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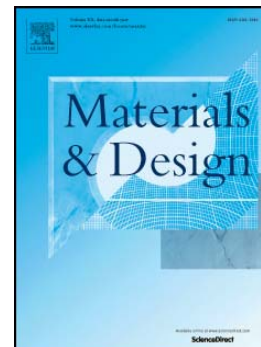
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**Preparation of plasticized Wheat Gluten/Olive Pomace powder
biocomposite: effect of powder content and chemical modifications**

Yasmina Hammoui^{a,*}, Sonia Molina-Boisseau^b, Antoine Duval^b, Nabila Djerrada^a, Nawel Adjeroud^a, Hocine Remini^a, Farid Dahmoune^{a,c}, Khodir Madani^a

^a *Laboratoire de Biomathématiques, Biophysique, Biochimie, et Scientométrie (L3BS), Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algérie*

^b *Univ. Grenoble Alpes, CNRS CERMAV, F-38000 Grenoble, France*

^c *Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Bouira, 10000 Bouira, Algérie*

* Laboratoire de Biomathématiques, Biophysique, Biochimie, et Scientométrie (L3BS), Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algérie

Corresponding author. Tel: +213 7 78 60 76 74

E-mail address: yasmina.hammoui@univ-bejaia.dz

ABSTRACT

During the last decades, a lot of interest was given to the biocomposites development, due to the low cost and degradability of waste food industries. In this study, biocomposites were developed from Wheat Gluten plasticized by 35% of glycerol, containing 0-20% of Clean Olive Pomace (COP), Esterified Clean Olive Pomace (ECOP), or Mercerized Clean Olive Pomace (MCOP) powders, which were prepared by conventional blending. Chemical modifications of the powders were characterized by Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and Thermogravimetric Analysis (TGA). Mechanical properties, water absorption, mass loss and thermal properties of biocomposites were detailed. It was found that thermal stability of powder was improved after mercerization. Incorporation of COP powder (20%) in plasticized wheat gluten (WG) improved significantly Young's modulus compared to that of WG films. Water absorption was decreased significantly at 20% of reinforcement and was 29% less than that of WG films. Young's modulus and tensile strength decreased at 5% of ECOP powder, contrary to MCOP biocomposite. Elongation at break of biocomposites was decreased with increasing powder content. Incorporation of COP powder (20%) decreased thermal stability of the biocomposite, nevertheless chemical changes did not affect the thermal stability of the ECOP and MCOP biocomposites.

Keywords: Wheat gluten, olive pomace powder, esterification, mercerization, mechanical properties.

1. Introduction

The petroleum depleting resources coupled with environmental compliance needs have ignited the interest in the use of renewable polymers from different resources for making new materials [1]. During the last few decades, extensive researches have been carried out [2], biodegradable materials from renewable agricultural resources such as carbohydrates, starch and proteins have attracted much attention for sustainable development and environmental conservation.[3]. Among candidate proteins, wheat gluten with its unique viscoelastic properties and its water insolubility is of particular interest [4]. It is a byproduct of the wheat starch industry that is commercially available at low cost in large quantities [5].

Protein fractions of gluten are traditionally classified according to their solubility in gliadins and glutenins, respectively, soluble in alcohol or diluted acid solutions. Gliadin is constituted of polypeptides in which cystein residues are connected by intra-chain disulfide bonds, glutenin is formed by discrete polypeptides (sub-units), which are linked together by inter-chain disulfide bonds to form high molecular-weight polymer [6]. Gluten-based materials can be prepared using common thermoplastic processing [7], usually by mixing. In this process, both mechanical shearing and heating are involved, gluten proteins is modified and undergoes an aggregation [8], then three-dimensional macromolecular network is formed [9]. As a plasticizer, glycerol is usually used to reduce intermolecular forces, it has high mobility and would dissociate some of the linkages within protein chains promoting hydrogen bonds between the plasticizer and the protein[10, 11]. The obtained films are excellent oxygen and carbon dioxide barriers at low relative humidity with selective permeability to gases [12]. Application of protein-based bioplastics is severely limited due to their lower stiffness, and their strong tendency to absorb moisture [3].

There are many approaches to improve the mechanical properties and water resistance of wheat gluten film [13], the use of agrowaste materials as a source of fillers or reinforcements provides a renewable source, and could generate a non-food source of economic development for farming and rural areas [14]. Among these materials, natural fibers were widely used as fillers, such as *Pine Needles* fiber [15] and *Saccharum Cilliare* fiber [16] Olive pomace is an industrial byproduct of the olive oil production process, obtained by squeezing the olive pulp without any chemical treatment [17]. In Algeria, huge amounts of olive pomace are generated; it represents 10^5 tons per year, this amount of agrowaste is usually burned [18].

These agricultural wastes possess a lot of industrial potential, but their value in non-food area and bioprocessing is still underestimated.

Olive pomace contains a great amount of cellulose, hemicelluloses, lignin [19], and oily wastes and phytotoxic substances such as polyphenolic compounds [20]. Different surface modification techniques of lignocellulosic filler were used, as graft copolymerization [21], chemical treatments with maleic anhydride [22] and alkali treatment [23] were applied to promote a strong interface adhesion between polymers and fillers.

The present work aims to elaborate biocomposites based on plasticized wheat gluten with glycerol containing Clean Olive Pomace (COP), Esterified Clean Olive Pomace (ECOP) or Mercerized Clean Olive Pomace (MCOP) powders obtained by conventional blending. The biocomposite properties in function of COP, ECOP or MCOP powder contents was studied. The chemical modifications efficiency (either with maleic anhydride or with sodium hydroxide) on powders was characterized by Fourier Transform Infrared (FTIR) spectroscopy, Particle Size Distribution (PSD), Scanning Electron Microscopy (SEM), and Thermogravimetric Analysis (TGA). Cryo-fractured surface of selected biocomposites was observed using SEM. The mechanical properties, water absorption, micropore ratio, mass loss and thermal properties were investigated.

2. Experimental work

2.1. Chemicals and reagents

Analytical grade glycerol ($\geq 99\%$), sodium azide ($\geq 99\%$) with FTIR grade potassium bromide (KBr) ($\geq 99\%$) were purchased from Sigma Aldrich (Saint-Louis, United States). Maleic anhydride ($\geq 99\%$), acetone ($\geq 99\%$) sodium hydroxide pellets were purchased from Biochem Chemopharma (Cosne-Cours-sur-Loire, France). Acetic acid (100%) was purchased from VWR International (Pennsylvanie, United States).

2.2. Raw materials

Wheat gluten was obtained from Tereos Syral (Marckolsheim, France). Chemlal Olive Pomace (OP) was obtained from a local olive refinery in the area of Fenaia-Ilmaten (Bejaia, North-east of Algeria), and washed with hot tap water to remove all water-soluble impurities, followed by drying at room temperature. The product was ground using an electrical grinder (IKA model-A11, Staufen, Germany) and was sieved using standard 125 μm sieve. Olive

Pomace (OP) powder was washed with organic solvent (acetone) using soxhlet extractor (Behrotest model-R106S, Düsseldorf, Germany) for 16 h to remove remaining organic impurities. The recuperated Clean Olive Pomace (COP) powder was dried in oven at 105 °C until constant weight was obtained. COP powder will be used for chemical modifications and manufacturing of biocomposites. The moisture content of the powders was measured by thermal drying method at 105 ± 2 °C for 3 h, it was respectively 6.2% for OP and 4.4% for COP powders.

2.3. Particle size distribution (PSD)

The particle size distribution of powders were determined using a laser-light diffraction unit (Mastersizer S, Malvern Instruments Ltd., Worcestershire, UK) equipped with 300 RF lens. The diameters $D(v, 0.10)$, $D(v, 0.50)$ and $D(v, 0.9)$ at 10% (small particles), 50% (medium size particle), 90% (large coarse particle), respectively and volume mean diameter ($D[4.3]$) were computed. A one-way analysis of variance (ANOVA) followed by a Tukey's test were performed using JMP (Ver. 7, SAS, North Carolina, USA) software to show if there is significant differences between COP, ECOP and MCOP powders in term of their particle size distribution ($p < 0.05$).

2.4. Chemical treatments

2.4.1. Esterification by maleic anhydride

According to Cantero et al. [22], COP powder (1 g) was esterified with 25 ml of maleic anhydride at 5% (w/v) (dissolved in boiling acetone at 50 °C), during 25 h. Then, the product was washed several times with acetone followed by distilled water ($0.5 \mu\text{S cm}^{-1}$) and then dried in the oven at 105 °C until constant weight. The powder obtained was labelled Esterified Clean Olive Pomace (ECOP).

2.4.2. Mercerization by sodium hydroxide at room temperature

COP powder (1 g) was dipped in 10% (w/v) of NaOH solution (50ml) at 25 °C for 24 h. Mercerized Clean Olive Pomace (MCOP) powder was neutralized with 5% (v/v) of acetic acid solution and thoroughly washed with distilled water. Then, MCOP powder was dried at 105 °C until constant weight.

2.5. FTIR spectroscopy characterization of the powders

Fourier Transform Infrared (FTIR) spectrometer (Perkin-Elmer Paragon 1000, Waltham, United States), equipped with Spectrum V 5.0.1 software (Waltham, United States) was used to characterize COP, ECOP and MCOP powders. The KBr pellets of samples were prepared by mixing 1 mg of sample (dried previously overnight in oven at 105 °C), with 100 mg KBr, and the prepared pellets were stored in oven at 60 °C before analysis. Spectra were recorded in the range of 400–4000 cm^{-1} using 32 scans with a resolution of 4 cm^{-1} .

2.6. Preparation of biocomposites

Processing of Duval et al. [24] was adopted to elaborate biocomposites, wheat gluten with dried COP, ECOP or MCOP powders were firstly hand mixed to the desired proportions (Table 1). Then, the resulting powder was mixed with glycerol (35%, based on total dry weight) in a two blade counter-rotating batch mixer, turning at 3:2 differential speed (Brabender, Duisburg, Germany). The mixture was performed by mixing at a speed of 100 rpm during 15 min at 70 °C. Glycerol was introduced into the chamber, as it provides more homogeneous blends. The blends were then thermo-moulded in a heated press (Carver hot press model-2629, Wabash, United States) at 120 °C. Approximately, 4 g of the blends were placed between two aluminum sheets in a rectangular mold (80 × 40 mm) for 10 min without pressure, followed by 3 min under a pressure of 15 MPa. Then they were removed from the mold and cooled at room temperature. The thickness of the resulting films was approximately 0.5 mm. Prior to tensile test, the films were conditioned into a desiccator producing 43% relative humidity at 24 °C for one week.

Table. 1

2.7. Scanning electron microscopy (SEM)

The micrographs of morphology observation of COP, ECOP and MCOP powders, WG films and biocomposites (OPC_20, OPCE_20, OPCM_20), were obtained using 8 kV secondary electrons microscopy (JEOL JSM-6100, Tokyo, Japan). Each material sample was frozen under liquid nitrogen and fractured. All samples (powders and cryo-fractured biocomposites) were coated with gold/palladium on a JEOL JFC-1100E ion sputter coater (Tokyo, Japan) before observation.

2.8. Micropore ratio

The micropore ratio (%) of biocomposites were derived from images processing of three similar images in term of magnifications ($\times 250$), and contrast of each films by ImageJ software (ver. 1.49, NIH, Maryland, USA) and the results was reported as mean \pm standard deviations (S.D). The statistical analysis with one-way analysis of variance (ANOVA) followed by Tukey's test using JMP software (Ver. 7, SAS, North Carolina, USA), were used to show if there is significant differences between COP, ECOP and MCOP powders in term of their micropore ratio ($p < 0.05$).

2.9. Tensile test

Tensile test was performed using universal testing machine (AGS-X, Kyoto, Japan) equipped with trapezium x software (Kyoto, Japan), equipped with a 500 N load cell at room temperature and with crosshead speed of 10 mm min^{-1} . Before testing, biocomposites containing 0 to 20% of COP, ECOP or MCOP powders were cut into dumbbell-shaped ($10 \times 5 \text{ mm}$), the thickness was measured in three different places with a caliper. Young's modulus, tensile strength and elongation at break were evaluated from eight replicates for each sample, the mean value and standard deviation were determined for each of these parameters. A one-way analysis of variance (ANOVA) followed by a Tukey's test using JMP software (Ver 7, SAS, North Carolina, USA), were used to show if there is significant differences between COP, ECOP and MCOP biocomposites in term of Young's modulus, tensile strength and elongation at break. Evaluations were based on the $p < 0.05$ significance level.

2.10. Water absorption and Mass loss

Before water absorption tests, all biocomposite samples containing 0 to 20% of COP, ECOP or MCOP powders were cut into square form at approximately $10 \times 10 \text{ mm}$, and then placed in desiccator containing silica gel until dryness. Three replicates for each sample with near weight (W_i) were selected, and immersed in flasks containing 50 ml of distilled water (containing 0.05% 'w/v' of sodium azide) at $25 \text{ }^\circ\text{C}$. The swollen samples were carefully wiped and weighed (W_{eq}), then they were dried over silica gel in desiccator until constant weight (W_f) and mass losses were calculated. The statistical analysis was assessed according to one-way analysis of variance (ANOVA) followed by a Turkey's test using JMP software (Ver 7, SAS, North Carolina, USA). Evaluations were based on the $p < 0.05$ significance

level. The Water Absorption (%) and Mass Loss (%) in water were evaluated by Eq.(1) and Eq.(2), respectively.

$$\text{Water Absorption (\%)} = \frac{W_{eq} - W_i}{W_i} \times 100 \quad (1)$$

$$\text{Mass Loss (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (2)$$

2.11. Thermogravimetric analysis (TGA)

The thermograms of weight loss and derivative weight loss of powders, WG films and selected biocomposites (COP_20, ECOP_20 and MCOP_20) were carried out with a thermogravimetric analyzer (SETARAM 92-12, Frankfurt, Germany) equipped with software Setsoft 2000 (Frankfurt, Germany). Thermogravimetric analysis (TGA) was done under nitrogen flux at 1.8 L h⁻¹. Approximately, 20 mg of each sample were placed in crucibles and analyzed over a temperature range of 20–500 °C with a heating rate of 5 °C min⁻¹. All samples were dried about one week in desiccator containing silica gel before testing.

3. Results and discussion

3.1. FTIR spectra of powders

The Fig. 1a shows the comparison between spectra of COP and ECOP powders. The major change observed after esterification was the increase of stretching of carbonyl groups at 1746 cm⁻¹, which represents the C=O of esterified powder [25]. The esterification reaction is also confirmed by the absorbance at 1166 cm⁻¹ (C—O stretching) [26, 27], and by the enhanced carbonyl absorption peak at 1381 cm⁻¹ (—C—CH) [28]. The appearance of a new band at 822 cm⁻¹ is attributed to out of plane deformation for carboxyl groups [29].

Fig. 1b shows the spectra of COP and MCOP powders, the major modification observed after mercerization was the decrease of absorbance at 3400 cm⁻¹, the disappearance of the peaks at 1246 cm⁻¹ (C—H stretching) and at 1746 cm⁻¹ (C=O stretching). The intensity of the peaks at 2942 cm⁻¹ (C—H stretching) and at 1600 cm⁻¹ (C—O stretching) also decreases. The absorbance bands at 1378 cm⁻¹ (C—H stretching) and at 1032 cm⁻¹ assigned respectively to aromatic C—H— in plane deformation and C—O deformation for primary alcohol in lignin are also weakened after mercerization. These modifications indicate

the removal of most hemicellulose and lignin after mercerization [30-34]. Furthermore, it is difficult to analyze the spectrum because olive pomace is a complex lignocellulosic materials containing cellulose, hemicellulose and lignin, however chemical modifications of the powders were confirmed by FTIR characterization.

Fig. 1

3.2. Scanning Electron microscopy (SEM)

3.2.1. SEM of powders

SEM observation of powders is presented in Table 2. It shows the morphology and the effect of chemical modifications on powder surfaces. COP powder Micrograph (Table 2) showed granules with flake-like shape and thin particles attached to their surfaces were observed, this might be attributed to the applied treatments. After chemical modifications, the ECOP powder (Table 2) presents a rather smooth surface, while the surface of MCOP powder (Table 2) appears rough. It can be noted that most of the fine particles were detached from the surface of ECOP and MCOP powders after applying chemical treatments without affecting the size of particles (Table 2).

Table 2

3.2.2. SEM of cryofractured biocomposites

Table 3 shows the cryofractured WG films, they were rather smooth and homogenous. COP_20 Biocomposite (Table 3) presented rougher and less homogenous surface that characterizes the brittle fracture. Micrograph of ECOP_20 biocomposite (Table. 3) shows a smooth surface, probably due to the plasticizing effect of maleic anhydride, also several holes were observed. The surface of MCOP_20 biocomposite (Table 3) was rough and also several holes were noticed.

Table 3

3.3. Mechanical properties

In function of the powder content and the presence or not of chemical modifications, the aspect of the curves changes. In case of biocomposites containing 0 to 20% of COP, ECOP or MCOP powders, curves representing the relationship between stress and elongation

(Fig. 2a, b and c) were typical of brittle materials. Parameters related to mechanical properties of these biocomposites (Young's modulus, tensile strength and elongation at break) are detailed in Fig. 2A, B and C.

Young's modulus (Fig. 2A) of COP biocomposite was increased with increasing powder content. At 20% of reinforcement, Young's modulus increased significantly ($p < 0.05$). The tensile strength (Fig. 2B) of COP biocomposite decreased significantly until to 15% of reinforcement ($p < 0.05$). The increase of Young's modulus and the decrease of tensile strength of COP biocomposite as a function of powder content were due to the powder content and their rigidity, which increased the stiffness of biocomposites, also the presence of several holes (Table 3) indicates an insufficient adhesion between WG and COP powder. These properties induced a reduction of films extensibility and elongation at break was decreased dramatically. This compartment characterized all filled materials, similar observations were reported [35, 36]. According to Bergeret et al. [37] a decrease in tensile strength with increase powder content indicate inefficient stress transfer between plasticized Wheat Gluten and COP powder.

The Young's modulus (Fig. 2A) and tensile strength (Fig. 2B) of ECOP_5 biocomposite decreased significantly ($p < 0.05$), then increased until 20%. The reduction in Young's modulus and tensile strength of biocomposite containing 5 to 15% of ECOP powder was probably due to plasticization effect of ester groups. For ECOP_20 biocomposite, filler effect was more important (Young's modulus and tensile strength increased).

The Young's modulus (Fig. 2A) of MCOP biocomposite rised with increasing powder content. At 15% of reinforcement, Young's modulus increased significantly ($p < 0.05$). Tensile strength (Fig. 2B) decreased with incorporation of 5% MCOP powder without significant differences compared to that of WG films ($p < 0.05$), then remained almost constant with the increase of powder content. At low MCOP powder content (from 5 to 15%) Young's modulus and tensile strength were improved with the increase of surface roughness. Biocomposite contained 20% of MCOP powder Young's modulus and tensile strength remained almost unchanged compared to those of COP biocomposite, this may be due to the filler effect and its rigidity which interfere with the improvement of adhesion interface by mercerization. The presence of holes can also affect these properties (Table 3).

The elongation at break (Fig. 2C) of all biocomposites decreased over the range of 0 to 20% of reinforcement, according to Mohanty et al. [38], this is may be attributed to the

reduction in deformability of a rigid interface between filler and the matrix component, which is also reflected in the increase of stiffness.

Fig. 2

3.4. Water absorption and mass loss

Fig. 3 shows the evolution of water absorption of biocomposites containing 0 to 20% of COP, ECOP or MCOP powders. Plasticized Wheat Gluten (WG) films has strong water absorption (40%) , which is due to the hydrophilic nature of the polar peptides [39], also the plasticizer increases affinity to moisture [40].

Water absorption of COP biocomposite, was decreased significantly with the increase of powder content. At 20% of reinforcement, water absorption decreased significantly and was 29% less than that of WG films ($p < 0.05$). This could be explained either by the presence of lignin in powder which increases with increasing powder content, their complex structure obstructs the water penetration in the biocomposite, similar observations were reported [7, 41], or / and by the decrease of glycerol proportion. Given that, the glycerol content is fixed at 35%, while the proportion of glycerol relative to wheat gluten decreased with the increases of powder content.

The values of water absorption of ECOP biocomposite (Fig. 3) containing 5 to 10% of powder remained near to that COP biocomposite. For 15 to 20% ECOP powder content, water absorption increases in contrast with that was observed by Marcovich et al. [42]. It may be due to the presence of holes (Table 3) and to the low efficiency of esterification, which was influenced by treatment conditions and concentration of maleic anhydride.

The water absorption of MCOP biocomposite (Fig. 3) remained almost unchanged with increase of powder content and appeared higher than that of COP biocomposite. Very close levels of water absorption were observed for MCOP_15 and MCOP_20 biocomposites. In this fact, the mercerization increased the accessibility of water molecules to surface particles.[43], with partial removal of lignin and hemicellulose. In addition, the presence of several holes (Table 3) could promote water penetration in biocomposites.

Fig. 3

For all biocomposites, a decrease of the samples weight, namely ‘mass loss’ was observed (Table 4), can be attributed to the glycerol loss from the material to water [7]. Mass loss of all biocomposites decrease significantly with a decrease of glycerol proportion and an increase of powder content. Wheat gluten and olive pomace powders are insoluble in water;

however, the increase of mass loss in OPCE biocomposite may be due to hydrophobic properties of the ester groups grafted on the powder, which accelerate the migration of glycerol from the material to water by a repelling effect on glycerol. This was suggested by Duval et al. [24] for wheat gluten material reinforced with kraft lignin.

Table. 4

3.5. Thermogravimetric analysis

Fig.4a and a', shows the thermograms of weight loss and derivative weight loss of COP, ECOP and MCOP powders. The initial stage of weight loss for COP powder between 25 and 150 °C was the same for all powders, assigned primarily to water evaporation. The second and the third stage of weight loss from 150 to 314 °C and from 314 to 380 °C, respectively, are due to the decomposition of hemicellulose and cellulose [44]. Lignin degradation occurred over a wide range of temperatures [45], ranging from 230 to 500 °C. The maximum degradation temperature rates (T_{max} (°C)) of each stage and the corresponding weight losses (*Wt.loss* (%)) are presented in Table 5. The thermal stability of ECOP powder (Fig. 4a and a') remained very close to that of COP powder. It implies that esterification did not affect thermal stability of ECOP powder. It has been noticed that the peak of hemicellulose of MCOP powder (Fig. 4a and a') was disappeared after mercerization (Table 5). It is known, that hemicellulose can be removed by mercerization treatment [46, 47].

Table 5

The thermograms of weight loss and derivative weight loss of WG films, COP_20, ECOP_20 and MCOP_20 biocomposites are shown in Fig.4b and b'. The thermal degradation of WG (Fig.4b and b') under nitrogen shows four-stages decomposition. The first stage corresponds to the water loss between 25 and 150 °C. The second stage between 150–270 °C related to the volatilization of glycerol. The third stage consists in the breakage of the covalent peptide bonds in the amino acid residues, varied from 270 to 360 °C. The last stage is due to the cleavage of S—S, O—N, and O—O bonds from proteins molecules, was located between 360 to 384 °C [48, 49].

Fig. 4b and b' shows that with an incorporation of 20% of COP powder, wheat gluten is degraded between 270 and 300 °C followed by powder components degradation from 300 to 360 °C. The thermal stability of COP_20 biocomposite was decreased (Table. 5), this might be due to the powder components which are degraded at low temperature, such as

hemicellulose [44]. The thermal stability of ECOP_20 and MCOP_20 biocomposites were unaffected by the chemical modifications of powder.

Fig. 4

4. Conclusion

This work focused on manufacturing of novel biocomposites by reinforcement of plasticized wheat gluten using glycerol with Clean Olive Pomace (COP), Esterified Clean Olive Pomace (ECOP) or Olive Pomace Clean Mercerized (OPCM) powders. FTIR spectroscopy confirms the chemical modifications of the powders, while SEM showed a smooth surface of esterified powder, and rough surface of mercerized one. TG Analysis reveals that mercerization treatment improves the thermal stability of powder, while the esterified powder was unaffected.

The chemical modifications did not improve significantly the mechanical properties of biocomposites, while at powder content under 15% all biocomposites were less stiffness. Except for COP biocomposite, chemical treatments did not affect the thermal stability of biocomposites but their water absorption was increased. The micropore ratio in the produced biocomposites influences the mechanical strength and water absorption.

The reinforcement of plasticized wheat gluten with olive pomace powder could be a good way to produce bio-based materials, but their properties were affected by the powder loading. However to improve the interfacial adhesion, further physicochemical modifications of surface powder must be investigated. The studied biocomposites can be used to produce containers and packagings for dry or partially dry foodstuffs. We are currently exploring the possibility of using these materials in fruits, vegetables or eggs packaging, they could be useful as biodegradable containers and also as insulation materials.

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Figure captions

Fig. 1. FTIR spectra of COP (a and b), ECOP (a) and MCOP (b) powders.

Fig. 2. Stress–Elongation relationship of COP (a), ECOP (b) and MCOP (c) and Young's modulus (A), tensile strength (B) and elongation at break (C) biocomposites containing 0-20% of powders.

Fig. 3. Water absorption of COP, ECOP and MCOP biocomposites containing 0-20% powders. Bars with the same letter (a-d) refer to means not statistically different according to ANOVA and Tukey's test ($p < 0.05$); the results are ranked in decreasing order: $a > b > c > d > e$.

Fig. 4. Thermograms of weight loss and derivative weight loss of COP, ECOP and MCOP powders (a, a'), and of WG material, COP_20, ECOP_20 and MCOP_20 biocomposites (b, b').

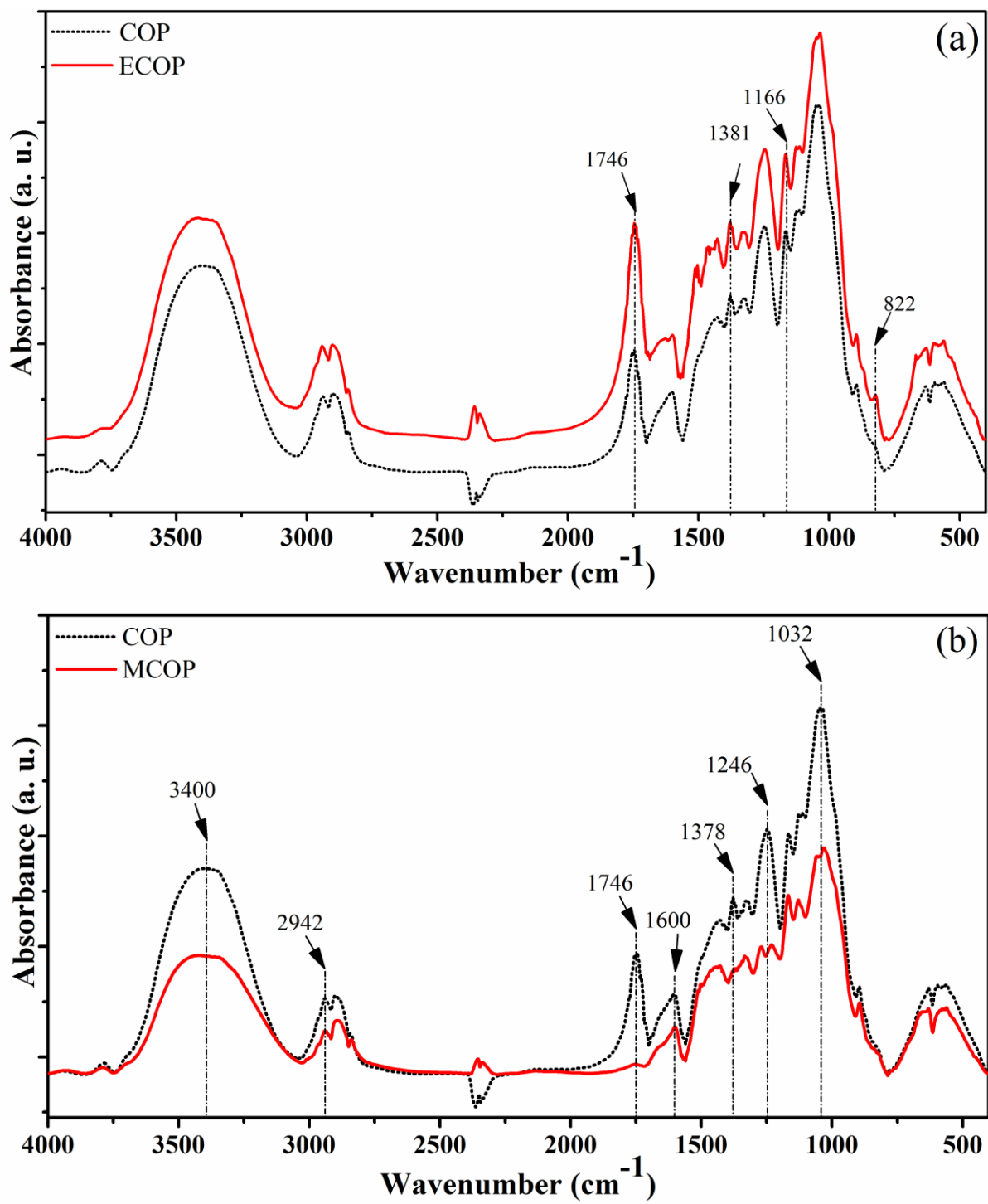


Fig. 1

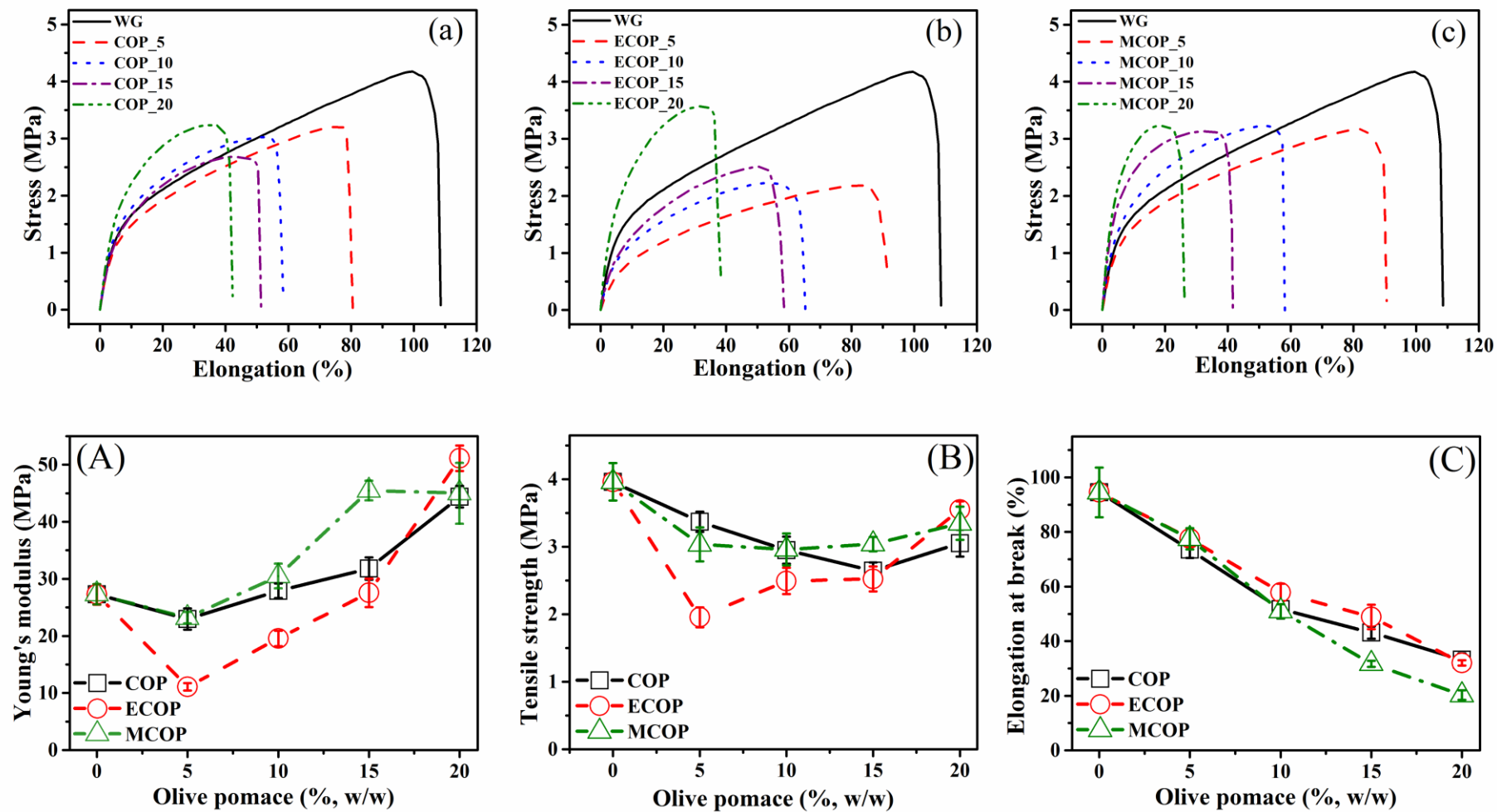


Fig.2

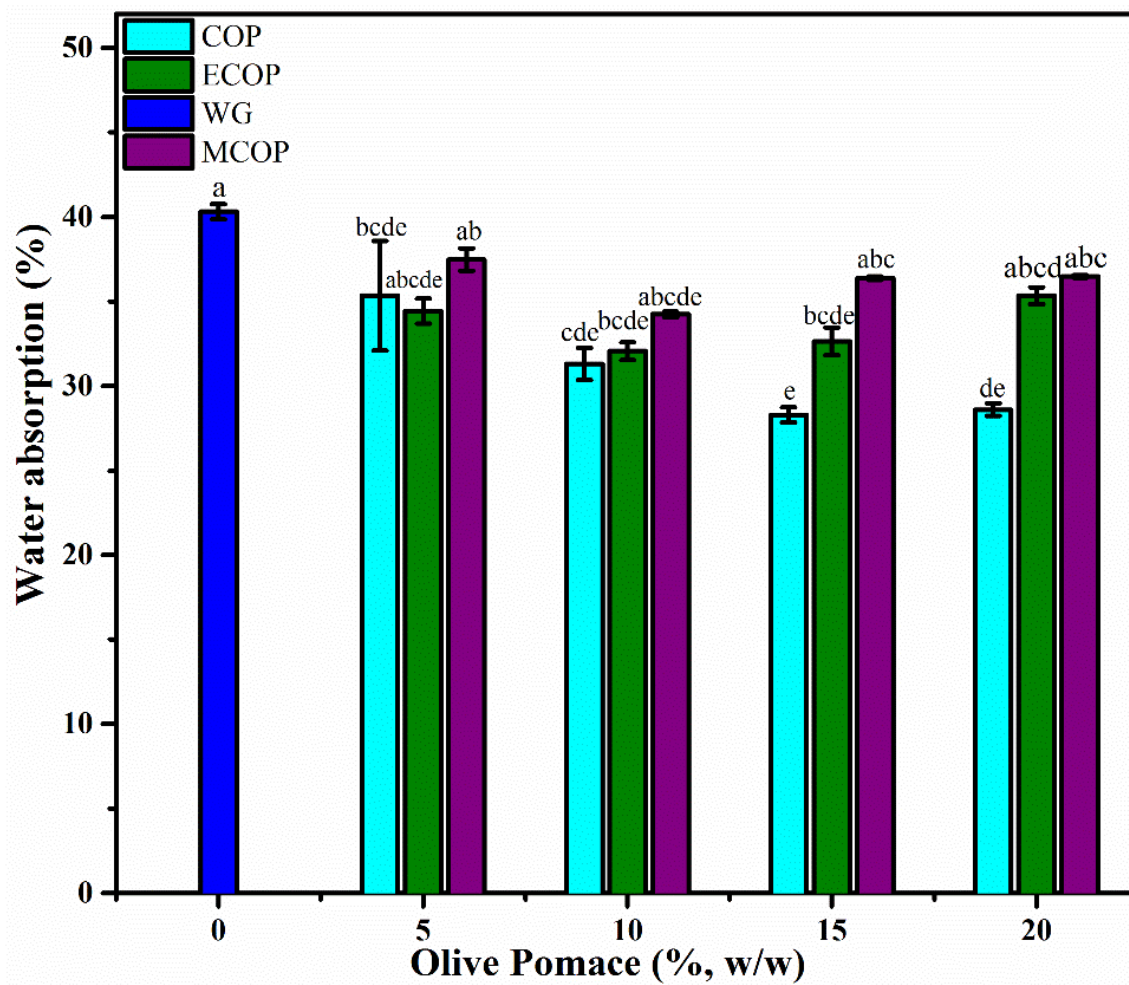


Fig.3

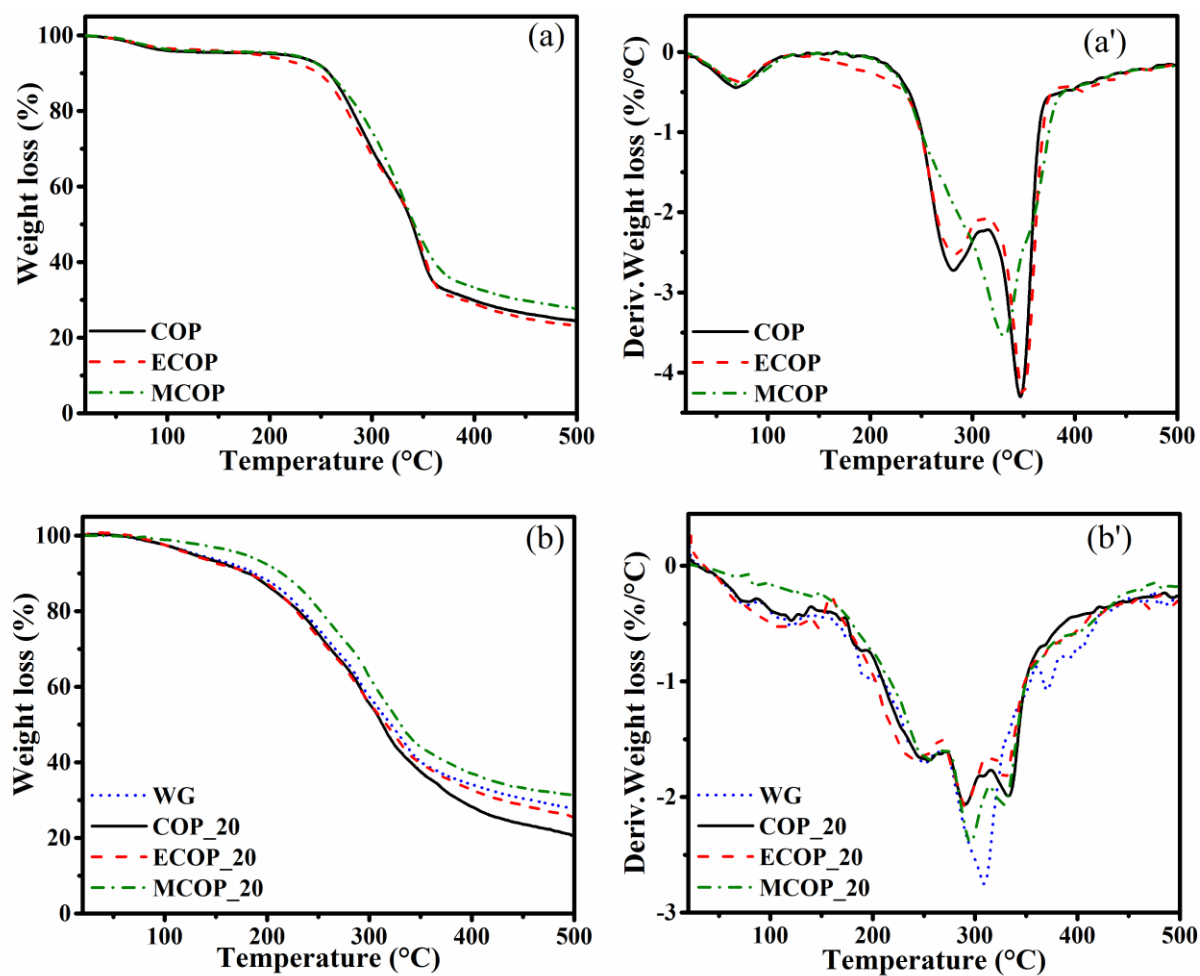


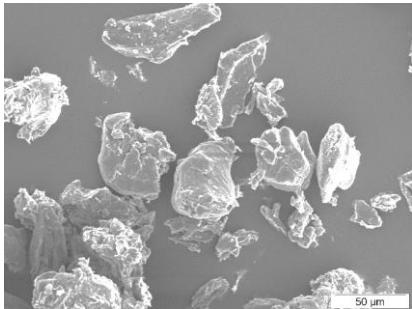
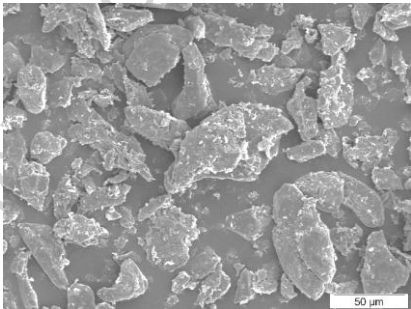
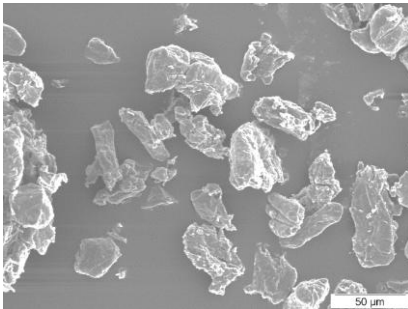
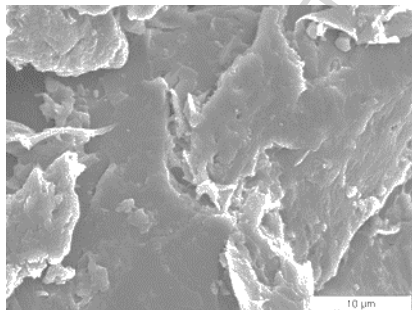
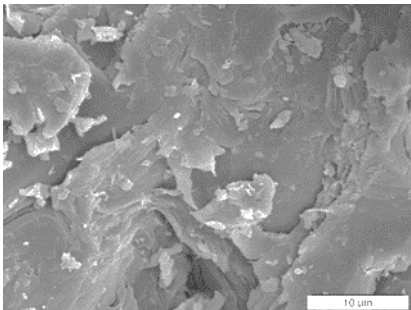
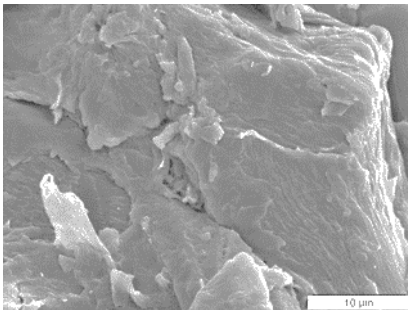
Fig. 4.

Table 1. Composition of plasticized Wheat Gluten (WG) and biocomposites, all percentages were calculated on a dry weight basis.

Biocomposite names*			Sample compositions (% w/w)	
			Wheat gluten	Powder (filler)
WG			65	0
COP_5	ECOP_5	MCOP_5	60	5
COP_10	ECOP_10	MCOP_10	55	10
COP_15	ECOP_15	MCOP_15	50	15
COP_20	ECOP_20	MCOP_20	45	20

* **WG**: plasticized Wheat Gluten. All materials were plasticized with 35 (% w/w) of glycerol, and the composition of WG and biocomposites were calculated on a dry weight. The indexes 5, 10, 15, 20 represent the percentage of powder. **COP**: Clean Olive Pomace powder, **ECOP**: Esterified Clean Olive Pomace powder, **MCOP**: Mercerized Clean Olive Pomace powder.

Table 2. Micrographs at two magnifications ($\times 300$ and $\times 1000$) of COP, ECOP and MCOP powders, and their particle size distribution. The results are reported as means \pm S.D. Same letters (a-d) in the same line refer to the means which are not statistically different according to ANOVA and Tukey's test ($p < 0.05$); the results are ranked in decreasing order: a > b > c > d).

Parameters		Powders		
		COP	ECOP	MCOP
Micrographs of powders	Magnification ($\times 300$)			
	Magnification ($\times 1000$)			
PSD (μm)	D (v, 0.10)	7.83 ± 1.05^a	7.67 ± 0.95^a	9.08 ± 0.88^a
	D (v, 0.50)	56.02 ± 0.91^b	55.63 ± 1.03^b	55.57 ± 1.07^b
	D (v, 0.9)	122.59 ± 0.48^c	119.31 ± 1.02^c	121.32 ± 0.80^c
	D [4.3]	61.75 ± 0.36^d	62.83 ± 0.78^d	60.26 ± 1.06^d

PSD (μm): Particle Size Distribution of powders; **D (v, 0.10)**, **D (v, 0.50)** and **D (v, 0.9)**: are the particle diameters at 10% (small particles), 50% (medium size particle) and 90% (large coarse particle), respectively. **D [4.3]**: volume mean diameter; **COP**: Clean Olive Pomace powder; **ECOP**: Esterified Clean Olive Pomace powder; **MCOP**: Mercerized Clean Olive Pomace powder.

Table 3. Micropore ratio of all biocomposites and micrographs at one magnifications ($\times 250$) of WG, COP_20, ECOP_20 and MCOP_20 biocomposites fractured in liquid nitrogen. The results are reported as means \pm S.D. Same letters (a-c) in the same column refer to the means which are not statistically different according to ANOVA and Tukey's test ($p < 0.05$); the results are ranked in decreasing order: $a > b > c$.

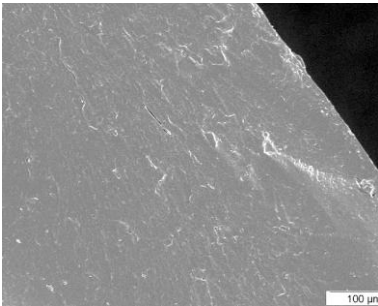
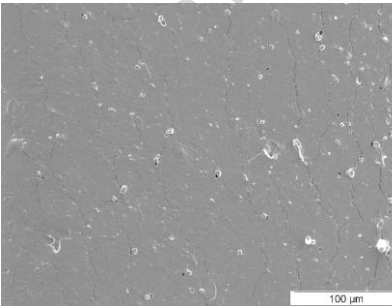
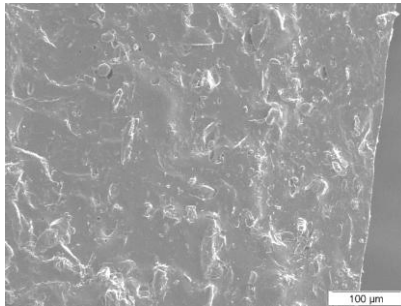
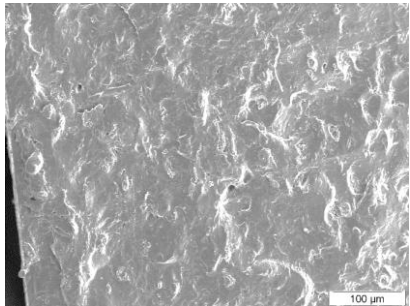
Parameters		Biocomposites						
Micropore ratio (%)	WG	1.06 \pm 0.45 ^{abc}	COP_5	0.82 \pm 0.58 ^a	ECOP_5	0.99 \pm 0.38 ^b	MCOP_5	1.02 \pm 0.32 ^c
			COP_10	0.79 \pm 0.30 ^a	ECOP_10	0.96 \pm 0.06 ^b	MCOP_10	0.99 \pm 0.57 ^c
			COP_15	0.75 \pm 0.56 ^a	ECOP_15	1.04 \pm 0.38 ^b	MCOP_15	1.05 \pm 0.12 ^c
			COP_20	0.72 \pm 0.63 ^a	ECOP_20	1.13 \pm 0.33 ^b	MCOP_20	1.08 \pm 0.64 ^c
Micrographs of cryo-fractured biocomposites								
		WG	COP_20	ECOP_20	MCOP_20			

Table 4. Mass loss of biocomposites containing 0 to 20 (% w/w) of powder during absorption water experiments. Results are reported as means \pm S.D. Same letters (a-e) in the same column and same line refer to means not statistically different according to ANOVA and Tukey's test. values ($p < 0.05$); the results are ranked in decreasing order: $a > b > c > d > e$.

Powder content (% w/w)	Mass loss (%)		
	COP	ECOP	MCOP
0	30.97 \pm 0.13 ^{abcd}	30.97 \pm 0.13 ^{abcd}	30.97 \pm 0.13 ^{abcd}
5	27.92 \pm 1.96 ^{bcd}	34.65 \pm 0.20 ^a	31.35 \pm 0.34 ^{abcd}
10	28.41 \pm 1.84 ^{cde}	33.37 \pm 0.25 ^{ab}	31.14 \pm 0.13 ^{abcd}
15	28.77 \pm 0.12 ^{cde}	32.14 \pm 0.22 ^{abc}	28.26 \pm 0.12 ^{cde}
20	26.42 \pm 0.01 ^e	29.62 \pm 0.51 ^{bcd}	28.01 \pm 0.12 ^{de}

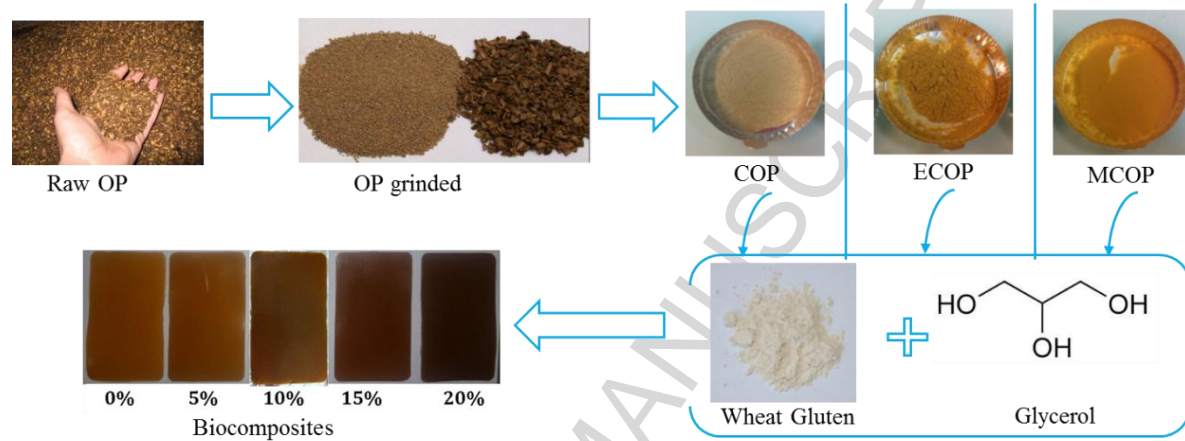
COP: Clean Olive Pomace powder; **ECOP:** Esterified Clean Olive Pomace powder; **MCOP:** Mercerized Clean Olive Pomace powder.

Table 5. Selected biocomposites, WG and powders maximum degradation temperature (T_{max} , °C) and weight loss percentages (*Wt. loss*, %) of each thermal degradation stage.

Samples name	Stage 1		Stage 2		Stage 3		Stage 4		
	T_{max} (°C)	<i>Wt. loss</i> (%)	T_{max} (°C)	<i>Wt. loss</i> (%)	T_{max} (°C)	<i>Wt. loss</i> (%)	T_{max} (°C)	<i>Wt. loss</i> (%)	
Powders	COP	69	4	281	32	347	36	—	—
	ECOP	69	4	283	33	349	32	—	—
	MCOP	70	4	—	—	331	32	—	—
Biocomposites	WG	120	7	252	26	309	32	370	5
	COP_20	121	6	255	25	291	12	332	19
	ECOP_20	110	8	242	26	291	11	329	18
	MCOP_20	86	3	256	23	296	11	328	20

WG: plasticized Wheat Gluten. **COP:** Clean Olive Pomace powder; **ECOP:** Esterified Clean Olive Pomace powder; **MCOP:** Mercerized Clean Olive Pomace powder. The indexed biocomposite names in 20 represent the percentage of powder.

Graphical abstract



Highlights

- Wheat gluten plasticized by glycerol (35%) was reinforced by olive pomace powder.
- Chemical modification does not affect the particles size distribution of powders.
- The presence of the holes affect the properties of biocomposites.