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Novel acrylic polymers for food packaging: Synthesis and antioxidant properties

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ABSTRACT

In the present work, a strategy for the synthesis of novel polyacrylates covalently linked to natural antioxidants was accomplished in a two-step process. First, monomers were prepared *via* lipase-catalyzed transesterification of acrylic acid methyl ester with tyrosol (T) or hydroxytyrosol (HT). Then, tyrosyl acrylate (TA) and hydroxytyrosyl acrylate (HTA) were subjected to radical homopolymerization to give poly(tyrosyl)acrylate (PTA) and poly(hydroxytyrosyl)acrylate (PHTA), respectively. The monomers and the corresponding homopolymers were characterized with FT-IR and NMR techniques and by Folin-Ciocalteu method to give an estimation of the available phenolic groups, as T and HT equivalents, linked to the polymers. The results of DPPH radical scavenging assay indicate that the free radical scavenging activity of tyrosol and hydroxytyrosol was almost completely retained in the corresponding monomers and polymers. In addition, polyacrylate films did not exhibit any cytotoxic activities *in vitro* on RAT1 normal fibroblast cells, using MTT assay.

1. Introduction

The principal function of packaging is protection and preservation from external contamination (Robertson, 2006). This function involves retardation of deterioration, extension of shelf life, and maintenance of quality and safety of packaged food. Packaging protects food from environmental influences such as heat, light, the presence or absence of moisture, oxygen, pressure, enzymes, spurious odors, microorganisms, insects, dirt and gaseous emissions. Traditional food packages are passive barriers designed to delay the adverse effects of the environment on the food product. Otherwise, “active packaging” goes beyond the traditional role of packaging by imparting specific, intentional functionality to the packaging system. An active packaging presents on its surface active functional groups that interact with the food therein contained inhibiting its deterioration and preserving its organoleptic characteristics (Català & Gavara, 2001). Developments in active packaging have led to advances in many areas, including delayed oxidation and controlled respiration rate, microbial growth, and moisture migration. (Day, 2008; Kerry, O'Grady, & Hogan, 2006; Ozdemir & Floros, 2004). Antioxidant packaging includes antioxidant substances in food packaging systems to impart antioxidant activity. These agents can be applied into the packaging systems in different forms, mainly including independent sachet packages, adhesive-bonded labels, physical adsorption/coating on packaging material surface, being incorporated into packaging polymer matrix, multilayer films, and covalent immobilization onto the food contact packaging surface (Tian, Decker, & Goddard 2013). Both synthetic and natural antioxidant compounds are widely used in food and personal care products to increase stability, shelf life and preserve nutritional quality. There has been a growing interest in the substitution of synthetic food antioxidants like butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) by natural ones because they are suspected carcinogens and the US Food and Drug Administration (FDA) has therefore, restricted their use (Code of Federal Regulations, 2012; Kim & Lee, 2004; Mandlekar & Kong, 2000). Therefore, the use of natural antioxidants, such as hydroxy and polyhydroxy derivatives of cinnamic acids, is to be preferred to synthetic ones. Hydroxytyrosol (2-(3,4-dihydroxyphenyl)ethanol) and tyrosol (2-(4-hydroxyphenyl)ethanol) are phenolic compounds naturally present in olive trees (*Olea europaea* L.), fruits, olive oil and olive mill waste (‘alperujo’), both in their molecular form or as part of more complex molecules, mostly as esters of elenolic acid (Bendini et al., 2007; Silva, Gomes, Leitao, Coelho, & Boas, 2006). Several studies have reported various biological activities of hydroxytyrosol and tyrosol, such as antimicrobial (Bisignano et al., 1999), anti-

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carcinogenic (Owen et al., 2000) and anti-inflammatory (Haloui, Marzouk, Marzouk, Bouraoui, & Fenina, 2011), but especially as powerful antioxidant agents (Aeschbach et al., 1994; Visioli, Poli, & Galli, 2002; Perez-Bonilla, Salido, van Beek, & Altarejos, 2014). In fact, the antioxidant capacity of hydroxytyrosol is higher than that of other phenolic compounds with similar structures and other natural antioxidants such as vitamin C, vitamin E or resveratrol (Garcia-Garcia, Hernandez-Garcia, Sanchez-Ferrer, & Garcia-Carmona, 2013). In addition, a direct dissolution of the antioxidant in the food matrix is sometimes inconvenient because it would result in undesirable alteration in the product composition. In this perspective, the development of polymeric materials containing the active moiety covalently bound to their surface is an interesting alternative. Among the main advantages of synthetic polymers utilized in packaging industries such as polypropylene, poly(vinyl chloride), and polyethylene, their excellent physicochemical properties as well as the possibility of their being processed and their low cost can be mentioned. Poly(methyl methacrylate) (PMMA), owing to its excellent surface hardness, UV and abrasion resistance, and a myriad of coloring options from transparent to deep color, has been widely used in various sectors including transportation, architecture, electronics, and health (Unnikrishnan, Smita, & Nayak, 2014).

However, these commodities present an inert surface. Therefore, one of the approaches to obtain active packages from synthetic polymers is carried out using surface modification techniques through chemical processes (Arrua, Strumia, & Nazareno, 2010). Recently, there has been an increasing interest in the immobilization of functional compounds onto the food contact surface of packaging by covalent linkages (Tian, Decker, & Goddard 2012). Covalent bonds can provide the most stable linkage between substrate film surface and active agents, a potential regulatory benefit. The active agents should not be labeled as food additives, as they are not likely to migrate from the package to the food (Tian, Decker, McClements, & Goddard 2014).

Taking into account the above-mentioned precedents the main aim of this work was the synthesis and characterization of novel polyacrylates covalently linked to natural antioxidants, tyrosol (T) and hydroxytyrosol (HT). The active polymers could be used for food packaging applications as active monomaterial in direct contact with the food. In this perspective, the efficiency of the new antioxidant materials described herein in protecting a real food sample was also evaluated. Moreover, screening the polyacrylate derivatives for cytotoxicity properties on RAT1 fibroblast cell proliferation provides some level of assurance of their safety for food application.

2. Materials and methods

2.1. Materials

Immobilized *Candida antarctica* lipase B (CALB, Novozym®435) was supplied by Novozymes A/S. Methyl acrylate (99%), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), tyrosol (98%) were purchased from Sigma Aldrich (Italy). All chemicals were used as received. The synthesis of 2-(3,4-dihydroxyphenyl)ethanol was performed according to literature method (Capasso, Evidente, Avolio, & Solla, 1999). Dimethylformamide (DMF) was freshly distilled.

2.2. Characterization

All NMR spectra were recorded on a Bruker Avance 300 Ultrashield spectrometer equipped with a 5-mm probe. Proton (^{1}H) and carbon (^{13}C) measurements were performed in $CDCl_3$ solutions at 300 and 75 MHz, respectively. All chemical shifts are reported in parts per million (δ, ppm), downfield to tetramethylsilane (Me_4Si) as an internal standard (δ = 0.00 ppm), or referenced to residual solvent $CHCl_3$ (^{1}H NMR 7.27 ppm and ^{13}C NMR 77.0 ppm); coupling constants J are given in Hertz. The FTIR spectra were performed using a Perkin-Elmer Paragon 1000 PC FT-IR spectrometer. Quantitative analysis of ascorbic acid in natural juice samples was performed by high-performance liquid chromatography (HPLC). The determinations were carried out using a Shimadzu HPLC system, equipped with two SCL-10-AVP pumps, an SLC-10-AVP controller, and an SPD-20A UV–vis detector: the column used was a discovery HS C18 (Supelco) (250 mm × 4.6 mm id, 5 μm particle size).

2.3. Synthesis of the monomers tyrosyl acrylate (TA) and hydroxytyrosyl acrylate (HTA)

The reaction was an enzymatic transesterification where the primary hydroxyl group of tyrosol and hydroxytyrosol was regioselectively acylated by acrylic acid methyl ester *via* the acyl enzyme complex. In a typical reaction, antioxidant compound (1.6 mmol), acrylic acid methyl ester (16 mL, 176 mmol), *Candida antarctica* lipase B (200 mg, immobilized) were stirred for 24 h at 50 °C. The enzyme was filtered off, the solvent was evaporated under reduced pressure and the product was purified by column chromatography (SiO_2, hexane: acetone = 9:1; TA yield 96%, HTA yield 75%). TA and HTA were analyzed by GC–MS, FTIR, ^{1}H and ^{13}C NMR.

2.3.1. Characterization of tyrosyl acrylate (TA)

MS *m/e* 192 (M^+, 1), 121 (11), 120 (100), 107 (36), 77 (11), 55 (18); IR (neat) 3392 (br, s), 3021 (w), 2960 (w), 1704 (s), 1615 (m), 1516 (m), 1411 (m), 1302 (m), 1220 (s), 1064 (m), 983 (m), 812 (m) cm^{-1}; ^{1}H NMR (300 MHz, CDCl3) δ (ppm) 7.12-7.04 (m, 2H, on phenyl ring), 6.82-6.75 (m, 2H, on phenyl ring), 6.40 (dd, *J* = 17.3, 1.4 Hz, 1H, C*H*H=CH), 6.11 (dd, *J* = 17.3, 10.4 Hz, 1H, CHH=C*H*), 5.83 (dd, *J* = 10.4, 1.4 Hz, 1H, CH*H*=CH), 5.77 (s, 1H, OH), 4.33 (t, *J* = 7.1, 2H, CH_2CH_2OC=O), 2.90 (t, *J* = 7.1, 2H, CH_2CH_2OC=O). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 165.5, 154.5, 130.9, 130.1, 129.7, 128.5, 115.5, 65.4, 34.3.

2.3.2. Characterization of hydroxytyrosyl acrylate (HTA)

MS *m/e* 208 (M^+, 1), 137 (10), 136 (100), 123 (28), 77 (8), 55 (18); IR (neat) 3388 (br, s), 2958 (w), 2921 (w), 1699 (s), 1615 (m), 1520 (m), 1446 (m), 1286 (m), 1197 (s), 1065 (m), 983 (m), 812 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 6.82–6.71 (m, 2H, on phenyl ring), 6.66–6.58 (m, 1H, on phenyl ring), 6.40 (dd, *J* = 17.3, 1.4 Hz, 1H, C*H*H=CH), 6.11 (dd, *J* = 17.3, 10.4 Hz, 1H, CHH=C*H*), 5.83 (dd, *J* = 10.4, 1.4 Hz, 1H, CH*H*=CH), 4.31 (t, *J* = 7.1, 2H, CH_2CH_2OC=O), 2.84 (t, *J* = 7.1, 2H, CH_2CH_2OC=O) ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 165.9, 142.8, 141.5, 130.3, 129.3, 127.2, 120.2, 114.9, 114.4, 64.5, 33.3.

2.4. Polimerization of tyrosyl acrylate (TA) and hydroxytyrosyl acrylate (HTA)

In a typical polymerization procedure, the monomer (0.25 mmol) was dissolved in 250 μL of dry DMF (1 M) followed by the addition of the initiator AIBN (0.018 mmol). Polymerization was conducted at 70 °C for 48 h with continuous stirring. PTA yield was 84%, PHTA yield was 91%. PTA and PHTA were analyzed by FTIR, ^{1}H and ^{13}C NMR.

2.4.1. Characterization of poly(tyrosyl acrylate) (PTA)

IR (KBr) 3412 (br, s), 3021 (w), 2962 (w), 1729 (s), 1616 (m), 1519 (m), 1453 (m), 1263 (m), 1166 (m), 1104 (m), 808 (m) cm-1; ^{1}H NMR (300 MHz, CD_3OD) δ(ppm) 7.1–6.9 (m, 2H, on phenyl ring), 6.8–6.6 (m, 2H, on phenyl ring), 4.3–3.9 (m, 2H, CH_2CH_2OC=O), 3.0–2.6 (m,