterial strains, especially for *Salmonella enteritidis* and *Escherichia coli*, without affecting the host cells.

3.6. Microbiological Assessment on Fresh Curd and Fresh Beef

Enterobacteria are a sterile barometer for white cheeses, in which microbiological spoilage during storage is caused mainly by yeasts and molds. Hence, we investigated microorganisms' growth on fresh white cheese in contact with unmodified and vegetable-oil-modified paper. The fresh curd used for testing was obtained from cow milk by adding rennet as a coagulant. The examined white curd was characterized by low contamination with the *Enterobacteriaceae*, their number in the fresh control sample being less than 10 CFU/g, with a pH of 5.0.

Meat can be subjected to microbial contamination when animals are butchered or during various procedures. The number of bacterial strains in meat shortly after slaughter is a major factor in the determination of shelf life. The surface of beef carcasses can contain anywhere from 10 to 107 cfu/cm^2 bacteria colonies. Meat processing can lead to increased

microbial load as the exposed surface area is larger. Yeasts and molds grow quite slowly on fresh meat compared to bacteria, being a minor component of spoilage flora [39].

For meat, the tests were performed on fresh beef supplied by a local slaughterhouse, delivered within 4 h of slaughter. The tested fresh beef was red, odorless, moist, springy in appearance, and presented an initial pH of 5.5.

Observations based on *in vitro* studies have been extended to *in vivo* tests to explore the potential use of essential clove oil and cold-pressed grape seed oil to delay food spoilage.

Figure 5 presents the population of microorganisms related to cheese and meat spoilage after 24 h and 48 h in terms of Total Viable Counts.

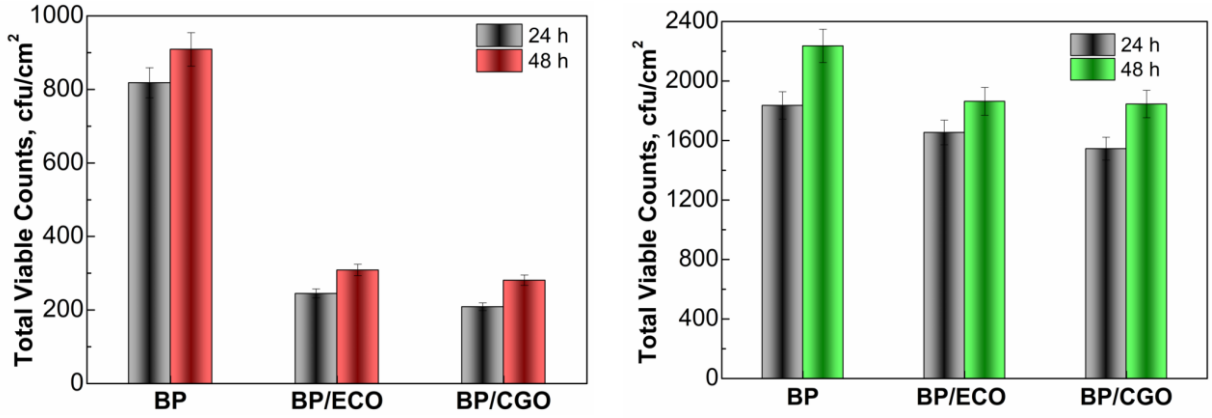

Figure 5. Total Viable Counts of control and plant-oil-modified bleached Kraft paper substrates for (**a**) fresh curd and (**b**) fresh beef.

Modification of Kraft paper with vegetable oils resulted in a reduction in microbial growth for both fresh curd and fresh beef, as exhibited by decreased Total Viable Counts.

Differences were noticed between the two plant oils; cold-pressed grape seed oil had a somewhat greater impact compared with essential clove oil. This effect was expected given that this oil has been shown to have better *in vitro* antimicrobial activity.

It appeared that microbial growth highly relies on the type of food. Both vegetable oils presented better microbial inhibition on curd, with a sudden decrease of growth percentage below 30% after 24 h and below 35% after 48 h. However, cell growth values remained high for beef, at over 80% after 48 h. These results indicate that plant-oil-modified paper is more suitable for extending the shelf life of foods with relatively low initial bacterial content, such as fresh cheese.

4. Conclusions

The present work showed a way to develop new bioactive materials by immobilization of plant oils with antimicrobial and antioxidant properties on a bleached Kraft paper surface, using cellulase enzyme as the substrate activation method. Modifications in the structural and morphological properties of recently obtained materials have been evidenced by ATR-FTIR, SEM-EDX, and water contact angle data. Depending on the type of oil used, modified Kraft paper exhibited distinct antioxidant and antibacterial properties. Essential clove oil has proven superior antioxidant activity, while cold-pressed grape seed oil was more efficient on *Escherichia coli* (−), *Salmonella enteritidis* (−), and *Listeria monocytogenes* (+), in lowering bacterial growth on fresh beef and fresh curd. All these tests recommend these materials for use in the food industry for prolonging food shelf-life.

Author Contributions: Conceptualization, A.I.; methodology, A.I.; formal analysis, A.I. and C.-M.P.; investigation, A.I. and C.-M.P.; resources, A.I.; data curation, A.I. and C.-M.P.; writing—original draft preparation, A.I. and C.-M.P.; writing—review and editing, A.I. and C.-M.P. All authors have read and agreed to the published version of the manuscript.

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