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Mechanism of action of an antioxidant active packaging prepared with Citrus extract

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ABSTRACT

Active packaging consisting of polyethylene terephthalate (PET) trays coated with a Citrus extract, without and with plasma pre-treatment, can reduce lipid oxidation in cooked meat. The mechanism of action of the packaging was investigated by quantifying the extent of transfer of antioxidant components from the active packaging into cooked turkey meat. Kinetic studies revealed the affinity for water of phenolic compounds and carboxylic acids in the Citrus extract, suggesting their diffusion into the water phase of the meat facilitated their antioxidant effect. Analysis by high-performance liquid chromatography permitted the identification of carboxylic acids and flavanones as major components of the extract. Their quantification in meat after contact with the trays revealed a release of 100% of the total coated amount for citric acid, 30% for salicylic acid, 75% for naringin and 58% for neohesperidin, supporting the release of these components into cooked meat as a mechanism of action of the antioxidant active packaging.

1. Introduction

Interest in active packaging as an approach to improve the quality and increase the shelf-life of food products has grown (De Kruijf et al., 2002; Kerry, O'Grady, & Hogan, 2006); antioxidant active packaging has been a particular focus of attention (Camo, Beltrán, & Roncalés, 2008; Pettersen, Mielnik, Eie, Skrede, & Nilsson, 2004). The amount and rate of release of the antioxidant compounds are fundamental to the extent and duration of the protective effect of the packaging; studies have been undertaken on the production of controlled-release packaging that optimizes the characteristics of the plastic polymer to regulate the release of the active substances (Chen, Lee, Zhu, & Yam, 2012; Nerín, Tovar, & Salafranca, 2008).

As cooked meat is highly susceptible to lipid oxidation (Gray, & Pearson, 1987; Ruban, 2009), an immediate interaction of the active compounds with the food could be advantageous to protect the product. A coating of antioxidants on the surface of the packaging in contact with the food can satisfy this requirement and permit an immediate protection against lipid oxidation. An antioxidant active packaging developed in our laboratory by coating polyethylene terephthalate (PET) trays with a Citrus extract has already been shown to reduce lipid oxidation in cooked turkey meat (Contini et al., 2012). The natural antioxidant used is a mixture of carboxylic acids and flavanones and has also been shown to exhibit antimicrobial activity in meat products (Mexis, Chouliara, & Kontominas, 2012). The aim of the present study was to identify the active components in the extract and investigate the mechanism of action of the active packaging. Our hypothesis was that active components in the Citrus extract coated on PET trays exert their antioxidant activity by migrating from the packaging surface into cooked meat.

2. Materials and methods

2.1. Meat

Turkey breasts (~1.2 kg) were obtained from IGWT Poultry Service Ltd, County Monaghan, Ireland. For the preparation of cooked meat, fresh turkey breast was wrapped in aluminium foil, cooked for ~2 h to an internal temperature of 73 °C and immediately cooled at 4 °C in an ice bath, as described in Contini et al. (2012).

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2.2. Reagents

Citrus extract in powder form containing a mixture of flavanones and carboxylic acids was obtained from Citrox Biosciences, Kimbolton, Cambridgeshire, England. A generic composition of the Citrus extract, as per the manufacturer's specification, was naringin 3.6 g, neohesperidin 1.9 g, rhoifolin 0.4 g, poncirin 0.3 g, naringenin 0.2 g, hesperidin 0.2 g, malic acid 15 g, ascorbic acid 15 g and citric acid 15 g in 100 g of Citrus extract. Chloroform (≥99%), gallic acid (≥98%), methanol (≥99%), phosphoric acid (≥99%), sodium carbonate (≥99.5%), ascorbic acid (≥99%), citric acid (≥99%), malic acid (≥99%), salicylic acid (≥99%), hesperidin (≥95%), naringin (≥95%), neohesperidin (≥90%), poncirin (≥95%) and rhoifolin (≥99%) were obtained from Sigma–Aldrich Ltd, Dublin, Ireland. Recycled polyethylene terephthalate (PET) trays (100 × 150 × 25 mm) were supplied by Holfeld Plastic, Wicklow, Ireland and low-density polyvinylchloride (PVC) catering film (thickness 7.0 μm; O_2 transmission 2000 $cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$) was supplied by Western Plastic Ltd, Galway, Ireland. Screw-cap plastic tubes (50 ml) were supplied by Sarstedt Ltd, Wexford, Ireland. Membrane filters (0.2 μm) were supplied by Pall Life Sciences, Cork, Ireland.

2.3. Preparation of the PET trays coated with Citrus extract

PET trays were coated with Citrus extract (PET-CIT) by spraying a methanolic solution of the extract onto the polymer surface through a Teflon nebulizer mounted on a computer numerical control system cncGraf (Boenigh Electronics, Bonn, Germany), following the procedure described in Contini et al. (2012). A further set of trays was prepared by a different procedure that involved a pre-treatment of the PET surface, consisting of plasma activation of the tray surface (PET-PA) with an atmospheric pressure plasma jet system (PlasmaTreat GmbH, Steinhagen, Germany). Compressed air was used as reagent gas and the plasma procedure carried out using the conditions described in Contini et al. (2013). After the pre-treatment, Citrus extract was nebulized onto tray surface (PET-PA-CIT) as above.

2.4. Measurement of total phenolic components of Citrus extract in meat

Our hypothesis was that if the antioxidant effect of the active packaging required migration of antioxidants from the tray surface into meat then it should be possible to detect Citrus extract components in the meat stored on Citrus coated surfaces. Since phenolic components with known antioxidant effects (Nijveldt et al., 2001) are constituents of Citrus extract, an important first step was to establish if it would be possible to detect these phenolics in meat at a level equivalent to that obtained if all the phenolics on the tray surface migrated into the meat. To do this, Citrus extract dissolved in methanol (13.5 mg ml^{-1}) was added to both raw and cooked turkey muscle at a level of 1.35 mg g^{-1}. This level of addition was calculated from the density of the coating applied to the PET tray area in contact with a 30 × 30 × 5 mm slice (3 g) of turkey meat (Contini et al., 2012). Citrus extract was also added to distilled water for comparison (1.35 mg g^{-1}).

A second experiment was carried out to quantify the release of phenolic compounds into cooked turkey meat in contact with PET, PET-PA, PET-CIT and PET-PA-CIT trays. The meat was cut into 5 mm thick slices using a meat slicer (Medoc, Logroños, Spain) and subsequently cut into 3 g square (30 × 30 mm) pieces which were placed on the trays and stored at 4 °C for 2 days. Meat pieces were removed immediately (day 0) and after 1 and 2 days of storage for measurement of total phenol (TP) content.

The extraction of phenol components from meat was performed following the procedures described by Jang et al. (2008). Meat samples were homogenized in 15 ml of distilled water for 1 min at 8000 rpm using an Ultraturrax T25 (IKA-Labortechnik) and subsequently 9 ml of chloroform were added. The mixture was shaken vigorously and centrifuged for 10 min at 3000 rpm for phase separation. The quantification of TP content in the upper aqueous phase was performed by the Folin-Ciocalteu (FC) procedure described by Harbourne, Marete, Jacquier, & O'Riordan (2009). This involved adding 0.2 ml of aqueous meat extract to 0.5 ml of FC reagent, 1.5 ml of 20% sodium carbonate and 7.8 ml of distilled water. The solution was mixed and left for 2 h for colour development. The absorbance was measured using a Shimadzu UV-1240 spectrophotometer (Kyoto, Japan) at a wavelength of 760 nm. Quantification was done based on a standard curve generated with gallic acid (0.01–0.5 mg ml^{-1}) and TP content was expressed as mg gallic acid equivalent (GAE) g^{-1} of meat.

2.5. Kinetics of release of total phenolic components from Citrus extract-coated trays into water

Square pieces of the PET trays (30 × 30 mm), corresponding to the surface in contact with the meat slices, were cut from uncoated (PET and PET-PA) and coated (PET-CIT and PET-PA-CIT) trays. Each piece was then placed in a weighing boat with 2.1 ml of water which corresponded to the moisture content of 3 g of cooked turkey meat (McCance, & Widdowson, 2002). The samples were then stored at 4 °C for 2 days, to mimic the conditions of meat storage. The weighing boats were covered with PVC catering film to prevent evaporation of the water during storage. The TP content in water was determined immediately (day 0), after 10, 20, and 40 s, after 1, 2, 5, 10, 20 and 40 min, after 1, 2, 3, 4 and 6 h and after 1 and 2 days, using the FC procedure described in Section 2.4.

2.6. Identification and quantification of the Citrus extract components

The identification of Citrus extract components was performed by high-performance liquid chromatography (HPLC) analysis of a solution of the extract and comparing the retention times of the peaks obtained with those of pure standards of the components declared by the provider of the Citrus extract. The Citrus extract (in powder form) was dissolved in methanol (5 mg ml^{-1}), filtered through a 0.2 μm membrane filter and analysed by HPLC, following the method described by Harbourne et al. (2009) with modifications to the mobile phase. The analysis was carried out using an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent Synergi Hydro-RP 80A analytical column (250 mm × 4.60 mm, 4 μm particle size) and a C18 guard column (Phenomenex, Chesire, UK). The mobile phase was (A) 0.1% phosphoric acid in water and (B) methanol. The separation was carried out at 37 °C at a flow rate of 0.8 ml min^{-1} with the following gradient: 0–2 min, 90% A; 2–16 min, 90% A to 10% A; 16–22 min, 10% A to 90% A. The detector used was a diode array detector (DAD) at a wavelength of 210 nm. For the quantification of the main Citrus extract compounds identified, the instrument was calibrated with 6 point calibration curves of their pure standards.

For confirmatory purposes, liquid chromatography-mass spectrometry (LC–MS) analysis was performed using a Waters Acquity HPLC system (Milford, MA, USA), coupled with a triple-quadrupole mass-spectrometer Xevo TQ Waters-Micromass (Manchester, UK) in negative electrospray ionization (ESI^-) with the same chromatogram conditions described for HPLC–DAD analysis.

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