applied sciences

MDPI

Article

Zein and Spent Coffee Grounds Extract as a Green Combination for Sustainable Food Active Packaging Production: An Investigation on the Effects of the Production Processes

Emanuela Drago [1,†], Margherita Pettinato [1,†], Roberta Campardelli [1,*], Giuseppe Firpo [2,*], Enrico Lertora [3] and Patrizia Perego [1]

[1] Department of Civil, Chemical and Environmental Engineering, Polytechnic School, University of Genoa, Via all'Opera Pia 15, 16145 Genoa, Italy
[2] Department of Physics, Nanomed Lab, University of Genoa, Via Dodecaneso 33, 16146 Genoa, Italy
[3] Department of Mechanical, Energy, Management and Transport Engineering, Polytechnic School, University of Genoa, Via all'Opera Pia 15, 16145 Genoa, Italy
* Correspondence: roberta.campardelli@unige.it (R.C.); giuseppe.firpo@unige.it (G.F.)
† These authors contributed equally to this work.

Abstract: In this work, the effect of different production techniques was evaluated on the physical and antioxidant properties of bio-based packaging intended to prevent the premature oxidation of packaged foods. Spent coffee ground extract, rich in antioxidant molecules, obtained through high pressure and temperature extraction, was loaded on zein polymeric matrices. The techniques adopted in this work are particularly suitable due to their mild conditions to produce active packaging completely based on natural compounds: electrospinning, solvent casting, and spin coating. The novelty of this work lay in the investigation of the dependance of the properties of active packaging on the adopted production techniques; the results clearly indicated a strong dependence of the features of the films obtained by different production processes. Indeed, spin coated samples exhibited the best oxygen barrier properties, while a higher tensile strength was obtained for the casted samples, and the fastest release of active compounds was provided by electrospun mats. The films produced with different methods had different physical properties and the release of extract bioactive compounds can be tunable by varying the production technique, dependent on the variable to be considered. The products developed offer an alternative to traditional packaging solutions, being more eco-sustainable and promoting waste valorization.

Keywords: biomaterial; antioxidant; food waste valorization; green process; shelf-life preservation; green solvent

Citation: Drago, E.; Pettinato, M.; Campardelli, R.; Firpo, G.; Lertora, E.; Perego, P. Zein and Spent Coffee Grounds Extract as a Green Combination for Sustainable Food Active Packaging Production: An Investigation on the Effects of the Production Processes. *Appl. Sci.* **2022**, *12*, 11311. https://doi.org/10.3390/app122211311

Academic Editor: Francisco Javier Gutiérrez Ortiz

Received: 14 October 2022
Accepted: 6 November 2022
Published: 8 November 2022

1. Introduction

In the past, plastics of fossil origin have allowed rapid innovation in the food packaging sector through the development of convenient packaging systems, both in terms of cost and use, that are adaptable to any type of food. However, behind the increasingly attractive designs of disposable packaging, there is the need to consider the serious problem of the environmental impact [1]. The current interest of the scientific community is, therefore, aimed at researching new biodegradable materials of natural origin according to a circular economy approach. A valid alternative to plastics can be represented by biodegradable polymers synthesized from bio-derived monomers such as polylactic acid (PLA) and polyhydroxyalcanoate (PHA) [2,3], chemically synthesized from fossil resources, such as polycaprolactone (PCL) [4], or extracted from biomass such as zein [5,6]. The main limitations of most biopolymers are related to the poorer physical properties compared to conventional plastics [7]. Zein, for example, is a biopolymer deriving from the processing of corn, which is enjoying great interest for its eco-sustainability and its versatility, but

which has poor mechanical properties. However, these problems can be overcome using polymeric blends and nanofillers such as inorganic particles [8–10]. Sometimes, even the addition of natural additives with antimicrobial or antioxidant properties can lead to an improvement of these characteristics. In fact, innovation in this field is not represented only by biodegradable materials, but also by their functionalization through the addition of natural and non-toxic additives for the design of so-called active packaging, aimed to prolong food shelf-life. The natural active substances most investigated in the literature as antimicrobial and antioxidant agents to be loaded into biopolymeric matrices are essential oils, such as from sage, thyme and oregano loaded in zein films [11,12]. More recently, natural extracts have drawn attention as interesting sources of antioxidant and antimicrobial compounds able to act synergistically and providing higher activities than single compounds [13]. In this context, spent coffee grounds (SCG) represent a useful and sustainable source for bioactive natural compounds recovery, containing significant amounts of high added-value compounds, which can be potentially applied in several industrial sectors. By considering a logical approach in agreement with the biorefinery concept, first this biomass can be exploited to recover high-added value compounds like chlorogenic acid and other phenolic acid and polyphenols, as well as caffeine, and melanoidins, which can find widely applications especially in the food and cosmetic fields. After this first step employing quite polar solvents for the extraction step, the residual biomass can be further used for the recovery by non-polar solvents of the so-called coffee oil, which can be used as a plasticizer, an antioxidant agent, the starting material for biodiesel production, and an alternative and cheap substrate for the cultivation of microorganisms to produce chemicals and polymers. Moreover, the solid residue of this second step can be hydrolyzed and used for fermentation purposes, such as to produce bioethanol, or the solid can be directly used as solid fuel. Other applications concern the use of spent coffee grounds as filler, as natural soil improver, as substrate for vermicomposting.

A few studies have been conducted so far regarding the loading of natural extracts recovered from agri-food waste into biofilms as a valid alternative to synthetic additives for food active packaging production and to potentially contribute to the improvement of physical properties of bio-based packaging [14–16]. Despite this, no work has yet investigated the potential use of spent coffee ground extract to functionalize zein films [15,16].

The solvent casting technique was used to produce PLA/diatomite-based materials enriched by the lipid fraction extractable from spent coffee grounds [17], while subcritical water extract from spent coffee grounds was added to fish skin gelatin to fabricate edible active packaging films by the same production technique [18]. A different approach was followed by the direct addition of defatted spent coffee ground as a filler to produce SCG/polybutylene succinate bio-composites by reactive extrusion [19]. The literature about zein-based active packaging often reports electrospinning as the preferential technique to fabricate mats enriched by different kinds of natural extracts, such as *Salvia officinalis* L. extract [11], thyme essential oil [20], or pure antioxidant compounds such as gallic acid [21], vanillin [6], and carvacrol [22], albeit studies of casted zein-based films functionalized with pure antimicrobial compounds and essential oils [23] were also available.

Therefore, in this work, the effect of the fabrication techniques and extract loading on the features of the zein-based active packaging was investigated. The high pressure and temperature extraction process (HPTE) was selected for the recovery of active ingredients from spent coffee grounds. This technique is characterized by high extraction yield without degradation and loss of the functionality of bioactive molecules [24]. Regarding the polymer processing, electrospinning, solvent casting, and spin coating were used for packaging fabrication. All these processes can work in mild temperature conditions, in order to preserve the nature of the processed compounds, and enable obtaining films with different properties that were the objective of study and comparison in this work, with the final aim of providing a guide for the identification of the best technique to be used to obtain the desired final film properties.

2. Materials and Methods

In this work, three different production techniques were employed to produce zein-based food packaging loaded with spent coffee grounds extract to provide antioxidant properties to the biobased material. The aim of the work was to evaluate the effects of the production techniques and of the extract loading on the features of the obtained products in terms of morphological properties, release rate of active agents, gas barrier and mechanical properties. The results of the physical characterization of the obtained films allowed a comparison of product characteristics as a function of the production technique and extract loading. In this section, the methods adopted to obtain zein-based packaging enhanced with the antioxidant properties of spent coffee ground extract are reported. Section 2.2 deals with the protocols for the extract and polymeric solution preparation, while Section 2.3 describes the operating conditions employed for the fabrication of the active films by the three different techniques, i.e., electrospinning, solvent casting, and spin coating. Finally, the methods used for active films characterization and data analysis are reported in Sections 2.4–2.8.

2.1. Materials

Purified zein, ethanol, acetonitrile, methanol, glycerol and 2,2′-azino-bis(3-ethylbenzo thiazolin-6-sulfonic acid) diammonium salt (ABTS), and chlorogenic acid standard were purchased from Sigma–Aldrich (Milan, Italy). The caffeine standard was purchased from VWR Chemicals (Milan, Italy). Spent coffee grounds were recovered from the vending machine of the Department of Civil, Chemical and Environmental Engineering of the University of Genoa. Oxygen gas was used with purity grade N5.0.

2.2. Extract and Polymeric Solution Preparation

The extract was produced starting from dried spent coffee grounds (SCG) by high pressure and temperature extraction (HPTE) in ethanol 54% (v/v), following the protocol reported in Pettinato et al., 2020 [25]. The extract was characterized by High Performance Liquid Chromatography (HPLC), according to the protocol reported in Section 2.5, and in terms of total polyphenol content and antiradical power, using the Folin–Ciocalteu's protocol and the ABTS assay reported in [25]. Because the investigated techniques envisage starting from a polymeric solution, the polymer and the extract were combined by optimizing the preparation of the polymeric solution. In detail, the addition of the hydroalcoholic extract into the zein solution was carried out maintaining a fixed ratio between the polymer and solvent. The polymeric solution for active packaging productions was prepared by dissolving zein in 80% v/v ethanol aqueous solution under stirring, at 70 °C, up to complete polymer solubilization. The solution was then cooled to 40 °C [26]. In the case of the polymeric film loaded with SCG extract, the extract from SCG of *Coffea canephora* was added to the solution to have different theoretical loading from 0 (unloaded samples) to 45% w/w with respect to zein content.

2.3. Preparation of Zein-Based Films Loaded with SCG Extract

Zein-based films and zein loaded SCG films were produced by three different techniques: electrospinning, solvent casting, and spin coating, for comparison purposes. Electrospun mats were obtained by feeding 6 mL polymeric solution (40% w/w zein with respect to the solvent content) in the electrospinning apparatus (Spinbow, Bologna, Italy) equipped by a high voltage supplier (Spellman, NY, USA) working at 17.0 kV during the experiment, a syringe pump (KD Scientific, Holliston, MA, USA), providing a flow rate set at 1.2 mL/min, and an injection needle of 21 gauge (anode), whose distance from the planar collector (cathode) was 16.5 cm. The employed experimental conditions were based on preliminary studies [6,26]. After deposition, mats were left for 2 h in the desiccator to ensure complete drying. The films were also produced by the solvent casting process. In detail, 2.5 mL of zein-based (20% w/w of zein with respect to the solvent) solution were mixed with the extract and glycerol (0.24 mL) was used as plasticizer and left under stirring

for 8 min. The obtained solutions were poured onto glass Petri dishes (9 cm diameter), dried in an oven at 60 °C for 2 h, and cooled in a desiccator before peeling off the films. The spin coating process was performed by pouring 4 mL of zein-based (20% w/w of zein with respect to the solvent) solutions loaded with SCG extract onto Petri dishes placed on a rotating disk in a spin coater (SPS-Europe, Spin 150 APT GmbH, Ingolstadt, Germany). The film was obtained by rotating the disk at 100 rpm for 10 s. The polymeric solutions were prepared as described for the solvent casting technique. After the deposition, samples were placed in an oven at 60 °C for 2 h and cooled in a desiccator before peeling off the films.

2.4. Morphology Characterization

An ultra-high resolution Field Emission Scanning Electron Microscope (UHR-FE-SEM, CrossBeam 1540 XB, Zeiss GmbH, Oberkochen, Germany) was used to analyze the morphology of all the samples. The thickness of the films obtained by the three production techniques was determined by optical microscopy (Olympus BX51, Life Science Solution, Segrate, Italy) with at least five measurements for each sample. For electrospun mats, the mean size of the fibers was evaluated using the ImageJ software (NIH, Bethesda, MD, USA), randomly analyzing about 300 fibers per sample.

2.5. Release Tests

The ability of the produced active packaging to release the bioactive compounds with antioxidant properties was evaluated by release tests in a food simulant. Sample disks of 9 cm diameter were submerged in 20 mL of ethanol 10% v/v as a food simulant (food simulant A), gently stirred by an orbital shaker at 250 rpm at room temperature [27]. Samples of the food simulant were collected over time, centrifuged at 35,273× g, 4 °C, for 10 min (Alliance Bio Expertise, MF 20-R, Seine-Port, France) and analyzed by High Performance Liquid Chromatography (HPLC) for caffeine concentration analysis and by UV-vis spectrophotometry to assess the scavenging ability of the active agent released. An HPLC Agilent 1100 Series (Palo Alto, CA, USA), coupled with a Diode Array Detector and equipped with a C18 reverse-phase column (Vydac, Hesperia, CA, USA) was used for caffeine detection. Samples of 100 µL of the supernatant of the centrifuged food simulant were injected without dilution for each run. Mobile phases water/acetic acid (99:1%, v/v) (solvent A) and methanol/acetonitrile (50:50%, v/v) (solvent B) were used and the solvent gradient was set as follows: 100% A for 5 min, from 5% to 30% B for 25 min, from 30% to 40% B for 10 min, from 40% to 48% B for 5 min, from 48% to 70% B for 10 min, from 70% to 100% B for 5 min, isocratic at 100% B for 5 min, followed by returning to the initial conditions (10 min) and column equilibration (12 min). A flow rate of 1.0 mL/min was used at 30 °C.

Caffeine concentration in the samples was determined from the area under the caffeine peak on the chromatograms by the calibration curve obtained from standard solutions of caffeine (0.0256–0.256 mg/mL), reported in Equation (1) (R^2 = 0.9995).

$$C\left(\frac{mg}{mL}\right) = \frac{Area}{105644} \quad (1)$$

The radical scavenging capacity of the SCG extract released from the polymeric support was determined by the $ABTS^{\bullet+}$ assay and expressed as percentage inhibition [28]. The $ABTS^{\bullet+}$ working solution was prepared by adding $K_2S_2O_8$ to an aqueous ABTS 7 mM solution up to a final concentration of 2.45 mM. The absorbances of the working solution (A_{blank}) and of 50 µL of sample mixed for 2 min with 1 mL of working solution (A_{sample}) were measured by an UV–vis spectrophotometer (Lambda 25, Perkin Elmer, Wellesley, MA, USA) at 734 nm. The percentage inhibition was calculated according to Equation (2).

$$\text{Inhibition } (\%) = \frac{\left(A_{blank} - A_{sample}\right)}{A_{blank}} \quad (2)$$

2.6. Permeability Properties

Assuming the validity of Fick's and Henry's laws for the permeability coefficient P, the following Equation (3) defines the permeability as:

$$P = \frac{JL}{\Delta p} = DS \quad (3)$$

where J is the rate of transfer per unit area through a sample cross section, L is the membrane thickness, D is the diffusivity, and S is the solubility, considering gas diffusion through a membrane, assumed as an infinite plate, due to a pressure difference Δp across the two sides of the membrane. Therefore, the oxygen barrier property of food packaging materials was quantified by the Oxygen Transmission Rate (OTR) and by the Oxygen Permeability Coefficient (OPC), whose correlation is reported in Equation (4).

$$OPC = OTR \times L \times \Delta p^{-1} \quad (4)$$

The measurements were performed using a homemade laboratory ultra-high vacuum instrument, described in [29] according to the procedure defined by ASTM D1434-82 [30].

2.7. Mechanical Properties

The tensile strength (TS), elongation at break (EB), and Young's modulus (YM) values of the samples, were evaluated using the Instron 8802 Machine according to ASTM638 standard method and UNI EN ISO 527 for polymeric materials [31]. The samples of 40 × 90 mm^2 were mounted between the grips of the instrument and tested using a 5 kN load cell and a cross-head speed of 1 mm/min. At least five specimens were tested for each measurement.

2.8. Statistical Analysis

Statistical evaluations of the experimental data were conducted using the Statistica v8.0 software (StatSoft, Tulsa, OK, USA). Analysis of variance (ANOVA) and Tukey's post-hoc tests were performed to assess the significance of differences among groups with statistical significance considered at a probability value $p < 0.05$.

3. Results

As a potential alternative to conventional plastic-based packaging, a biobased polymer, i.e., zein was used and loaded with a naturally derived extract to provide an antioxidant action to the packaging to prolong food shelf life. The experimental session analyzed the characteristics of the extract obtained by a non-conventional extraction technology (HPTE) and evaluated three different processes used to produce the antioxidant packaging to analyze the dependencies of the product characteristics as a function of the production method and the amount of extract loaded into the zein-based material. Below, the results of the experimental campaign are reported and compared. In Section 3.1, the results of the extract characterization by High performance liquid chromatography in terms of total polyphenol content and anti-radical power of the extract are reported. In Section 3.2, the results comparing the production of the active packaging films by the different process and extract loading are described. A detailed comparison of the samples is given in the Sections 3.3–3.6 analyzing the product characteristics in terms of their morphological aspects, release rate of the active agent incorporated within the bio-based material into food simulants, gas barrier, and mechanical properties.

3.1. Spent Coffee Ground Extract

Several antioxidant compounds in SCG extracts were identified in the literature, whose activity can contribute to prolong food shelf-life. Particularly, chlorogenic acid is one of the main phenolic compounds in SCG, but also protocatechuic acid, caffeic acid, ferulic acids, melanoidins, proteins, carbohydrates, alkaloids, showed antioxidant activity by different mechanisms (H-donor mechanisms, hydroxyl free radical scavenging, etc.) and

antimicrobial activities, making the extract a potential active agent for food packaging purposes [32–37].

The antioxidant-rich extract from SCG was produced by HPTE at 150 °C using ethanol 54% v/v as the solvent [25].

In Figure 1, the HPLC chromatogram of the produced extract is reproduced. It shows that caffeine (1.032 ± 0.018 mg/mL) was the main peak in the extract profile. In addition, chromatograms related to the release tests on unloaded zein films (control samples) did not show any interferences at the retention time of caffeine peak (23.7 min). For these reasons, caffeine was selected as the representative compound of the extract for the release tests.

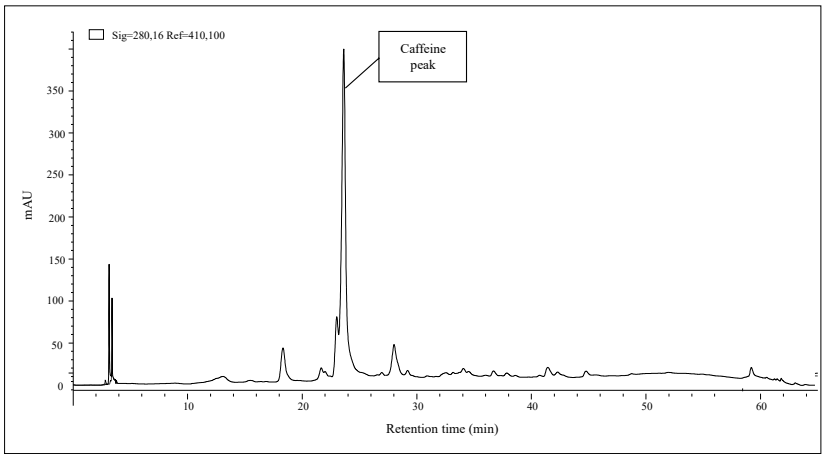

Figure 1. SCG extract chromatogram obtained via HPLC.

The action of other classes of compounds contributing to the antioxidant activity of the selected active agent was considered by the ABTS assay. Indeed, even if this test is less suitable for the detection of caffeine antioxidant activity, on the other hand it can highlight the radical scavenging activity of class of compounds like polyphenols [38]. Total polyphenol content in the obtained extract was of 4.96 ± 0.25 mg caffeic acid equivalents/mL of extract, exhibiting an antiradical power of 46.2 ± 5.2 $\mu g_{TE}/L_{EXTRACT}$.

3.2. Zein-Based Films Production Outcomes as Function of Production Technique and Extract Loading

Films enriched with antioxidants from SCG were produced by electrospinning, solvent casting, and spin coating processes. As a reference, unloaded films were also fabricated by means of the same techniques. All the selected methods enable polymeric support operating at mild temperatures and use green solvents, i.e., ethanol aqueous solutions, enabling the loading of thermosensitive compounds without remarkable losses of their antioxidant activity. The SCG extract was added at different percentages from 15, 20, 35 and 45% w/w with respect to the polymer content. The used of hydroalcoholic solvents for both HPTE (ethanol 54%, v/v) and polymer dissolution (ethanol 80%, v/v) represented a remarkable advantage for the production process, avoiding solubility problems of the involved components.

Furthermore, the volatility of the hydroalcoholic mixture was sufficient to perform successfully solvent evaporation by electrospinning process and not so high as to create sudden changes in polymer solution viscosity during pouring and spin coating. Finally, hydroalcoholic solvents could be supplied by biorefineries treating agri-food waste, increasing the process sustainability.

The mechanisms involved in the polymeric film productions provided products with different features. In particular, through the electrospinning technique (Figure 2a,b), opaque structures composed of fibers (Figure 3a) resulted, while isotropic, transparent, and continuous films were produced by solvent casting (Figure 2b,e) and spin coating (Figure 2c,f).

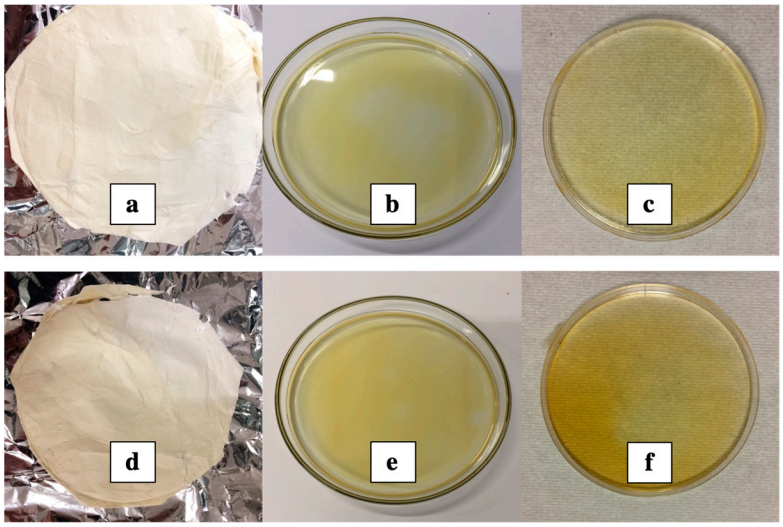

Figure 2. Unloaded zein films obtained through (**a**) electrospinning, (**b**) solvent casting, and (**c**) spin coating, SCG 45% *w*/*w* loaded zein films obtained through (**d**) electrospinning, (**e**) solvent casting and (**f**) spin coating.

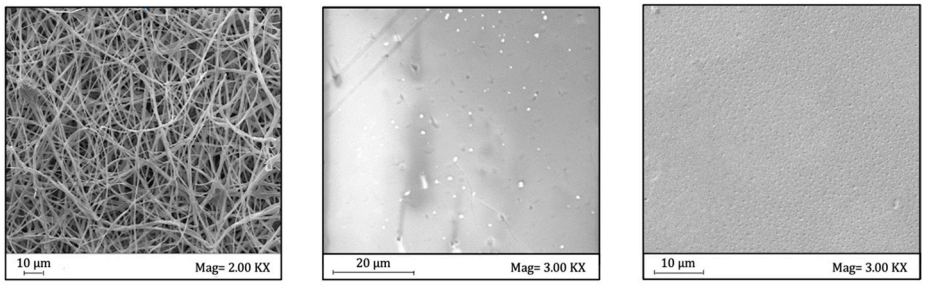

Figure 3. (**a**) SEM image of the fibrous structure obtained by the unloaded electrospun mat, (**b**,**c**) SEM images of the continuous structure obtained by the unloaded casted and spin coated samples, respectively.

As can be observed, the fibrous structure of electrospun mats conferred a white color to the product, while the typical yellowish shade of zein was maintained by casted and spin coated films. The addition of the brown extract at 15, 20, 35, and 45% (*w*/*w*) concentrations did not change significantly the color of electrospun film as shown in Figure 2d relating to the higher SCG concentration, while a more amber tonality was assumed by the casted and spin coated films by increasing the extract concentration, as shown in Figure 2e,f, referred to the samples loaded at 45% *w*/*w* of SCG extract.

3.3. Morphology Characterization

The macroscopic differences observed between the fibrous structures obtained by electrospinning and the continuous structures provided by solvent casting and spin coating is shown by the SEM images reproduced in Figure 3, which shows the three representative unloaded samples.

In particular, electrospinning produced mats composed of a network of randomly arranged fibers, while dense films were obtained by the other techniques tested. However, spin coating provided a regular rugosity of the surface (Figure 3c), due to the support rotation, compared to casted samples (Figure 3b) where superficial random defects were noticed.

The details of the films thickness and specific weight, as a function of the technique, are reported in Table 1. The lower specific weight of the electrospun films is due to the greater surface of the collector (25 cm × 25 cm) compared to the plates used for the other two techniques. The fiber deposition occurs randomly due to the static collector, affecting the thickness uniformity as is evident from the standard deviations.

Table 1. Thickness and specific weight of the zein samples produced by electrospinning, solvent casting, and spin coating by varying the SCG extract concentration.

SCG Extract% *w/w*	ELECTROSPINNING		SOLVENT CASTING		SPIN COATING	
	Thickness (μm)	Specific Weight (g/m^2)	Thickness (μm)	Specific Weight (g/m^2)	Thickness (μm)	Specific Weight (g/m^2)
0%	54.0 ± 7.52	33.37 ± 0.63	51.23 ± 9.47	73.23 ± 1.51	55.80 ± 3.24	61.22 ± 0.96
15%	35.8 ± 2.95	35.12 ± 10.6	37.27 ± 7.28	76.34 ± 6.38	53.50 ± 3.11	62.86 ± 2.89
20%	27.4 ± 6.43	26.50 ± 3.89	37.93 ± 9.49	80.62 ± 1.03	54.25 ± 2.98	63.26 ± 2.50
35%	31.2 ± 4.44	23.92 ± 4.33	43.57 ± 11.47	93.29 ± 0.92	50.75 ± 1.50	46.24 ± 1.04
45%	14.4 ± 3.36	19.47 ± 3.30	50.80 ± 11.48	66.96 ± 5.08	51.28 ± 3.33	46.29 ± 0.56

The results are expressed as mean value ± standard deviation.

Regarding the samples loaded with the extract, its presence tended to decrease the thickness of electrospun samples. This effect also influenced the micrometric and submicrometric fiber size. Indeed, as seen in Figure 4, increasing the extract loading up to 20% *w/w*, the fiber mean size remained quite constant (1.10 ± 0.40 μm for 0% *w/w*, 1.46 ± 0.70 μm for 15% *w/w*, and 1.44 ± 0.53 μm for 20% *w/w* of extract loading), while a lower fiber mean diameter was detected at the higher loadings, i.e., 0.88 ± 0.38 μm and 0.72 ± 0.19 μm, respectively, at 35 and 45% *w/w*.

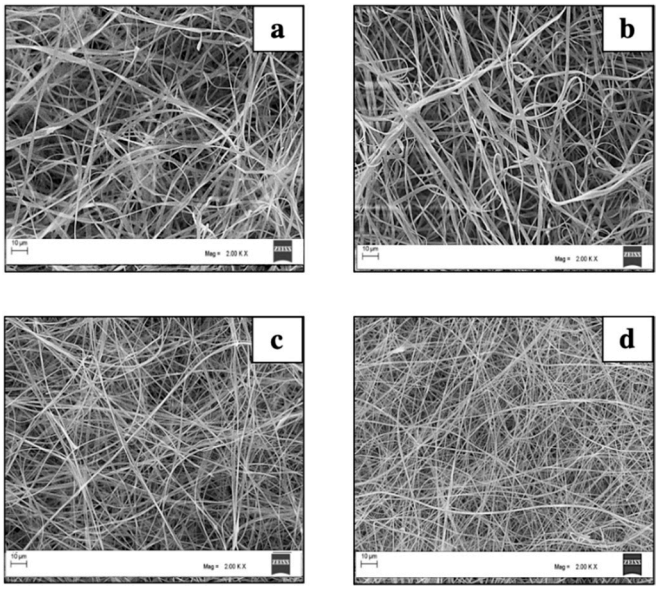

Figure 4. SEM images, at the same magnification (×2000), of electrospun zein fibers loaded with (**a**) 15%, (**b**) 20%, (**c**) 35%, (**d**) 45% SCG extract.

Furthermore, the shape of the fibers changes as the concentration of the extract increases, passing from a ribbon-like structure, typical of the reference sample, to a more filiform one.

No remarkable effects as a function of the SCG extract increasing was noticed for continuous samples produced by casting and spin coating. The last process outcome was more reproducible due to the macroscopic characteristics of the film, such as the thickness of the film and the specific weight, due to the equalizing effect provided by the rotation of the support.

3.4. Release Test

The samples produced were compared for the release of active ingredients at room temperature (25 °C). Such tests were performed by the complete immersion of samples in ethanol 10% (*v/v*) as a food simulant.

Figure 5a shows the caffeine migration from the polymer obtained by casting to the liquid medium over time, reported as mg of migrated caffeine per kg of polymer. In the 48 h of test, a continuous incremental release can be observed, which tended to a plateau not reached during the experimental time. The amount of caffeine released in a fixed time was strongly dependent on the extract loaded into the polymeric film.

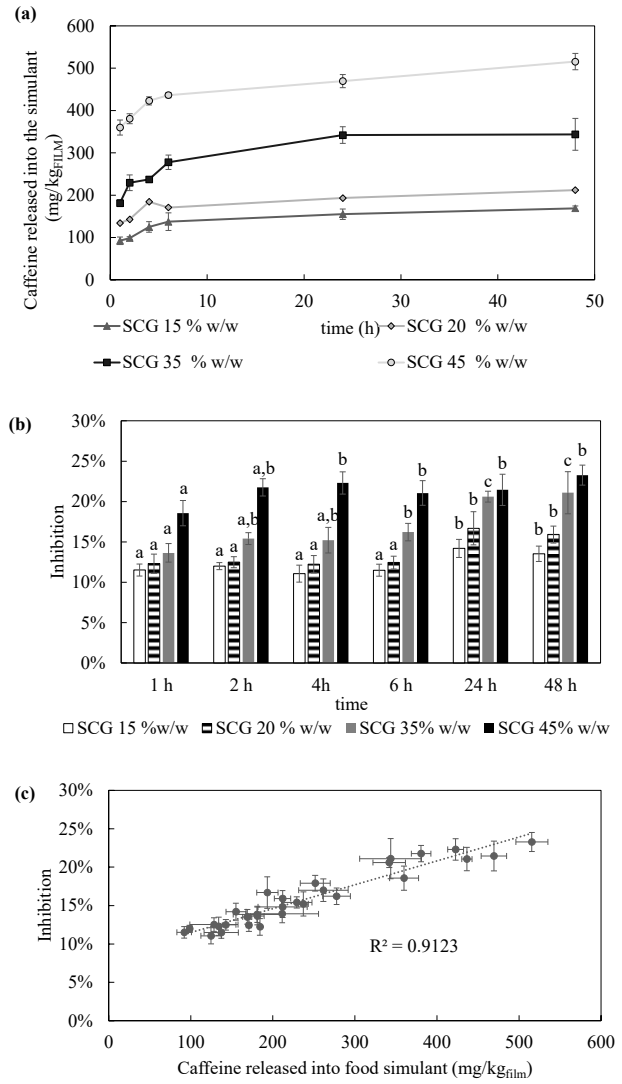

Figure 5. Results of the release tests on films obtained by solvent casting in ethanol 10% (v/v), in terms of: (**a**) caffeine released per kg of film, (**b**) radical ABTS$^{\bullet+}$ inhibition, and (**c**) linear correlation between the caffeine concentration released and radical inhibition power of the extract migrated into the food simulant. Different symbols indicate statistically significant differences among values over time ($p < 0.05$) of the dataset with the same extract loading.

Figure 5b reports the radical ABTS$^{\bullet+}$ inhibition exerted by aliquots of the food simulant sampled over time, which showed antiradical power due to the extract release.

As a general observation, the higher was the extract loading, the higher was the ability to scavenge the radical in a fixed time. Moreover, at the lowest extract loadings (15 and 20%, w/w), the inhibition values remained as constant up to 6 h, while significant increase was observed after 24 h. Conversely, at the highest extract loadings (35 and 45%, w/w), in 2 h was recorded a significant increase in the inhibition action, which maintains a constant value in the following observed time. The antiradical power determined by the ABTS assay was not related to the caffeine concentration, whose antioxidant activity cannot be detected by the employed colorimetric method but to the other antioxidant compounds,

mainly polyphenols and melanoidins [38,39]. Figure 5c shows that a linear correlation (R^2 = 0.9123) can be found between the caffeine concentration released and the inhibition power, demonstrating that caffeine can be used as a marker for the entire extract behavior for what concerns the migration into the selected simulant.

Films obtained by spin coating showed a faster caffeine release than casted packaging, especially in the first hours of release. Dependence on the concentration of the loaded SCG extract was not regular as for casted films (Figure 6a), especially for samples loaded with the 20% *w*/*w* concentration of extract, which exhibited fast delivery of caffeine after 6 h, reaching the performance of samples with 45% *w*/*w* of extract. The spin coating process, involving centrifugal forces during the fabrication, could have provided an extract distribution near the external surface, which could explain the faster migration compared to solvent casting samples. The higher rate of extract release was also confirmed by the greater inhibition effect of aliquots of food simulant tested by ABTS assay (Figure 6b). Indeed, only at the lowest concentration of extract loaded, inhibition observed was comparable with that of casted samples, while for loadings higher than 15% *w*/*w*, the observed values were immediately above 40%, increasing up to 4 h of release. Such fast migration made the extract available for degradation reactions, which explains the lower inhibition effect encountered at times longer than 4 h. Moreover, plasticizing effects are associated to the introduction of phenolic compounds and lipids into biopolymer-based packaging [40,41]. The different distribution of loaded compounds can have affected the overall structure of the films and the release of the active agents. The different behavior encountered for casted and spin coated films was also observed by López-Rubio et al., 2020, who compared the two production processes in the fabrication of amaranth protein isolate-based thin films, finding a strong dependance of the physical properties of the product on the production method [42]. Consequently, the relationship between the released caffeine (and extract) and inhibition power was no longer linear, as for solvent casting, indicating the occurrence of activity losses of the antioxidants.

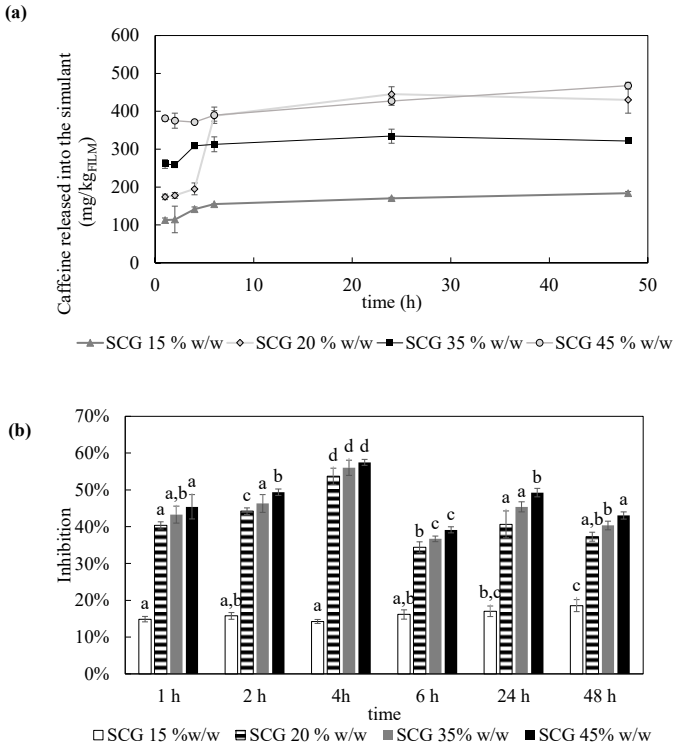

Figure 6. Results of the release tests on films obtained by spin coating in ethanol 10% (*v*/*v*), in terms of: (**a**) caffeine released per kg of film, (**b**) radical $ABTS^{\bullet+}$ inhibition. Different symbols indicate statistically significant differences among values over time ($p < 0.05$) of dataset with the same extract loading.

Electrospun mats are characterized by a fibrous structure, which facilitates the release of active components due to the larger surface available for mass transfer. Consequently, the released mass of caffeine plateaued very fast (Figure 7a) for all the extract loadings tested. Therefore, the extract was almost completely delivered in the early hours, implying its availability to degradation reactions, as can be observed through Figure 7b, where the decrease over time of the inhibition ability is shown for all the tested samples.

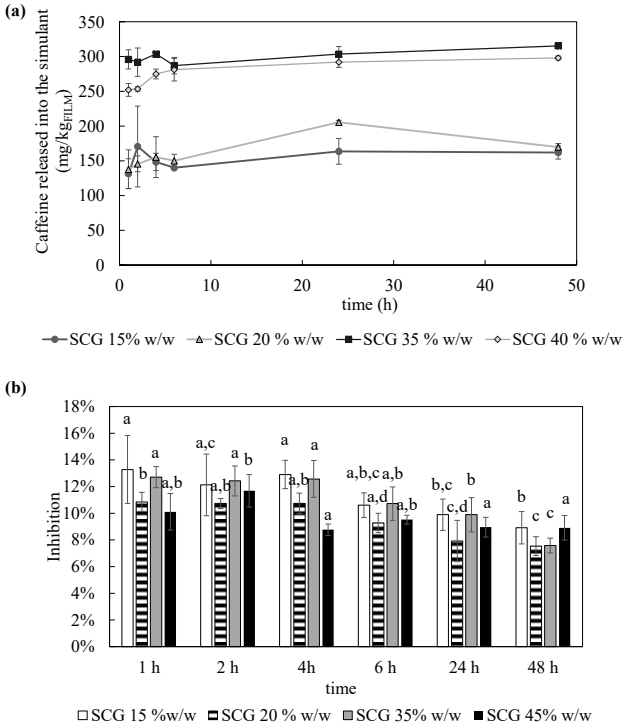

Figure 7. Results of the release tests on films obtained by electrospinning in ethanol 10% (*v*/*v*), in terms of: (**a**) caffeine released per kg of film, (**b**) radical ABTS$^{\bullet+}$ inhibition. Different symbols indicate statistically significant differences among values over time ($p < 0.05$) of dataset with the same extract loading.

This evidence shows that the technique adopted for loaded film production can affect the properties of the active system, allowing the most sustained release in solvent casted packaging and the fastest in electrospun mats. The different production methods can be thus applied to tune the rate of migration for the active agent, according to the specific application, planned shelf-life of the food, and the velocity of food spoilage, and the degradation reaction. Active packaging by solvent casting ensure protection against food oxidation less intense initially but increasing and sustained over time, while electrospun mats allows an immediate and intense action to counteract oxidation for those foods that rapidly tend to modify their organoleptic properties. Furthermore, an intermediate action can be achieved by spin coated active films. This finding supported the importance of further investigations into the effect of the production technique on the antioxidant activity of active packaging, as also highlighted by Domínguez et al., 2018 [43].

3.5. Permeability Properties

In general, if the permeability of the active packaging to oxygen from the external environment increases, perishable foods will be more rapidly subjected to oxidative and degradative phenomena, including microbial proliferation, with a strong reduction of food shelf-life. On the other hand, for those products packaged in a modified atmosphere, the packaging must prevent a fast release of the inert gases from the environment surrounding food (having protecting effects on food) to the external environment.

The oxygen permeability measurements were initially carried out on pure polymer samples, to conduct a feasibility study of the measurements. The zein films made by

electrospinning, being porous materials, were found to be completely transparent to the gas flow. The tests carried out on the samples produced by solvent casting, on the other hand, were strongly affected by the inhomogeneity of thickness. Therefore, after having optimized the last aspect by associating the solvent casting technique with that of spin coating, the oxygen permeability measurements were carried out systematically on these latter samples. The OPC and OTR were measured from the pressure rise in a known volume applying 1 atm of differential pressure of O_2 across the zein film at different percentual composition of SCG, from 0 to 45% [30]. In Table 2 the results are reported for films produced by spin coating with zein enriched with different concentration of SCG.

Table 2. OPC and OTR measurements results for different packaging films at different SCG concentration, from 0% to 45%.

Measurement	SCG 0%	SCG 15%	SCG 20%	SCG 35%	SCG 45%
OPC ($m_{STP}{}^3$ m/(m^2 s Pa)) × 10^{20}	470 ± 70	200 ± 30	5.8 ± 0.9	1.4 ± 0.2	4.9 ± 0.7
OTR ($m_{STP}{}^3/m^2$ s) × 10^{11}	940 ± 122	480 ± 62	20 ± 2.6	4.7 ± 0.6	16 ± 2
OTR (cc/m^2 day)	202 ± 26	86 ± 11	25 ± 3	6 ± 0.8	21 ± 3

Units' conversion: 1 $m_{STP}{}^3$ m/(m^2 s Pa) = 4.4 × 10^3 mol/(m s Pa), 1 $m_{STP}{}^3$/s = 4.4 × 10^3 mol/(m^2 s) = 2.2 × 10^{10} cc/(m^2 day).

In Table 2, OPC is reported in $m_{STP}{}^3$ m/(m^2 s Pa), OTR in $m_{STP}{}^3/m^2$ s and cc/(m^2 day) as they are units most often used in film packaging technology. However, a unit conversion in SI is reported as the Table note. As for all polymers, zein suffers from physical aging depending on the quantities of the adsorbed gas, especially for condensable gases such as CO_2 or O_2 that can induce plasticization. Plasticization is based upon molecular chain reorganization of a polymer induced by high sorption of a condensable gas [44]. This phenomenon ensues instability of the permeation properties over time. Considering the experimental procedure adopted, the obtained data are free from artefacts with respect to the physical aging of zein [45].

The value of OPC for pure zein film agrees with literature results [45]. As expected, these values decrease by increasing the percentage of SCG extract, consequently improving the oxygen barrier action. The trend changes increasing quantity of extract over 35%. At values equal or higher than 45% w/w, it is possible that the quantity of SCG extract generates a swelling of the polymer allowing it to increase gas diffusion.

Comparing our permeability data with those of other materials often used in food packaging, such as low-density polyethylene (LDPE) or Polyethylene terephthalate (PET), the spin coating films can provide an equal, or increased oxygen barrier [46].

3.6. Mechanical Properties

The mechanical performances of the zein films were evaluated through the characterization of tensile strength, elongation at break, and Young's modulus values. The films obtained from electrospinning showed evident brittleness in handling, a fact confirmed by other authors who have investigated how to improve the mechanical properties of electrospun materials deriving from natural proteins, for example by using polymeric blends [47]. For this reason, the samples obtained by electrospinning were not subjected to mechanical tests. Instead, the mechanical properties were evaluated for continuous samples produced through the other two techniques. The results of the tests conducted on the samples produced by solvent casting are shown in Table 3.

Table 3. Mechanical properties of zein samples produced by solvent casting in terms of tensile strength (TS), elongation at break (EB), and Young's modulus (YM) values.

SCG Extract % *w*/*w*	TS (MPa)	EB (%)	YM (MPa)
0%	13.04 ± 3.10	0.63 ± 0.13	1860.12 ± 368.22
15%	10.64 ± 2.06	0.92 ± 0.32	1403.52 ± 456.16
20%	14.09 ± 1.76	0.59 ± 0.12	1846.83 ± 237.96
35%	13.66 ± 1.61	0.76 ± 0.20	1895.32 ± 168.18
45%	14.21 ± 3.99	0.58 ± 0.15	2128.25 ± 661.24

The results are expressed as mean value ± standard deviation.

In particular, all samples had a tensile strength higher than those reported in other works and higher than the limit set for packaging materials equal to 3.5 MPa [48,49]. Moreover, all the films presented poor elongation at break and high Young's modulus values. It is interesting to note that the presence of the SCG extract did not particularly affect the results. Only at 45% *w*/*w* of SCG extract were the mechanical properties generally worse, in fact the elongation at break was lower, indicating greater fragility of the samples, while the Young's modulus value was higher, indicating greater rigidity of the material. The reason can be attributed to the presence of a higher dry solid fraction of extract inside the samples, compared to the extract loaded in lower concentrations, which involves the addition of irregularities in the material with a consequent decrease in mechanical performance. This brittleness, which is known to be the greatest intrinsic weakness in vegetable proteins, could also be attributed to the amount of glycerol used. In fact, it has been studied by Zhang et al., 2015, that glycerol can act both as an anti-plasticizer, worsening the mechanical properties and as a plasticizer, improving them depending on the quantity used [50].

Furthermore, as regards the films obtained by spin coating, the blank samples of zein were preliminarily compared with those produced by solvent casting, to investigate the influence of the production technique on the mechanical properties and it emerged that the spin coated samples did not meet the minimum requirements, having presented a tensile strength of 1.13 ± 0.18 MPa, while the elongation at break was comparable with solvent castings 0.76 ± 0.12% and the Young's modulus value was lower and equal to 152.05 ± 16.76 MPa. These results are clearly different from those of the samples obtained by casting, and it could be because of the centrifugal force imparted by the rotation of the spin coater support on the arrangement of both polymer and plasticizer molecules. Furthermore, unlike solvent casting, for which the evaporation of the solvent occurs at a constant temperature of 60 °C, in the case of spin coating, the evaporation begins during the deposition of the solution on the rotating support at room temperature and then ends in an oven at 60 °C. Hence, the different evaporation rates and conditions evidently negatively affected the mechanical properties of the samples produced by spin coating.

4. Conclusions

Concluding, a novel bio-active material composed of zein and SCG extract derived antioxidant molecules can be produced combining a high pressure and temperature extraction method and innovative film forming processes.

The films produced by solvent casting and spin coating, given their transparency and the greater mechanical and barrier properties compared to electrospun samples, could be used as primary packaging materials or as an internal layer in a multilayer film to carry out the antioxidant action on food with lipidic composition. In contrast, the materials produced by electrospinning could be used as antioxidant patches to be inserted inside the packages to prevent degradation phenomena, such as in packages of red fruits, on hamburgers or in packages of chopped fruit with a shelf-life of a few hours. As reported in the work, the different production methods tested can be applied to tune the rate of migration of the active agent, according to the specific application, planned shelf-life of the food, and the speed of food spoilage and degradation reactions. Active packaging made

by solvent casting ensures protection against food oxidation is less intense initially but increases and is sustained over time, whereas electrospun mats allows an immediate and intense action to counteract oxidation for those foods that rapidly tend to modify their organoleptic properties. Furthermore, an intermediate action can be achieved by spin coated active films.

Therefore, this work allows the identification of the potential and limits of three different film manufacturing techniques to produce antioxidant food packaging materials from agri-food wastes using green solvents. Furthermore, it has demonstrated their potential as alternative materials to non-degradable plastics and synthetic additives. Finally, this study demonstrated how by varying the production process, starting from the same raw materials, properties of the product can be drastically changed and adapted according to the intended specific application.

These materials, being made using not only a biopolymer of natural origin but also natural compounds deriving from food waste, green solvents, and green processes, would allow contributing to the reduction of the volumes of plastic waste. Currently, the packaging sector contributes enormously to such waste and current management, especially in the agri-food sector, leads to huge greenhouse gases emissions and to pollution of soil and water. Therefore, active packaging that can be obtained from natural biopolymers combined with natural antioxidant agents, can counteract and slow down food wastage, which accounts for about 1.3 billion tons of waste per year of the total 1.6 billion tons of waste per year produced by the agri-food sector along the whole supply chain.

Author Contributions: Conceptualization, R.C. and G.F.; methodology, E.D., M.P., G.F. and E.L.; formal analysis, E.D. and M.P.; investigation, E.D. and M.P.; resources, R.C. and P.P.; data curation, E.D. and M.P.; writing—original draft preparation, E.D. and M.P.; writing—review and editing, E.D., M.P. and R.C.; visualization, P.P., R.C. and G.F.; supervision, P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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

References

1. Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J.H.; Abu-Omar, M.; Scott, S.L.; Suh, S. Degradation Rates of Plastics in the Environment. *ACS Sustain. Chem. Eng.* **2020**, *8*, 3494–3511. [CrossRef]
2. Kabir, E.; Kaur, R.; Lee, J.; Kim, K.H.; Kwon, E.E. Prospect of biopolymer technology as an alternative option for non-degradable plastics and sustainable management of plastic wastes. *J. Clean. Prod.* **2020**, *258*, 120536. [CrossRef]
3. Sharma, V.; Sehgal, R.; Gupta, R. Polyhydroxyalkanoate (PHA): Properties and Modifications. *Polymer* **2021**, *212*, 123161. [CrossRef]
4. Ding, Y.; Zhou, Q.; Han, A.; Zhou, H.; Chen, R.; Guo, S. Fabrication of Poly(ε-caprolactone)-Based Biodegradable Packaging Materials with High Water Vapor Barrier Property. *Ind. Eng. Chem. Res.* **2020**, *59*, 22163–22172. [CrossRef]
5. Nechita, P.; Roman, M. Review on Polysaccharides Used in Coatings for Food Packaging Papers. *Coatings* **2020**, *10*, 566. [CrossRef]
6. Campardelli, R.; Pettinato, M.; Drago, E.; Perego, P. Production of Vanillin-Loaded Zein Sub-micron Electrospun Fibers for Food Packaging Applications. *Chem. Eng. Technol.* **2021**, *44*, 1–8. [CrossRef]
7. Vinod, A.; Sanjay, M.R.; Suchart, S.; Jyotishkumar, P. Renewable and sustainable biobased materials: An assessment on biofibers, biofilms, biopolymers and biocomposites. *J. Clean. Prod.* **2020**, *258*, 120978. [CrossRef]
8. Yu, H.; Li, W.; Liu, X.; Li, C.; Ni, H.; Wang, X.; Huselstein, C.; Chen, Y. Improvement of functionality after chitosan-modified zein biocomposites. *J. Biomater. Sci. Polym. Ed.* **2016**, *28*, 1–15. [CrossRef]
9. Yuan, Y.; Zhang, X.; Pan, Z.; Xue, Q.; Wu, Y.; Li, Y.; Li, B.; Li, L. Improving the properties of chitosan films by incorporating shellac nanoparticles. *Food Hydrocoll.* **2021**, *110*, 106164. [CrossRef]
10. Zubair, M.; Ullah, A. Recent advances in protein derived bionanocomposites for food packaging applications. *Crit. Rev. Food Sci. Nutr.* **2019**, *60*, 1–29. [CrossRef]
11. Salević, A.; Stojanović, D.; Lević, S.; Pantić, M.; Đordević, V.; Pešić, R.; Bugarski, B.; Pavlović, V.; Uskoković, P.; Nedović, V. The Structuring of Sage (Salvia officinalis L.) Extract-Incorporating Edible Zein-Based Materials with Antioxidant and Antibacterial Functionality by Solvent Casting versus Electrospinning. *Foods* **2022**, *11*, 390. [CrossRef] [PubMed]
12. Sedlarikova, J.; Janalikova, M.; Peer, P.; Pavlatkova, L.; Minarik, A.; Pleva, P. Zein-Based Films Containing Monolaurin/Eugenol or Essential Oils with Potential for Bioactive Packaging Application. *Int. J. Mol. Sci.* **2022**, *23*, 384. [CrossRef] [PubMed]

13. Troilo, M.; Difonzo, G.; Paradiso, V.M.; Summo, C.; Caponio, F. Bioactive Compounds from Vine Shoots, Grape Stalks, and Wine Lees: Their Potential Use in Agro-Food Chain. *Foods* **2021**, *10*, 342. [CrossRef] [PubMed]
14. Pettinato, M.; Casazza, A.A.; Ferrari, P.F.; Palombo, D.; Perego, P. Eco-sustainable recovery of antioxidants from spent coffee grounds by microwave-assisted extraction: Process optimization, kinetic modeling and biological validation. *Food Bioprod. Process.* **2019**, *114*, 31–42. [CrossRef]
15. Nonthakaew, A.; Matan, N.; Aewsiri, T.; Matan, N. Antifungal Activity of Crude Extracts of Coffee and Spent Coffee Ground on Areca Palm Leaf Sheath (Areca catechu) Based Food Packaging. *Packag. Technol. Sci.* **2015**, *28*, 633–645. [CrossRef]
16. Ounkaew, A.; Kasemsiri, P.; Kamwilaisak, K.; Saengprachatanarug, K.; Mongkolthanaruk, W.; Souvanh, M.; Pongsa, U.; Chindaprasirt, P. Polyvinyl Alcohol (PVA)/Starch Bioactive Packaging Film Enriched with Antioxidants from Spent Coffee Ground and Citric Acid. *J. Polym. Environ.* **2018**, *26*, 3762–3772. [CrossRef]
17. Cacciotti, I.; Mori, S.; Cherubini, V.; Nanni, F. Eco-sustainable systems based on poly(lactic acid), diatomite and coffee grounds extract for food packaging. *Int. J. Biol. Macromol.* **2018**, *112*, 567–575. [CrossRef]
18. Getachew, A.T.; Ahmad, R.; Park, J.S.; Chun, B.S. Fish skin gelatin based packaging films functionalized by subcritical water extract from spent coffee ground. *Food Packag. Shelf Life* **2021**, *29*, 100735. [CrossRef]
19. Fang, Y.; Jiang, Z.; Zhao, X.; Dong, J.; Li, X.; Zhang, Q. Spent coffee Grounds/Poly(butylene succinate) biocomposites with Robust mechanical property and heat resistance via reactive compatibilization. *Compos. Commun.* **2022**, *29*, 101003. [CrossRef]
20. Ansarifar, E.; Moradinezhad, F. Encapsulation of thyme essential oil using electrospun zein fibers for strawberry preservation. *Chem. Biol. Technol. Agric.* **2022**, *9*, 1–11. [CrossRef]
21. Neo, Y.P.; Swift, S.; Ray, S.; Gizdavic-Nikolaidis, M.; Jin, J.; Perera, C.O. Evaluation of gallic acid loaded zein sub-micron electrospun fibre mats as novel active packaging materials. *Food Chem.* **2013**, *141*, 3192–3200. [CrossRef] [PubMed]
22. Altan, A.; Çayir, Ö. Encapsulation of carvacrol into ultrafine fibrous zein films via electrospinning for active packaging. *Food Packag. Shelf Life* **2020**, *26*, 100581. [CrossRef]
23. Pavlátková, L.; Sedlaríková, J.; Pleva, P.; Peer, P.; Uysal-Unalan, I.; Janalíková, M. Bioactive zein/chitosan systems loaded with essential oils for food-packaging applications. *J. Sci. Foo Agric.* **2022**. [CrossRef]
24. Fernandes da Silva, M.; Pettinato, M.; Casazza, A.A.; Sucupira Maciel, M.I.; Perego, P. Design and evaluation of non-conventional extraction for bioactive compounds recovery from spent coffee (*Coffea arabica* L.) grounds. *Chem. Eng. Res. Des.* **2022**, *177*, 418–430. [CrossRef]
25. Pettinato, M.; Trucillo, P.; Campardelli, R.; Perego, P.; Reverchon, E. Bioactives extraction from spent coffee grounds and liposome encapsulation by a combination of green technologies. *Chem. Eng. Process. Process Intensif.* **2020**, *151*, 107911. [CrossRef]
26. Pettinato, M.; Drago, E.; Campardelli, R.; Perego, P. Spent Coffee Grounds Extract for Active Packaging Production. *Chem. Eng. Trans.* **2021**, *87*, 583–588. [CrossRef]
27. The European Commission. European Commission Regulation 2016/1416 of august 24, 2016 amending and correcting Regulation (EU) no. 10/2011 concerning plastic materials and objects intended to come into contact with food. *Off. J. Eur. Union* **2016**, *230*, 22–42.
28. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 9–10. [CrossRef]
29. Firpo, G.; Setina, J.; Angeli, E.; Repetto, L.; Valbusa, U. High-vacuum setup for permeability and diffusivity measurements by membrane techniques. *Vacuum* **2021**, *191*, 110368. [CrossRef]
30. ASTM Standard Test Method for Determining Gas Permeability Characteristics of Plastic Film and Sheeting. ASTM International Designation: D1434-82 (Reapproved 2015). [CrossRef]
31. *ISO 527-1:2012*; Plastics-Determination of Tensile Properties-Part 1: Test General Principles. International Organization for Standardization: Geneva, Switzerland, 2012.
32. Mussatto, S.I.; Ballesteros, L.F.; Martins, S.; Teixeira, J.A. Extraction of antioxidant phenolic compounds from spent coffee grounds. *Sep. Purif. Technol.* **2011**, *83*, 173–179. [CrossRef]
33. Ramón-Gonçalves, M.; Gómez-Mejía, E.; Rosales-Conrado, N.; León-González, M.E.; Madrid, Y. Extraction, identification and quantification of polyphenols from spent coffee grounds by chromatographic methods and chemometric analyses. *Waste Manag.* **2019**, *96*, 15–24. [CrossRef] [PubMed]
34. de Cosío-Barrón, A.C.G.; Hernández-Arriaga, A.M.; Campos-Vega, R. Spent coffee (*Coffea arabica* L.) grounds positively modulate indicators of colonic microbial activity. *Innov. Food Sci. Emerg. Technol.* **2020**, *60*, 102286. [CrossRef]
35. Bandyopadhyay, P.; Ghosh, A.K.; Ghosh, C. Recent developments on polyphenol–protein interactions: Effects on tea and coffee taste, antioxidant properties and the digestive system. *Food Funct.* **2012**, *3*, 592. [CrossRef] [PubMed]
36. Ballesteros, L.F.; Teixeira, J.A.; Mussatto, S.I. Extraction of polysaccharides by autohydrolysis of spent coffee grounds and evaluation of their antioxidant activity. *Carbohydr. Polym.* **2016**, *157*, 258–266. [CrossRef]
37. Choi, B.; Koh, E. Spent coffee as a rich source of antioxidative compounds. *Food Sci. Biotechnol.* **2017**, *26*, 921–927. [CrossRef]
38. Brezová, V.; Šlebodová, A.; Staško, A. Coffee as a source of antioxidants: An EPR study. *Food Chem.* **2009**, *114*, 859–868. [CrossRef]
39. Petrucci, R.; Zollo, G.; Curulli, A.; Marrosu, G. A new insight into the oxidative mechanism of caffeine and related methylxanthines in aprotic medium: May caffeine be really considered as an antioxidant? *Biochim. Biophys. Acta Gen. Subj.* **2018**, *1862*, 1781–1789. [CrossRef]
40. Iskender, A.; Ahmet, Y. Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. *Food Res. Int.* **2011**, *44*, 550–556. [CrossRef]

41. Garcia, C.V.; Kim, Y.T. Spent Coffee Grounds and Coffee Silverskin as Potential Materials for Packaging: A Review. *J. Polym. Environ.* **2021**, *29*, 2372–2384. [CrossRef]
42. López-Rubio, A.; Blanco-Padilla, A.; Oksman, K.; Mendoza, S. Strategies to Improve the Properties of Amaranth Protein Isolate-Based Thin Films for Food Packaging Applications: Nano-Layering through Spin-Coating and Incorporation of Cellulose Nanocrystals. *J. Nanomater.* **2020**, *10*, 2564. [CrossRef] [PubMed]
43. Domínguez, R.; Barba, F.J.; Gómez, B.; Putnik, P.; Kovačević, D.B.; Pateiro, M.; Santos, E.M.; Lorenzo, J.M. Active packaging films with natural antioxidants to be used in meat industry: A review. *Food Res. Int.* **2018**, *113*, 93–101. [CrossRef] [PubMed]
44. Swaidan, R.; Ghanem, B.; Litwiller, E.; Pinnau, I. Physical Aging, Plasticization and Their Effects on Gas Permeation in "Rigid" Polymers of Intrinsic Microporosity. *Macromolecules* **2015**, *48*, 150829080103007. [CrossRef]
45. Seung, Y.C.; Seung, Y.L.; Chul, R. Edible oxygen barrier bilayer film pouches from corn zein and soy protein isolate for olive oil packaging. *LWT-Food Sci. Technol.* **2010**, *43*, 1234–1239. [CrossRef]
46. Mokwena, K.K.; Tang, J. Ethylene Vinyl Alcohol: A Review of Barrier Properties for Packaging Shelf Stable Foods. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 640–650. [CrossRef]
47. Mehri, K.; Milad, F.; Soleimanian-Zad, S. Incorporation of zein nanofibers produced by needle-less electrospinning within the casted gelatin film for improvement of its physical properties. *Food Bioprod. Process.* **2020**, *122*, 193–204. [CrossRef]
48. Bisharat, L.; Berardi, A.; Perinelli, D.R.; Bonacucina, G.; Casettari, L.; Cespi, M.; AlKhatib, H.S.; Palmieri, G.F. Aggregation of zein in aqueous ethanol dispersions: Effect on cast film properties. *Int. J. Biol. Macromol.* **2018**, *106*, 360–368. [CrossRef]
49. Hosseini, S.F.; Rezaei, M.; Zandi, M.; Farahmandghavi, F. Fabrication of bio-nanocomposite films based on fish gelatin reinforced with chitosan nanoparticles. *Food Hydrocol.* **2015**, *44*, 172–182. [CrossRef]
50. Zhang, Y.; Cui, L.; Che, X.; Zhang, H.; Shi, N.; Li, C.; Chen, Y.; Kong, W. Zein-based films and their usage for controlled delivery: Origin, classes and current landscape. *J. Control. Release* **2015**, *206*, 206–219. [CrossRef]