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# Release of volatile compounds and biodegradability of active soy protein lignin blend films with added citronella essential oil

Mirari Y. Arancibia [a,b], M. Elvira López-Caballero [a,*], M. Carmen Gómez-Guillén [a], Pilar Montero [a]

[a] Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), C/ José Antonio Novais, 10, 28040 Madrid, Spain[1]
[b] Technical University of Ambato (UTA), Av. Los Chasquis y Río Payamino, Ambato, Ecuador

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ABSTRACT

Active biodegradable bilayer films based on soy protein isolate, lignin and formaldehyde were developed. These films showed high water resistance and malleability, making them suitable for use in extreme environmental conditions. The presence of lignin in the formulation provided greater protection against light, especially UV. With the addition of 3% w/w citronella essential oil, the films showed good antifungal activity against *Fusarium oxysporum* pathogen microorganism in bananas. When the bananas were covered by the films during storage, there was a noticeable reduction in total aerobic mesophiles and moulds and yeasts. As the films aged (1, 3, 6 months), in both controlled and environmental conditions, the main active compounds in the citronella essential oil, citronellal and geraniol, were progressively released from the film matrix, citronellal more than geraniol. Over 30 days of soil degradation, the films lost around 30% of weight irrespective of the conditioning time.



## 1. Introduction

It is a common practice in agriculture to place bags over growing fruits to prevent damage from insects. This practice ensures that the product arrives at the market free of blemishes or bugs, and thus favours food quality and preservation (Tabilio et al., 2013). In this respect, for instance, it has been observed that storing fruits inside plastic bags prevents chilling injury (Fallik et al., 2009). However, when the bags are removed, they must either be taken away, which involves additional cost, or left on the ground—with consequent environmental damage if they do not degrade in a reasonable period of time.

Biopolymers offer potential as a way to overcome the limitations of synthetic plastics. They have good film forming properties, which makes them suitable for many applications, and as packaging material in general (Van de Velde & Kiekens, 2002). Biopolymers are much more biodegradable than plastics. Biodegradation rates of biopolymer based materials have been reported at between 3 and 33 days (Dalev, Patil, Mark, Vassileva, & Fakirov, 2000; González, Strumia, & Alvarez Igarzabal, 2011; Martucci & Ruseckaite, 2009; Patil, Dalev, Mark, Vassileva, & Fakirov, 2000). Biodegradation depends on various factors, some of them characteristics of the film such as type of biopolymer, structure, cross-linking, composition, chain mobility, crystallinity and hydrolytic enzymes (Rizzarelli, Puglisi, & Montaudo, 2004). Biopolymer degradation behaviour is thought to be determined by environmental conditions, especially soil composition, presence of microorganisms and moisture conditions (González et al., 2011).

Soy protein isolate, a biodegradable and environmentally friendly biopolymer, has potential for use as a packaging material in biodegradable films or coatings. Chemical treatments can be used to modify properties so as to produce films with good physicochemical characteristics and also allow them to act as carriers for active compounds. Essential oils have been used as antimicrobial compounds and insect repellents for these kinds of purposes. Citronella essential oil possesses insect repellent, herbicidal and antimicrobial properties and can therefore be used to replace chemical fungicides and so avoid the potentially harmful effects of the latter on aquatic life and human health (Moraes, Elfvendahl, Kylin, & Molander, 2003). Mosquito repellent and weed control formulations in organic agriculture look promising (Arancibia et al., 2013), but these natural herbicides act very rapidly and their efficacy is limited because of their rapid volatilization (Dayan, Cantrell, & Duke, 2009; Thorsell, Mikiver, Malander, & Tunón, 1998). On the

* Corresponding author. Tel.: +34 31 5492300; fax: +34 91 5493627.
*E-mail address:* elvira.lopez@ictan.csic.es (M.E. López-Caballero).

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other hand, essential oils can be trapped and released slowly from a film matrix to make "active packaging", one of the most promising innovations at this time (Licciardello, Muratore, Suma, Russo, & Nerín, 2013).

The aim of this work was to determine the release of active volatile compounds from films based on soy protein isolate-lignin-formaldehyde blends with added citronella essential oil. The antimicrobial activity during conditioning of active film and their biodegradation potential were also evaluated.

## 2. Materials and methods

### 2.1. Preparation of bilayer films

The films were prepared by the casting technique using a two-step process for obtaining a bilayer film as previously described by Arancibia et al. (2013). To the first layer, soy protein isolate (SPI) (PRO FAM, ADM, Decatur, IL, USA) was dissolved in distilled water (4 g/100 mL) at room temperature until complete dissolution, then glycerol was added (1% w/v) and pH was adjusted at 8.0 with 2 N NaOH, then formaldehyde (0.035% w/v) was added and stirred by 10 min and pH adjusted to 10.5 using 2 N NaOH. Another solution was prepared dissolving commercial lignin powder (Protobind 1000, Granit R&D SA, Lausanne, Switzerland) in distilled water to a final concentration of 0.6% (w/v) in the film forming solution. The mixture was stirred at 40 °C by 15 min and was alkalinized to pH = ~11.0 to obtain a blend with total solubility. Finally, the two solutions described above were mixed, and 25 mL of the final mixture were spread over a plexiglass plate (144 $cm^2$) and dried at 45 °C in a forced-air oven (Binder FD 240, Tutlingen, Germany) for 6 h. To prepare the second layer, the same method described above was followed, replacing the plasticizer (glycerol) by citronella (*Cymbopogon nardus*, Isabrubotanik S.A, Ambato–Ecuador) essential oil (EO) at 3% (w/w), and homogenizing with Ultra-Turrax (T25, IKA Werke GmbH & Co. KG, Staufen, Germany) 3 min at 17,500 rpm. Thereafter, the lignin solution was added and mixed with magnetic stirring to prevent bubbles formation. The film forming solution containing the essential oil (as second layer) was slowly poured over the first layer and dried at 45 °C in a forced-air oven (Binder FD 240, Tuttlingen, Germany) for 6 h. These films were called **SLC**. The second layer in the films used as control (without EO) were prepared following the same method described above with the addition of glycerol at 1% w/v. These films were called **SL**. The resulting bilayer films SLC and SL were conditioned over a saturated solution of KBr (58% RH) for 3 days prior to analysis.

### 2.2. Film determinations

#### 2.2.1. Thickness

The thickness of SLC film was measured using a micrometer (MDC-25M, Mitutoyo, Kanagawa, Japan), averaging the values of 4–6 random locations in 15 films.

#### 2.2.2. Water barrier properties

The moisture content of each preconditioned SLC film was determined by drying samples of around 0.5 g at 105 °C for 24 h, according to Association of Official Analytical Chemists (A.O.A.C, 1995), at least in triplicate. Water content was expressed as a percentage of total weight.

For the water solubility test SLC films were cut into 40 mm diameter circles and placed in plastic containers with 50 mL distilled water at 22 °C for 24 h. The solution was then filtered through Whatman #1 filter paper to recover the remaining undissolved film, which was desiccated at 105 °C for 24 h. Film solubility *FS* (%) was calculated using the expression $[(W_o - W_f)/W_o] \times 100$, where $W_o$ was the initial weight of the film expressed as dry matter and $W_f$ was the weight of the undissolved desiccated film residue (Blanco-Pascual, Fernández-Martín, & Montero, 2013). All tests were carried out at least in triplicate.

The water resistance was determined following the method described by Blanco-Pascual et al. (2013). SLC films were fixed onto calibrated cells (area 15.90 $cm^2$) and then placed in desiccators containing distilled water at 21°C ± 1 °C. Distilled water (5 mL) was poured over the film surface. The film deformation due to the water effect, the time when the water started to leak and the time when the film broke were annotated over a 30-days period. All tests were carried out at least in triplicate.

#### 2.2.3. Colour and light properties

The colour values $L^*$ (lightness), $a^*$ (redness/greenness) and $b^*$ (yellowness/blueness) of the SLC films were measured using a Konica Minolta CM-3500d colorimeter (Konica Minolta, Madrid, Spain). D65 illuminant (Daylight) and D10 standard observer were used. Chroma ($C^*_{ab}$) value was calculated using the equation: $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$ as was described by Nuñez-Flores et al. (2013). Measurements were taken at a number of 5 locations in different film portions and each reported value was the mean of at least 15 measurements.

The light barrier properties and transparency of the films were calculated at least in triplicate using a UV-1601 spectrophotometer (Model CPS-240, Shimadzu, Kyoto, Japan) at selected wavelengths from 200 nm to 800 nm. The films were cut into a rectangle piece and directly placed in the spectrophotometer test cell, using an empty test cell as the reference. Transparency (%*T*) was calculated following the method described by Ifuku et al. (2007).

#### 2.2.4. ATR-FTIR spectroscopy

Film infrared spectra between 4000 $cm^{-1}$ and 650 $cm^{-1}$ were recorded using a Perkin Elmer Spectrum 400 Infrared Spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) equipped with an ATR prism crystal accessory. For each spectrum 32 scans of interferograms were averaged and the spectral resolution was 4 $cm^{-1}$. Background was subtracted using the Spectrum software version 6.3.2 (Perkin–Elmer Inc.) Measurements were performed at 21°C ± 1 °C. Prior to the analysis the SLC films were stored for 1, 3, and 6 months at 58% RH in desiccators at 21°C ± 1 °C (batch: 58%). In order to simulate environmental conditions and changes that could suffer the films when were applied in an agricultural system, other batch with SLC films was stored outside under a shade cover for 1, 3 and 6 months (batch EC). The temperature and relative humidity data endured by this batch are indicated in Table 1. Each film was placed on the surface of the ATR crystal, and pressed with a flat-tip plunger until spectra with suitable and stable peaks were obtained. All experiments were performed at least in duplicate. Background subtraction and second derivative of amide I and amide II band were done using the Spectrum software version 6.3.2 (Perkin–Elmer Inc.).

#### 2.2.5. Release of citronellal and geraniol

The head-space volatiles were analysed by GC–MS (Agilent 6890N GC system, Palo Alto, CA, USA, coupled directly to a 5973 MS) with an integrated headspace autosampler Turbomatrix HS 40. Injector temperature was held at 220 °C. Split injection was conducted with a split ratio of 100:1. Injected volume was 0.2 μL. Carrier gas was helium at 1.3 mL/min. The GC system was equipped with a HP-5MS capillary column (30 m × 0.25 mm; 0.25 μm of film thickness). The oven temperature program was 45 °C for the first 2 min, rising to 210 °C at a rate of 15 °C/min and then rising to 240 °C at a rate of 50 °C/min and held for 5 min. Spectra were obtained on electron impact at 70 eV in the selective ion