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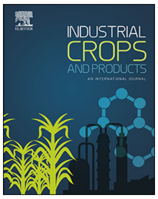

Active polylactic acid film incorporated with green tea extract: Development, characterization and effectiveness

Cristiana Martins[a,1], Fernanda Vilarinho[b,1], Ana Sanches Silva[c,d,*], Mariana Andrade[b], Ana V. Machado[e], M. Conceição Castilho[a], Arsénio Sá[e], Américo Cunha[e], M. Fátima Vaz[f], Fernando Ramos[a,g]

[a] Faculdade de Farmácia, Universidade de Coimbra, Coimbra, Portugal
[b] Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P., Lisboa, Portugal
[c] Instituto Nacional de Investigação Agrária e Veterinária, (INIAV), I.P., Vairão, Vila do Conde, Portugal
[d] Centro de Estudos de Ciência Animal (CECA), Universidade do Porto, Porto, Portugal
[e] Instituto de Polímeros e Compósitos/I3N, Universidade do Minho, Campus de Azurém, Guimarães, Portugal
[f] IDMEC, Instituto Superior Técnico, Departamento de Engenharia Mecânica, Universidade do Lisboa, Lisboa, Portugal
[g] REQUIMTE/LAQV (Rede de Química e Tecnologia/Laboratório Associado para a Química Verde), Faculdade de Farmácia da Universidade de Coimbra, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal

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ABSTRACT

A new antioxidant active packaging film has been developed based on polylactic acid (PLA) in which green tea extract (GTE) has been immobilized by extrusion. Two GTE concentrations were tested, 1% (w/w) and 2% (w/w). Four GT samples were compared regarding their antioxidant capacity (by DPPH radical scavenging method and β-carotene bleaching method), total phenolics content (TPC) and total flavonoids content (TFC). The commercial GTE presented with highest antioxidant capacity by the DPPH radical assay ($EC_{50} = 0.12 \pm 0.00$ mg/mL) and the highest TPC (416 ± 9.95 mg gallic acid equivalents (GAE)/g extract) and it was selected to be incorporated in the active film. Films were evaluated regarding their mechanical properties (*e.g.* tensile strength decreased 12% with the incorporation of GTE in the PLA matrix and strain at break increased 9.6 and 36% with the addition of 1 and 2% of GTE) and water vapour transmission rates (water-vapour barrier properties improved with the addition of higher amounts of GTE).

The study of the effectiveness of PLA/GTE films against lipid oxidation was performed by the following methods: peroxide value, *p*-anisidine value, thiobarbituric acid reactive substances (TBARS) assay and hexanal monitoring, after packaging smoked salmon slices during different storage times (0, 7, 15, 30, 45 and 60 days). The results showed that the incorporation of GTE in the PLA films protects the smoked salmon from lipid oxidation in the different storage times tested. However, additional studies should be performed to better understand the GTE mechanism of action as the results suggest a pro-oxidant effect of PLA/GTE 2% after 60 days of storage and to evaluate the potential antimicrobial activity of active films.

1. Introduction

The packaging allows preserving the safety and quality of food during storage, transportation, and above all, to prolong the shelf life of the food, avoiding unfavourable factors or conditions, such as deteriorating microorganisms, chemical contaminants, oxygen, moisture, light, external forces, among others (Yam et al., 2005; Marsh and Bugusu, 2007).

The use of biomaterials for food packaging is considered an environmentally friendly alternative, since it reduces the use of plastics from non-renewable resources or non-biodegradable and their accumulation in the environment (Jamshidian et al., 2010). PLA is produced from L-lactic acid, that is derived from the fermentation of corn or sugar beet. One of its main characteristics is to be biodegradable (Paul et al., 2003).

Changes in consumers' preference for safer, healthier and more convenient foods have led to innovations in packaging technologies. In this context, the concept of active packaging emerged, based on the

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[*] Corresponding author at: Instituto Nacional de Investigação Agrária e Veterinária, (INIAV), I.P., Vairão, Vila do Conde, Portugal.
E-mail address: ana.silva@iniav.pt (A. Sanches Silva).
[1] Authors contributed equality for this paper.

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positive interaction between the packaging and the food or its environment, in order to provide them with active protection (Biji et al., 2015). This is opposite to the main safety feature of conventional packaging intended to come into contact with food, which is to be as inert as possible (Silva et al., 2008; Sanches Silva et al., 2009). According to Regulation (EC) No. 1935/2004/EC (2004) and Regulation (EC) No. 450/2009 (2009), "*active materials and articles are intended to extend the shelf life or to maintain or improve the condition of packaged food*". They are designed to deliberately incorporate substances that can be released or absorbed into or from the packaged food or environment surrounding the food (Biji et al., 2015). In this sense, and seeking to satisfy the high concerns about consumer health and environmental problems, research on this type of packaging is currently focused on the use of natural components and biodegradable packaging materials (Carrizo et al., 2014). Some of these biodegradable polymers are also edible such as soy protein (Yu et al., 2018); cassava starch (Assis et al., 2017) and whey protein (Ribeiro-Santos et al., 2017). Substances with antioxidant or antimicrobial potential are available from a variety of natural sources, namely aromatic plants, fruits and vegetables (Yang et al., 2016a,b; Crizel et al., 2016; Cardoso et al., 2017).

Green tea is obtained from the leaves of *Camelia sinensis* L. and it is recognised by its antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory properties (Basnet et al., 2015; Perumalla and Hettiarachchy, 2011). Green tea is a rich source of polyphenol antioxidants, particularly catechins. GTE also contains other compounds, such as flavonoids and phenolic acids but in lower proportion (Lorenzo and Munekata, 2016). The GTE is reported to have antioxidant capacity, the main compounds responsible for this antioxidant capacity are gallic acid and eight major catechins: (+)-catechin (C), (–)-epicatechin (EC), (–)-catechin gallate (CG), (–)-epicatechin gallate (ECG), (–)-gallocatechin (GC), (–)-epigallo- catechin (EGC), (–)-gallocatechin gallate (GCG), and (–)-epigallocatechin gallate (EGCG) (López de Dicastillo et al., 2011).

Green tea extracts have already been incorporated in different polymeric matrices. For instance to polyethylene (López de Dicastillo et al., 2013); ethylene vinyl alcohol (López-de-Dicastillo et al., 2012; Muriel-Galet et al., 2015); agar and agar-gelatin (Giménez et al., 2013; López de Lacey et al., 2014); silver carp skin gelatin (Wu et al., 2013); chitosan (Siripatrawan and Harte, 2010; Siripatrawan and Noipha, 2012); *gelidium corneum*-gelatin (Hong et al., 2009) and soy protein (Theivendran et al., 2006).

Most of the studies focused on the optimization of the production conditions of the GTE active films and on the evaluation of its physical properties (thickness, tensile strength, elongation at break, light transmission, transparency, water vapour permeability, water resistance, water solubility, thermal stability) (Giménez et al., 2013; Wu et al., 2013) or on the release of active compounds from the film to food simulants (López-de-Dicastillo et al., 2012; Giménez et al., 2013; Muriel-Galet et al., 2015), evaluation of antioxidant activity (Giménez et al., 2013; Siripatrawan and Harte, 2010; López de Dicastillo et al., 2013; Muriel-Galet et al., 2015) and antimicrobial activity (Giménez et al., 2013; Muriel-Galet et al., 2015). GTE has been applied as a preservative treatment or incorporated in food packaging to different food matrices aiming to extend its shelf-life. These matrices include pork loins (Hong et al., 2009), sardines (López-de-Dicastillo et al., 2012); turkey frankfurters (Theivendran et al., 2006), pork sausages (Siripatrawan and Noipha, 2012), hake fillets (López de Lacey et al., 2014) and fresh-cut lettuce (Martín-Diana et al., 2008). The emerging technological field of nanotechnology is already applied in the production of active films. Wrona et al. (2017) have successfully developed a new active film material based on hydroxypropyl-methylcellulose (HPMC) containing poly(lactic acid) (PLA) nanoparticles (NPs) loaded with antioxidant (AO) green tea extract (GTE). The physico-chemical properties of the films and the antioxidant capacity of the GTE released from the active films was studied. The results suggested that the material could potentially be used to extend the shelf life of high fat food products.

Fish, in particular salmon, is very perishable, therefore several treatments have been applied to extend their shelf life such as cooling, super chilling, freezing, use of modified atmosphere packaging, smoking, irradiation or new technologies such as cold plasma or high pressure treatment (Broekaert et al., 2017; Albertos et al., 2016; Lyu et al., 2018). Smoked Atlantic salmon (*Salmo salar*) is highly appreciated cold ready-to-eat food mainly due to its flavour, colour, taste and associated health benefits due to their omega 3 fatty acids and protein content (Sampels, 2013; Martinsdóttir et al., 2014). Active packaging has recently been applied to smoked salmon. Baek and Song (2018) applied *Gracilaria vermiculophylla* extract films containing zinc oxide nanoparticles and they were able to obtain antibacterial activity and low degree of oxidation. Albertos et al. (2016) developed an olive leaf gelatin film which significantly reduced the growth of *Listeria monocytogenes* over storage.

The aim of this paper was to produce a biodegradable active film based on PLA, by blown film extrusion, incorporated with GTE. The GTE was characterized regarding its antioxidant capacity, total phenolics content (TPC) and total flavonoids content (TFC) and compared with other three non-commercial GTE. The new film was evaluated regarding its mechanical properties, water vapour transmission and effectiveness to inhibit lipid oxidation of model food. Smoked salmon was selected due to be a much appreciated high-fat content food with considerable high value in the market. To our knowledge this is the first time that an active PLA film containing GTE is evaluated regarding mechanical and barrier properties as well as its effectiveness in inhibiting lipid oxidation of smoked salmon.

2. Material and methods

2.1. Green tea samples

Four green tea samples were compared, two of them consisted of dried leaves of *Camellia sinensis* L., another comprised whole green leaf powder, and finally the fourth sample was a commercial GTE. Two varieties of green tea were obtained from dried leaves (*Hysson* and *Encosta de Bruma*) of the brand *Gorreana* (Azores, Portugal). *Hysson* green tea is produced from the first three leaves of the tea plant (*Camellia sinensis*) which are harvested in July and August, while the green tea "*Encosta da Bruma*" is produced exclusively from the terminal bud and the first leaf of the plant of tea, grown on the highest ridge of the Gorreana tea plantation and it is harvested in late July and early August. These samples were purchased from the online store of the company *Plantações de Chá Gorreana, Lda*. Green tea in capsules was purchased on a commercial surface in the region of Coimbra, whiles the commercial extract (Batch L704906709), was acquired from MyProtein.

The samples were stored at room temperature and protected from light and moisture.

2.2. Chemicals and reagents

Methanol (analytical grade) was purchased from VWR Chemicals (Fontenay-sous-Bois, France). Methanol and sulfuric acid (HPLC grade), anhydrous sodium sulphate, isooctane, glacial acetic acid, trichloroacetic acid, orthophosphoric acid (all analytical grade), sodium carbonate, absolute ethanol, sodium hydroxide, chloroform, all were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT), 2-(1,1-dimethylethyl)-1,4-benzenediol (TBHQ), (-)-epicatechin (purity ≥90%), (-)-epigallocatechin gallate (purity ≥80%), (-)-epigallocatechin gallate (purity ≥95%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (purity 97%), Tween® 40, linoleic acid, β-carotene, Phenol reagent Folin & Ciocalteu`s, sodium nitrite, aluminium chloride, potassium iodide, soluble starch, *p*-anisidine, thiobarbituric