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Effect of hydrolyzed protein-based mulching coatings on the soil properties and productivity in a tunnel greenhouse crop system

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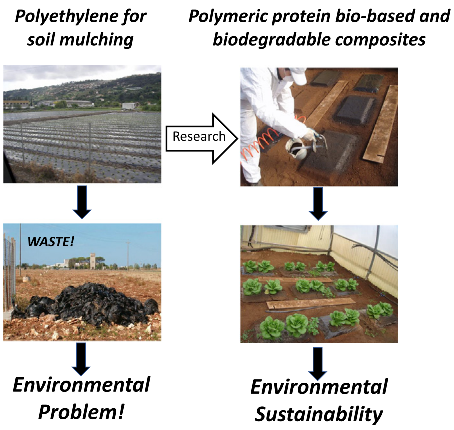
HIGHLIGHTS

- New biodegradable products from residues of leather industry and natural fillers from renewable resources were manufactured
- Biodegradable mulching coatings by means of spray technique were made
- Agronomic performances of biodegradable coatings are comparable to those of LDPE films

GRAPHICAL ABSTRACT



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ABSTRACT

Polymeric protein-based biocomposites were used in this work as water dispersions to generate, in situ, biobased mulching coatings by spray technique, as alternative to low density polyethylene films for soil mulching. At the end of their lifetime, these biodegradable coatings degrade in soil thank to the microbial community that mineralizes them.

Protein hydrolysates (PH) were derived from waste products of the leather industry, while poly(ethylene glycol) diglycidyl ether (PEG) and epoxidized soybean oil (ESO) were used to make the biodegradable spray coatings.

A study under greenhouse condition was carried out using seedling test plots in order to investigate the performance of the spray coatings and their possible influence on some aspects of leaf growth, functionality and nutritional quality of lettuce (*Lactuca sativa* L., Mortarella selection Romanella variety Duende) and on soil properties.

The biodegradable coatings showed the same good agronomic performances comparable with the ones of a commercial low density polyethylene mulching film, maintaining the mulching effect for the requested cultivation period and ensuring at the same time a similar rate of plant growth and dry matter accumulation. The research showed that 2 months after the tillage carried out at the end of the cultivation the amount of coating residues present in the soil was <5% of the initial weight of the biodegradable coatings.

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At the end of the field test, the soil mulched with the polyethylene film recorded an electrical conductivity value lower with respect to the soil mulched with the sprayed coatings, which release nutrients in the soil during their decomposition.

1. Introduction

The agricultural practice of soil mulching consists in laying a continuous coating over the soil in order to suppress weed, decrease the loss of moisture from the soil and keep plants and edible products clean. Low density polyethylene (LDPE) mulching films are worldwide used due to their mechanical properties appropriate to assure an easy handling and installation, to their functionality and resistance throughout the cropping cycle and to the low cost. LDPE mulching films are generally monolayer films with a thickness ranging from 10 μm to 100 μm and an average lifetime of 2–12 months (Briassoulis et al., 2004; Picuno, 2014; Picuno et al., 2012). Mulching films used for weed control must be opaque to prevent the passage of the photosynthetically active radiation (PAR) (Vox and Schettini, 2007). China, Japan and South Korea use about 80% of the worldwide mulching films production in horticulture, with a consumption of about 700,000 ton per year. After their use, the mulching films are dirty of soil, organic matter and agro-chemicals thus they need a correct collection, disposal or recycling process with high costs for the growers. Agricultural plastic waste is often abandoned in illegal dumps, in river basins, burned or shipped illegally or in non-compliance with the regulations (Picuno and Scarascia-Mugnozza, 1994). These illegal practices also result in feeding cross-border trafficking that concerns polyethylene wastes (www.polieco.it). As a consequence, plastic residues accumulate in the environment and toxic emissions give off in the atmosphere becoming a considerable threat to human life as well as for terrestrial and aquatic wildlife (Blanco et al., 2018; Lanorte et al., 2017; Mugnozza et al., 2016).

In order to overcome the problem of huge quantity of plastic wastes, biodegradable and renewable raw materials can be used to make mulches. At the end of their lifetime, biodegradable materials degrade in soil thank to the microbial community that mineralizes them (Kapanen et al., 2008; Tuominen et al., 2002). The lifetime of the biodegradable mulches can be tailored differently through the variation of the thickness in relation with the crop cycle and/or cultivation needs. Biodegradable mulches can be performed by employing thermo-plasticizing, casting and spraying processes using renewable and biodegradable raw materials such as starch, cellulose, chitosan, alginate and glucomannan (Malinconico et al., 2008; Briassoulis, 2007).

The most recent technique is based on spraying water solutions onto soil forming a biodegradable mulching coating directly in field. Presently, spray mulching coatings based on polysaccharides that are potentially polluting residues from marine and agricultural wastes and co-products can be obtained, even if they need to be improved mainly in their mechanical properties (Immirzi et al., 2009; Malinconico et al., 2008; Johnston et al., 2017; De Corato et al., 2018). Sartore et al. (2013) studied the possibility to obtain spray mulching coatings using protein hydrolysates (PH), derived from waste products of the leather industry, for their intrinsic agronomic values due to their high nitrogen content (Sartore et al., 2016b; Schettini et al., 2012). Biodegradable coatings can be obtained by the modification of PH with poly(ethylene glycol) diglycidyl ether (PEG), a water soluble and highly reactive epoxy compound largely used as a cross-linking and insolubilizing agent in water-based systems. They showed very good mechanical and agronomical performances in the field, maintaining their mulching effect for even 12 months (Sartore et al., 2013; Hiroyuki, 2014). PEG is a derivative of petroleum, therefore a sustainable approach can be its substitution with a renewable resource from biorefinery such as the epoxidized soybean oil (ESO). Although soybean oil is primarily used as edible oil, there is an increasing demand for its use in industrial applications, e.g. inexpensive and renewable plasticizer, stabilizer, reactive modifier and diluent (Niedermann et al., 2014).

In order to improve the thermo-mechanical properties and decrease water sensitivity preserving biodegradability, natural fillers from renewable resources, such as wood flour and lignin, were incorporated in mulching coatings obtaining biocomposite materials (Sartore et al., 2016a). Lignocellulosic products, mainly cellulose and lignin-based materials, have recently attracted much attention due to their renewable nature, wide variety of source materials available throughout the world, low cost and density, high surface functionality and reactivity. Lignin, a byproduct of important processes like paper or biodiesel production, is a natural polymer found in wood and in the secondary cell walls of plants and some algae; it is the second most abundant biopolymer on Earth (Holmgren et al., 2006). Because of its polyphenolic structure, lignin shows antimicrobial (Holmgren et al., 2006; Fortunati et al., 2016) and antioxidant properties (Lora and Glasser, 2002; Rahman et al., 2013) which can be very useful for agriculture applications or packaging with improved life-time (De Corato et al., 2018).

The aim of this paper is to describe the development and the application of novel biodegradable polymeric materials, based on protein hydrolysate and reagents from renewable sources, for the creation of mulching coatings alternative to LDPE films. Two blends using PH, PEG or ESO and natural fillers were developed. A study under greenhouse condition was carried out using lettuce (*Lactuca sativa* L., Mortarella selection Romanella variety Duende) plant test plots in order to investigate the performance of the spray coatings and their possible influence on some aspects of leaf growth, functionality and nutritional quality of the test-plants and on soil properties.

2. Materials and methods

2.1. Biodegradable coating materials preparation

The raw materials utilized for the preparation of the mulching coatings were: PEG (molecular weight = 526 Da), ESO, ethylene diamine (EDA) and pyromellitic dianhydride (PMDA), supplied by Sigma- Aldrich Co (Milan, Italy) and used as received. Carbon black (CB) was supplied by Degussa-Hüls (Dusseldorf, Germany) with an average primary particle size of 23 nm. CB is a commercial form of solid carbon that is manufactured in highly controlled processes to produce, specifically, black pigments and molecules absorbing detrimental UV light and convert it into heat during the production of polymers. CB shows toxicity for human beings only when a long-term inhalation occurs without adequate protection on the production sites and in several user industries (IARC, 2010). Absence of release of toxic substances in the soil was assessed after the use of biodegradable mulching films containing CB (Kapanen et al., 2008).

Protein hydrolysate (PH) was a slightly yellow, chromium-free, product supplied by Sicit Chemitech S.p.A (Vicenza, Italy) as a by-product of chemical hydrolysis in the leather production process. PH is generated from alkaline hydrolysis of the solid residues generated downstream of leather chrome tanning stage (leather shavings). PH is composed of a mixture of oligopeptides with the typical amino acid composition of collagen and an average molecular weight between 6000 and 8000 Da.

Lignin used was Indulin® AT, provided by MeadWestvaco Corporation (Charleston Heights, SC, USA), a commercially available purified powder

Table 1
Composition of PH-ESO and PH-PEG-ESO derivatives.

	PH-ESO	PH-PEG-ESO
PH [%]	41.2	41.4
ESO [%]	10.3	5.2
PEG [%]	–	5.2
PMD [%]	0.6	–
EDA [%]	–	0.3
Lign [%]	24.7	24.8
WF [%]	21	21
CB [%]	2	2.1

form of pine Kraft lignin, free of hemicelluloses, characterized by a relatively high degree of purity (97%). This type of softwood Kraft lignin has a specific density of 1.24 g cm^{-3} and an average particle size of 8 μm. Beech wood flour, characterized by particle size < 180 μm and apparent density equal to 160–210 kg m^{-3}, was supplied by La.So.Le Srl (Udine, Italy) and used as raw material.

Biodegradable polymeric materials were prepared starting from protein-based aqueous solutions, obtained by dissolving the proper amount of PEG or ESO in a 25% (w/v) solution of PH. The desired amount of lignin, natural fillers and water, up to a final 18 wt% concentration, were added under stirring at 50 °C. For both materials the final total natural fillers content was about 47%, which was recognized to enhance the environmental lifetime of proteinaceous derivatives (Sartore et al., 2016b).

Water suspensions were used to make the mulching coatings in field and the specimens for the laboratory tests (Table 1). The suspensions, kept under stirring for few minutes at 50 °C, were sprayed onto the soil surface after the addition of the proper crosslinking agent. Casted coatings were prepared by slow evaporation of the water suspension of the ingredients in glass trays at room temperature. The dry formulations were hot pressed at 50 °C to make 2 mm thick sheets, from which the specimens for mechanical and radiometric test were obtained.

2.2. Biodegradable coating materials characterization

Tensile tests were performed by an Instron Model 3366 Universal Testing Machine at the University of Brescia. Elastic modulus (E), strength (σ_b) and elongation at break (ε_b) were determined on 2 mm thick and 10 mm wide bars, with a gauge length of 80 mm; crosshead speed was 2 mm min^{-1}. Tensile tests were performed at room temperature after specimen conditioning at 55 °C for 2 h under vacuum.

The same tests were performed on the materials after immersion in distilled water for 30 min. At least 8 specimens were tested for each mulching composition.

Weight loss was evaluated measuring the weight of dry specimen after immersion in water for fixed intervals as:

$$\text{Weight loss} = \frac{W_1 - W_3}{W_1} 100 \tag{1}$$

where W_1 and W_3 denote the weight of the starting dry specimen and of the dry specimen after water immersion respectively.

Radiometric tests on the mulches were carried out at the University of Bari by means of spectrophotometers in order to evaluate the spectral transmissivity $\tau(\lambda)$, i.e. the fraction of the incident energy radiant flux that is transmitted at a specific wavelength λ. The total transmissivity, in the wavelength range between 200 nm and 2500 nm, was measured by means of a double beam UV-VIS-NIR spectrophotometer (Lambda 950, Perkin-Elmer Instruments, Norwalk, CT, USA) equipped with an integrating sphere (diameter 60 mm). Radiometric coefficients of the materials were calculated as average values of the spectral transmissivity over different wavelength bands: the solar wavelength range (300–2500 nm), the PAR range (400–700 nm) and the long wave infrared radiation range (LWIR; >3000 nm). The transmissivity coefficient in the solar range was calculated as the weighted average value of the spectral transmissivity using the spectral distribution of the solar radiation at the ground level as weighting function (Vox and Schettini, 2007). Transmissivity in the LWIR range between 2500 nm and 25,000 nm was measured by a FT-IR spectrophotometer (1760×, Perkin-Elmer Instruments, Norwalk, CT, USA). The LWIR transmissivity coefficient was calculated as the average value over the wavelength interval between 7500 nm and 12,500 nm (Vox and Schettini, 2007).

2.3. Bioassays under greenhouse

2.3.1. Mulching coatings

The test was carried out from November 2013 to January 2014 inside a North-South oriented tunnel greenhouse at the experimental farm of the University of Bari, Italy, latitude 41° 05′ N. The steel-constructed tunnel (length of 30.00 m, width of 8.00 m, ridge height of 3.20 m) was covered with an ethylene-vinyl acetate (EVA) film (PATILUX, P.A.T.I. Company, San Zenone degli Ezzelini, Treviso, Italy). The film had thickness of 200 μm and the following radiometric coefficients: solar total transmissivity equal to 90.9%, solar direct transmissivity equal to 56.7%, LWIR transmissivity equal to 22.5%.

Greenhouse air temperature was controlled by means of two electric fans positioned in the South end wall and of two sliding shutters in the North end wall; the system was automatically driven in order to maintain internal air temperature under the set point value of 27 °C.

Clay-loam textured soil plots, East-West oriented, each of 0.4 m^2 (0.8 m × 0.5 m), were prepared for transplanting lettuce seedlings (*Lactuca sativa* L., Mortarella selection Romanella variety Duende) used as test-plants for their growth readiness.

On 27 November 2013 two different aqueous dispersions, coded PH-ESO and PH-PEG-ESO (Table 1), were sprayed on the soil to form coatings resistant for the time required by the crop cycle (Fig. 1a). The quantity of the sprayed solution determined coating's lifespan. The side slope of the raised bed of each plot was limited in order to avoid possible sliding of the spray mulching coating at the liquid state before the dry process. The solutions, in quantity of 2.25 kg m^{-2}, were distributed on the top of the plots by an airbrush with a nozzle having an internal diameter of 3 mm, using a spray machine at 0.9 MPa of pressure. The obtained dry coating was continuous and regular with an average thickness of 0.71 ± 0.18 mm. Control plots, coded as LDPE, were mulched with a black LDPE film, characterized by a thickness of 40 μm.

The experiment was carried out one time using a randomised block design with three treatments (PH-ESO, PH-PEG-ESO and LDPE) and ten replications for each treatment.

During the field test, greenhouse air temperature and relative humidity were measured by means of a Hygroclip-S3 sensor (Rotronic, Zurich, Switzerland); solar radiation was measured in the wavelength range 0.3–3.0 μm by a pyranometer model 8104 (Schenk, Wien, Austria). The data, measured with a frequency of 60 s, were averaged every 15 min and stored in a data logger (CR10X, Campbell, Logan, USA).

2.3.2. Assessment of the test plant growth and quality

On 29th November 2013, two lettuce seedlings per replicate were transplanted. The cropping method was the same for all the plots. In particular, each plot received on the whole 8.0 g m^{-2} of N (N form: urea 11%, NO_3^- 3%, NH_4^+ 1%), 2.8 g m^{-2} of P_2O_5 and 16.8 g m^{-2} of K_2O. An automated drip irrigation system ensured water availability to the plants providing 1.5 L $plant^{-1}$ every two days.

In order to test the effects of the mulching treatments on leaf development and expansion, the number of leaves per plant, the leaf length and width for the 2 biggest leaves per plant were measured 7 times from the transplanting to the harvesting, that occurred on 27th January 2014.

At transplanting, the features of plants assigned to each treatment were recorded. Possible effects of mulches on soil and leaf temperature were also assessed, since they are critical in controlling plant growth through several biological processes, such as root growth and water

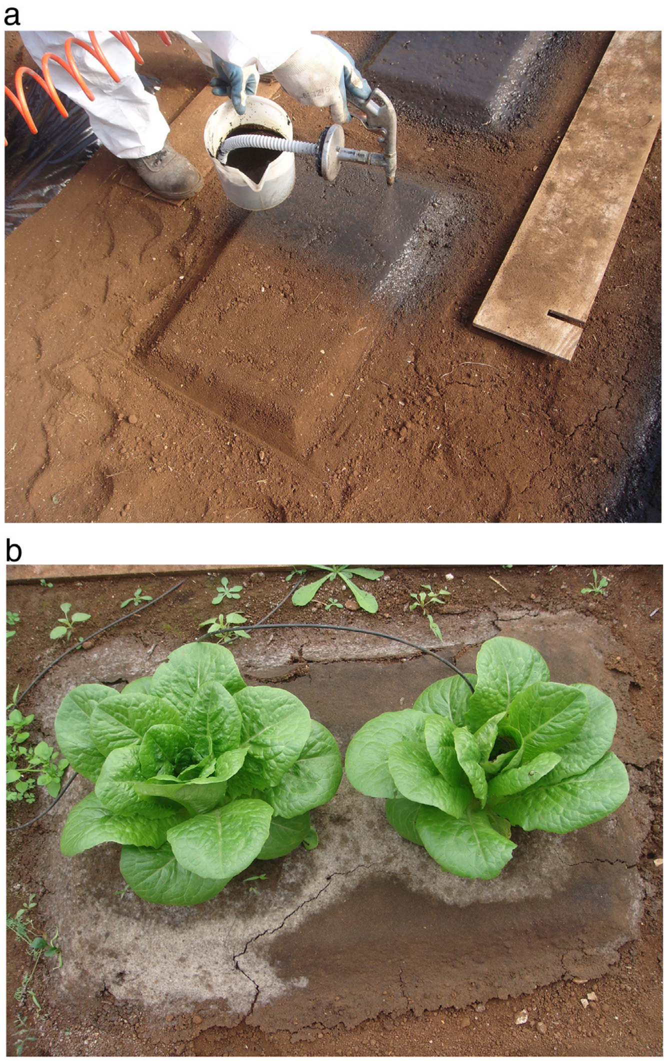

Fig. 1. Spray biodegradable coating at the experimental field of the University of Bari: (a) at the beginning of the test; 27/11/2013; (b) at harvest, 27/1/2014.

uptake, rates of water vapour loss, stomatal aperture, rate of net photosynthesis, etc. (He et al., 2009). From 10th December 2013 to harvest, leaf temperature was measured using an infrared thermometer (3 readings per plant on 1st-to-3rd fully expanded leaves), while soil temperature was measured at ~10 cm depth using a thermocouple thermometer (3 readings per plot). Readings were taken 6 times (at about 10-day intervals), around 10:00 a.m., under different weather conditions.

Possible indirect effect of mulches on leaf chlorophyll and nitrogen relative content was estimated, on the 2nd and 3rd leaves from the plant outside (Tsiakaras et al., 2014), using the SPAD 502-meter handheld device (Konica Minolta, Inc.): on the largest blade portion, two readings per leaf where taken symmetrically to the midrib.

At harvest, all plant heads were cut and transported to the “Laboratorio di Arboricoltura” of the University of Foggia. The first two leaves of each plant were collected for chemical analyses and weighted, the remaining portion was also weighed and used to assess the plant dry weight after oven-drying at 65 °C until constant weight.

To evaluate the possible influence of mulch types on total polyphenol content (TPP) and total antioxidant activity (AA), the first two leaves (previously used for field measuring of relative chlorophyll content) were trimmed using a tissue ruptor (Quiagen). Leaf extracts were obtained putting in contact 5 g of grinded fresh leaves with 20 mL of methanol solution (80 MeOH: 20 H_2O) for few minutes, and centrifuging for 8′ at a frequency of 66.67 Hz (Yurttas et al., 2000). The antioxidant activity was determined using ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid) method (Re et al., 1999). $ABTS^+$ solution was diluted with ethanol (1:88) which absorbance, at 30 °C and at 734 nm, is about 0.70 (±0.02) against ethanol as blank. 200 μL of the diluted sample were added to 2 mL of chromogen into a cuvette of p.l. 10 mm and mixed; after 15 min the absorbance at 734 nm was red. Percentage inhibition was calculated as follows:

$$\text{Inib.}\% = \left(1 - \frac{\text{Abs}_s}{\text{Abs}_b}\right) \cdot 100 \tag{2}$$

where: s = sample; b = blank.

The equivalent concentration (μ/L Trolox uM) was obtained replacing, in the Trolox standard curve, the percentage inhibition.

TPP was determined using the colorimetric Folin-Ciocalteu procedure. Into a 25 mL flask, 0.1 mL of extract was put followed by 1 mL of Folin-Ciocalteu reagent. After 3–4 min, 10 mL of $NaCO_3$ (7%) were added and the flask filled with water till the mark. The flask was tipped faster several times and then left in dark for 120′ (Slinkard and Singleton, 1977). The absorbance was red at 760 nm against a blank. The absorbance was entered into the standard curve to express values as $\mu\,mL^{-1}$ gallic acid (Oviasogie et al., 2009). Final data are expressed respect to the leaf dry matter.

2.3.3. Assessment of the soil properties

At the beginning of the experiment, the soil was sampled collecting five cores using a W scheme, air-dried and 2-mm sieved before laboratory analyses (Sparks et al., 1996).

In order to record any variation of selected properties, sprayed and LDPE mulched soils were also sampled at the end of the trial, when the amount of coatings residues presented into the soil was negligible. Therefore, soil sample sets were characterized by conventional analytical methods for their moisture content, pH (1:2.5 w/v), electrical conductivity (EC, 1:2 w/v), total nitrogen (TN) by the Kjeldahl method, organic carbon (OC) by the Walkley and Black method, available P and total carbonates (TC) (Sparks et al., 1996).

2.3.4. Data analyses

Mechanical properties of LDPE mulching film and of PH-ESO and PH-PEG-ESO derivatives samples were carried out after drying and in wet state considering 8 specimens for each state.

The radiometric coefficients were calculated as average values of the measurements carried out on 5 specimens for each material.

Data concerning the test plant growth and quality were taken in 10 replicates.

Soil parameters were calculated as means of 5 replicates.

All data were subjected to the analysis of variance (one-way ANOVA) at 95% probability level. The statistical analyses were carried out in order to assess the effects exerted by the coatings on the selected soil properties and on the tested parameters of leaf growth, functionality and nutritional quality. For each variable, when F test was significant at $p = 0.05$, means were compared by Duncan multiple range test (DMRT) at $p \le 0.05$. Data are presented as average of the replicates ± standard error.

2.3.5. Coating degradation

The degradation of the mulching coatings residues in the soil was assessed after the cultivation period. Soil samples were taken after tillage every 15 days from the soil surface covered with the PH-ESO and the PH-PEG-ESO coatings. The soil samples were collected up to a depth of 0.20 m, which was the depth to which the digging tools of the milling machine penetrates. The soil samples were sieved through a 1.8 cm mesh, placed on the ground at an angle of 45°. The residues of biodegradable coating that did not pass through the mesh were collected and weighted.

3. Results and discussion

3.1. Spray coatings characterization

Results of tensile test performed on dried and wet specimens, prepared in laboratory from PH-ESO and PH-PEG-ESO dispersions, are reported in Table 2. A stiffer response is found in both the dried specimens, which present higher Young's Modulus and lower strain at break, <1%. By contrast, wet samples (i.e. after immersion in water for 30 min) revealed a comparable strength, but a significantly higher strain at break, approximately 40%. These results suggest increased ductility due to the plasticizing effect of water. As reported by Sartore et al. (2016a), the addition of natural fillers to PH-PEG hardened the resulting biocomposites, in particular PH-PEG coating containing up to 30% different natural fibres maintained a ductile behavior, whereas severe embrittlement was observed for contents of 50%. Nevertheless, the introduction of natural fillers was recognized to enhance the environmental lifetime of proteinaceous derivatives. For this reason, in this research the selected natural filler total content was about 47%. After real spray deposition of a concentrated water dispersion of the materials, crosslinking and water evaporation took place simultaneously originating a regular continuous stiff and strong coating incorporating the roughness and inhomogeneity of the soil.

Concerning the behavior of the derivatives in the presence of water, the weight loss of the specimens immersed in distilled water at room temperature was monitored over a period of about thirty days. Unlike the reagents were completely soluble, both the biocomposites possessed limited solubility and degradation rate. PH-PEG-ESO as well as PH-ESO derivatives swelled but for >25 days, no significant differences were noticed on the integrity of the corresponding immersed specimens which, in addition, showed a slow release of material gradually increasing with time. This may suggest extensive crosslinking of the two systems and may promote slow release of proteinaceous materials.

Fulfilling the radiometric requirement of a black mulch of being opaque to the PAR radiation, the PH-ESO and PH-PEG-ESO coatings showed a PAR total transmissivity coefficient of 0.00%, as the LDPE black mulching film (Table 3), so that weed growth was inhibited in the period from transplanting to harvesting. The LDPE mulching films must satisfy the EN 13655 standard (EN 13655, 2002); independently of their thickness, black plastic mulches must have a PAR total transmissivity coefficient < 0.01%. Even if the spray coatings are materials at an experimental stage and their physical properties are not defined by international standards, the present paper reports very promising results about their PAR transmissivity coefficients. Malinconico et al. (2008) and Immirzi et al. (2009) tested different biodegradable spray coatings that showed a good capacity to reduce weeds growth, even if with different PAR transmissivity coefficient. The spray coatings prepared mixing Locust bean gum and Guar gum, agarose, glycerol and carbon black were substantially opaque to the PAR radiation. In contrast, the coatings made with sodium alginate, seaweeds and bran of wheat showed a PAR total transmissivity coefficient of 6.36%. The spray coatings realized with hydrolyzed proteins by Sartore et al. (2013), with a thickness ranging from 0.6 to 0.8 mm, showed a good capacity to reduce weed growth even with a PAR total transmissivity coefficient ranging from 0.2 to 4.1%.

Table 2
Mechanical properties of PH-ESO and PH-PEG-ESO derivatives after drying and in wet state, and of LDPE mulching film. Mean values ± standard deviation. At least 8 specimens for each state were considered.

		E [MPa]	σ_b [MPa]	ε_b [%]
PH-ESO	Dried	1030 ± 170	5.8 ± 0.7	0.7 ± 0.1
PH-ESO	Wet	593 ± 95	10 ± 3	41 ± 3
PH-PEG-ESO	Dried	2550 ± 190	7.6 ± 1.1	0.37 ± 0.1
PH-PEG-ESO	Wet	850 ± 77	11 ± 3	38 ± 2
LDPE		270 ± 135	20 ± 14	580 ± 170

Table 3
Radiometric properties of PH-ESO and PH-PEG-ESO derivatives and of LDPE mulching film. The radiometric coefficients were calculated as average values of the measurements carried out on 5 specimens for each material.

	PH-ESO	PH-PEG-ESO	LDPE
Solar total transmissivity (%)	0.10	0.09	0.02
PAR total transmissivity (%)	0.00	0.00	0.00
LWIR transmissivity (%)	0.01	0.01	11.17

3.2. Spray coatings performance

The biodegradable spray coatings kept their mulching effect for 2 months from application to harvesting, though some cracks appeared on the surface (Fig. 1b). The irregularities appeared on the surface of the coatings around the plants where water was delivered by the drip irrigation system. Higher humidity values of soil induce faster degradation of the biodegradable coating (Schettini et al., 2007). The LDPE film recorded no variation during the cultivation period.

During the experimental test, the mulches were subjected to a cumulative solar radiation equal to 272 MJ m^{-2}. During the cold period, inside the tunnel the air temperature ranged from a minimum value equal to 2.82 °C (recorded on 8/12/2013) to a maximum value equal to 29.56 °C (recorded on 16/01/2014). Greenhouse relative humidity ranged from 47% (recorded on 8/12/2013) to 100%, typical value in greenhouse during the night.

The amount of mulching coating residues in the soil was determined to assess the time frame necessary for degradation of the buried biodegradable coating residues after tillage. The soil sampling in the field showed that after 2 months after tillage the amount of coating residues present in the soil was <5% of the initial weight of the biodegradable coatings for both the coatings. It was impossible to separate out the mulching coating residues in the soil due to their reduced size. The time of degradation of biodegradable coatings is influenced by the dimension of the residue pieces and by the thickness of the coating: the smaller the pieces and the thickness of the buried coatings, the faster is the degradation rate.

The lifetime of these biodegradable spray coatings could be shortened if the spray coatings are used outdoor. The lifetime is influenced by the meteorological conditions of the site and by humidity and temperature of the soil. Coatings can be damage due to adverse climatic conditions such as heavy rain, heat wave or hail storm. The higher the ambient and soil temperature and humidity, the shorter is the coating lifetime duration.

The lifetime of these biodegradable spray coatings can be compared with literature. A water solution was composed of sodium salt of alginic acid, i.e. a polysaccharide coming from seaweeds; polyglycerol and hydroxyethylcellulose were added (Immirzi et al., 2009). The solution was sprayed on a fibrous bed of seaweeds and bran of wheat, deposited on the soil in a dry state before the spraying. This coating was characterized by inhomogeneous surface and irregular thickness that varied from 3 to 5 mm. The sodium alginate-based spray mulch was characterized by a lifetime inside a greenhouse of 6 months, subjecting to a cumulative solar radiation of 2705 MJ m^{-2}. Few cracks on the surface appeared within the first month (Immirzi et al., 2009).

A transparent spray glucomannan based mulching coating was applied by spraying a water solution of glucomannans enriched by polyamino-polymers as mulching filler (Schettini et al., 2007). It was characterized by a thickness of 50 μm. Some little cracks appeared on the surface within the first month, but it lasted for 5 months inside a greenhouse (Schettini et al., 2007).

A coloured synthetic latex mulch base product ('BN 1849'; BASF, Charlotte, NC, USA), diluted 1:2 (product:water), was used in open air

(Mahmoudpour and Stepleton, 1997). This mulching coating was damaged by a hail storm after two weeks from the application.

3.3. Influence of the mulches on biometrical traits, productivity and quality nutritional factors

Concerning the test-plant lettuce, the best dry matter accumulation is known to be obtained with 24/24 °C root media/daytime air temperature. Therefore, in the present trial, the soil temperature was sub-optimal at all measurements. However, the PH-PEG-ESO mulch warmed the soil more than PH-ESO coating. Hence, the two biodegradable spray coatings could be differentially applied to keep, respectively, warmer or cooler the root media, in order to match cultural purposes, and/or to favor climate control in greenhouse cultivation aiming to reduce costs and environmental impact of field production.

Leaf temperature averaged from 4.70 ± 0.03 °C (21/01/2014) to 15.15 ± 0.2 °C (10/12/2013) (Fig. 2). The mulch type can influence the surface radiation balance and thus the plant microclimate (Liakatas et al., 1986). In this trail, differences among treatments were very small (from 0.11 °C to 0.56 °C) and not statistically significant on each date of measurements. Thus, the tested mulch types did not affect the leaf temperature measured during the daylight hours. It can be deduced that this basic parameter of leaf functioning is not prone to be modified by the two innovative coatings, neither comparing them to one another, nor comparing them with the LDPE black mulching film.

The type of mulching did not influence the leaf development and expansion of the test-plant. At transplanting, the average leaf number ranged from 3.83 ± 0.17 in PH-PEG-ESO and LDPE to 4.00 ± 0.29 in PH-ESO (Fig. 3a), leaf length from 10.33 ± 1.17 cm in PH-ESO to 10.67 ± 0.33 cm in LDPE, and leaf width from 3.40 ± 0.30 cm in PH-PEG-ESO to 3.80 ± 0.40 in LDPE (Fig. 3b). After transplanting, seedlings grew up with similar number of leaves (Fig. 3a) and leaf dimensions (Fig. 3b). At harvest, the leaf number ranged from 27.33 in PH-PEG-ESO to 28.33 in PH-ESO (Fig. 3a), the leaf length from 23.5 cm in PH-PEG-ESO to 24.33 cm in PH-ESO, and the leaf width from 15.5 cm in LDPE to 16.33 cm in PH-ESO (Fig. 3b).

The SPAD readings, that are generally correlated to chlorophyll content and leaf nitrogen status (León et al., 2001), averaged from 32.55 ± 2.3 (recorded on 12/12/2013) to 40.82 ± 0.4 (recorded on 30/12/2013). Differences among treatments changed pattern during the growing cycle (Fig. 4). Nevertheless, PH-PEG-ESO leaves showed a tendency for higher readings and reached the significantly highest values on the first date of measurement and on each of the last two dates. At harvest, PH-PEG-ESO reading was about 42 SPAD units, while PH-ESO and LDPE readings were about 38–39 SPAD units. Nitrogen uptake is known to be stimulated by warmer daylight soil temperature (Verdial et al., 2001). However, in this trial, LDPE showed a higher soil temperature than PH-PEG-ESO (except for the first and last date of measurements), but not higher SPAD units. Hence, factors different from soil temperature

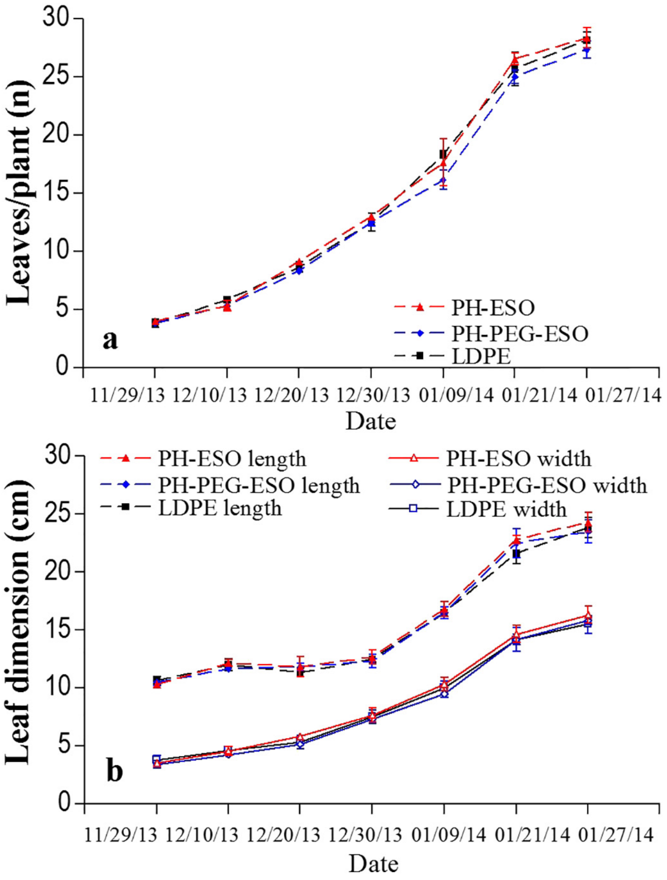

Fig. 3. Test-plant growth in terms of leaf number (a), leaf length and width (b), according to mulching treatments: PH-ESO, PH-PEG-ESO, LDPE (bars represent standard error). On each date of measurement, leaf number was assessed on 2 plants for each of 10 replicates, while leaf dimensions were assessed on 4 leaves for each of 10 replicates per treatment. All variables: on each date, not significant differences at ANOVA (F test at p = 0.05).

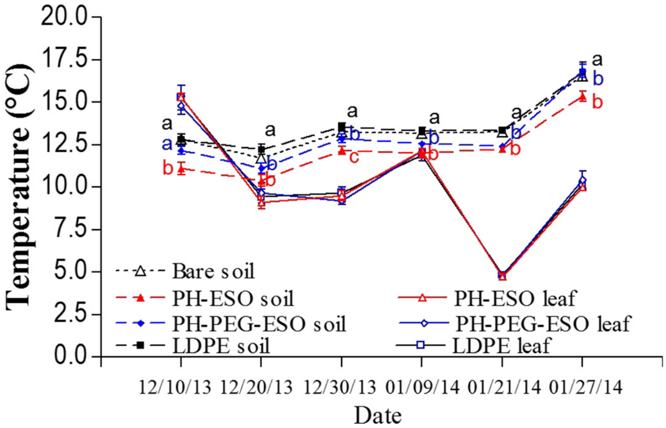

Fig. 2. Soil temperature and leaf temperature, measured at about 10:00 a.m., according to mulching treatments: PH-ESO, PH-PEG-ESO, LDPE (bars represent standard error). On each date of measurement, 6 leaf temperature readings and 3 soil temperature readings per replicate were taken on each of 10 replicates per treatment. Soil Temperature: on each date, different letters indicate significant difference at p ≤ 0.05 (Duncan test). Leaf temperature: on each date, not significant differences at ANOVA (F test at p = 0.05).

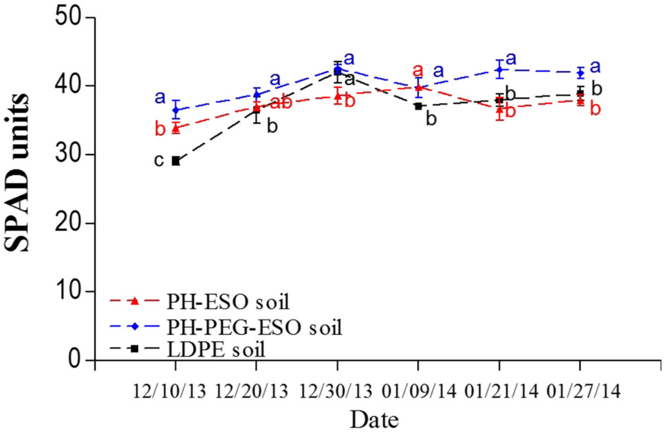

Fig. 4. Relative chlorophyll content (as SPAD Units) during test-plant growth, according to mulching treatments: PH-ESO, PH-PEG-ESO, LDPE (bars represent standard error). On each date of measurement, 8 readings were taken for each of 10 replicates per treatment. On each date, different letters indicate significant differences at p ≤ 0.05 (Duncan test).

Table 4
Dry matter percentage, index of total polyphenol content, and of antioxidant activity in leaves, according to the mulching treatments. Mean value ± standard error. Data were taken on 4 leaves for each of 10 replicates per treatment.

	PH-ESO	PH-PEG-ESO	LDPE
Dry matter (% fw)	5.01 ± 0.58a	5.00 ± 0.19a	4.94 ± 0.24a
TPP (mg gallic acid/100 g dry matter)	81.01 ± 6.511b	83.95 ± 7.01b	98.88 ± 7.45a
AA (mg Trolox/100 g dry matter)	1714.88 ± 40.79b	1683.33 ± 41.21b	1914.65 ± 87.54a

Within row, different letters indicate significant difference at $p \leq 0.05$ (DMRT).

should have influenced the SPAD readings, for example, the level of other soil nutrients, such as phosphorus and calcium, whose content in leaf tissues is subjected to decline as soil temperature increases (Verdial et al., 2001). Pitchay et al. (2014) found SPAD readings ranging from 30 to 34 units in mature lettuce leaves having normal concentration of nitrogen and other chemical elements, and readings ranging from 35 to 45 units in leaves having low potassium concentration, since the leaf color tuned darker. On the whole, as for the leaf nitrogen content, we can estimate that, at the harvest time, all treatments had a normal level, and that the innovative coatings did not exert any negative effect on leaf accumulation of this chemical element that plays key-role for both plant growth and consumer health.

Leaf dry matter assessed at harvest was about 5% of fresh weight in all treatments (Table 4), hence, its accumulation did not change using the two spray mulches alternative to the commercial black plastic film.

TPP varied in a range of about 80–100 mg gallic acid/100 g dry matter (Table 4). TPP content did not differ between PH-ESO leaves and PH-PEG-ESO leaves, but leaves of both these treatments had, on average, 17% less TPP than those of the LDPE one.

AA showed the same trend and was 11% lower for the two spray treatments than for the LDPE one (Table 4).

Mulch influence on TPP and AA was pointed out in other studies, but most of them were focused on reflective mulches, where the solar radiation environment was likely implicated. For not reflective mulches, Morra et al. (2016) found that polyphenols and other antioxidant compounds increased in strawberry fruits when MaterBi® biodegradable extruded film was used instead of LDPE, but they could not clear which type of environmental factor, or abiotic stress, was correlated to that result. It is well-known that both cultural system and environment affect production and accumulation of plant antioxidant compounds (Wang et al., 2002), but, under field conditions, it is hard to separate specific elicitor effects. Possibly, monitoring the evolution of phenolic contents throughout the growing cycle, in addition to taking into account more cultural and environmental variables, can provide further indications useful to understand more about the influence exerted by soil mulching on nutritional value of fruit and vegetables.

3.4. Influence of the mulches on soil physical and chemical parameters

Soil temperature under the mulches measured around 10:00 a.m. averaged from 11.32 ± 0.2 °C (recorded on 20/12/2013) to 16.25 ± 0.1 °C (recorded on 27/01/2014). At the same time, the temperature of the control soil without mulching coating ranged from 11.73 ± 0.1 °C (recorded on 20/12/2013) to 16.58 ± 0.1 °C (recorded on 27/01/2014). The temperature of the soil mulched with PH-ESO was colder than the temperature of the soil mulched with LDPE and PH-PEG-ESO; the difference ranged between 1.08 °C and 1.83 °C (Fig. 2). The LDPE film was particularly efficient in warming the substrate and retaining the thermal energy.

The soil at the beginning of the trial (T0) was characterized by sub alkaline pH, low EC, very low TC, good endowment of OC, TN and P_{av} (Table 5).

At the end of the trial, the soil mulched by LDPE showed a significant but slight increase of the pH while its EC value was significantly lower with respect to the other treatments. The pH could have been slightly modified because of the higher temperature reached by LDPE plots that enhanced the soil enzymatic activities, particularly the urease one, releasing higher amount of NH_4^+ from the urea added with the initial soil fertilization. The higher EC values recorded in the biopolymers mulched plots could be the results of their mineralization that released inorganic nutrients in the soil, increasing its EC values. In addition, the soil covered with PH-PEG-ESO showed, at the end of the experiment, a slight but significant increase of the OC, possibly due to the more complex composition of that material with respect to the PH-ESO (Table 1). In fact, the soil microbial community could have mineralized faster the organic matter of the latter biopolymer, while the more recalcitrant nature of PH-PEG-ESO results in a slower decomposition of the sprayed coating, possibly releasing organic moieties from that mulch that results, in the short period, in a higher soil OC content.

The TN of the PH-PEG-ESO soil resembled the same behavior of OC since that mulching coating showed more N compounds in its composition (Table 1).

The LDPE and PH-PEG-ESO soils showed the highest value of P_{av}. Even for this parameter, the soil temperature could have played a major role, enhancing the activities of phosphatases. In addition, a possible higher concentration of CO_2 occurred in the same soils due to the higher respiration of roots and soil microbial community because of the temperature and, according to Sposito (2008), that environmental condition induces an increase of P solubility in alkaline soil.

4. Conclusions

Our findings showed that novel polymeric protein-based biocomposites can be used as water dispersions to generate, in situ, biobased mulching coatings by the spray technique. The biodegradable coatings showed agronomic performances comparable with the ones of a commercial low density polyethylene mulching film, maintaining the mulching effect for the requested cultivation period and ensuring a similar rate of plant growth and dry matter accumulation. The study of the mulch influence on phytochemical content in lettuce seedlings should be deepened in order to identify eliciting environmental factors and, possibly, improve the new mulching materials looking also to their effect on the plant nutritional value.

The sprayed coatings, in short period, did not modify the pH of the soil, while increased the soil EC value due to their biodegradable nature. Apparently, for its chemical composition and performances, the PH-

Table 5
Main soil parameters at the beginning of the trial (T0) and after the crop cycle under each mulching coating. Mean value ± standard error. The values are means of 5 replicates.

	pH	EC ($dS\ m^{-1}$)	TC ($g\ kg^{-1}$)	OC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	C/N	P_{av} ($mg\ kg^{-1}$)
T0	7.80 ± 0.01b	0.74 ± 0.01a	14 ± 2.1a	19.0 ± 0.0b	1.86 ± 0.15ab	10.2	36.7 ± 0.9b
PH-ESO	7.85 ± 0.25b	0.83 ± 0.31a	15 ± 1.0a	18.7 ± 0.3b	1.97 ± 0.14ab	9.5	35.4 ± 0.3b
PH-PEG-ESO	7.85 ± 0.22b	0.64 ± 0.27a	18 ± 2.2a	19.7 ± 0.4a	2.05 ± 0.14a	9.6	41.3 ± 0.7a
LDPE	8.15 ± 0.03a	0.20 ± 0.01b	15 ± 1.1a	18.6 ± 0.5b	1.81 ± 0.07b	10.3	48.2 ± 6.4a

The values in each column when followed by a different letter are significantly different at $p \leq 0.05$ (DMRT).

PEG-ESO has to be preferred because it induced higher availability of N and P, and a temporary slight OC enrichment.

The use of biodegradable hydrolyzed proteins-based coatings could increase the sustainability of the agricultural commodities by using co-products and polluting wastes from the leather industry.

Nomenclature

AA	index of antioxidant activity
ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid
CB	carbon black
C/N	carbon/nitrogen ratio
dm	dry matter
DMRT	Duncan multiple range test
E	Young's modulus
EC	electrical conductivity
EDA	ethylene diamine
ESO	epoxidized soybean oil
EVA	ethylene-vinyl acetate
fw	fresh weight
LDPE	low density polyethylene
Lign	lignin
LWIR	long wave infrared radiation
OC	organic carbon
P_{av}	available phosphorous
PAR	photosynthetically active radiation
PEG	poly(ethylene glycol) diglycidyl ether
PH	protein hydrolysates
PMDA	pyromellitic dianhydride
T0	soil at the beginning of the trial
TC	total carbonates
TN	total nitrogen
TPP	index of total polyphenol content
UV	ultra violet
WF	wood flour
W_1	weight of the starting dry specimen
W_3	weight of the dry specimen after water immersion
ε_b	elongation at break
λ	wavelength
σ_b	tensile strength at break
$\tau(\lambda)$	spectral transmissivity at wavelength λ

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