Innovative Food Science and Emerging Technologies 12 (2011) 594–599

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Use of endospore-forming bacteria as an active oxygen scavenger in plastic packaging materials

Tom Anthierens [a], Peter Ragaert [a], Sam Verbrugghe [b], Assia Ouchchen [b], Bruno G. De Geest [c], Bert Noseda [a], Johan Mertens [d], Lynda Beladjal [d], Dirk De Cuyper [e], William Dierickx [e], Filip Du Prez [b], Frank Devlieghere [a,*]

[a] Ghent University, Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, Laboratory of Food Microbiology and Food Preservation, Member of Food2Know, Coupure Links 653, 9000 Ghent, Belgium
[b] Ghent University, Faculty of Sciences, Department of Organic Chemistry, Polymer Chemistry Research Group, Krijgslaan 281 (S4bis), 9000 Ghent, Belgium
[c] Ghent University, Faculty of Pharmaceutical Sciences, Department of Pharmaceutics, Laboratory of Pharmaceutical Technology, Harelbekestraat 72, 9000 Ghent, Belgium
[d] Ghent University, Faculty of Sciences, Department of Biology, Terrestrial Ecology Unit, Ledeganckstraat 35, 9000 Ghent, Belgium
[e] Resilux nv, Damstraat 4, 9230 Wetteren, Belgium

ARTICLE INFO

Article history:
Received 21 April 2011
Accepted 15 June 2011

Editor Proof Receive Date 14 July 2011

Keywords:
PET
Active packaging
Oxygen scavengers
Bacillus
Microorganisms
Spores

ABSTRACT

The incorporation of active oxygen scavengers in polymer packaging materials is essential to allow packaging of oxidation sensitive products. Opposed to the currently available chemical oxygen scavengers, systems based upon natural and biological components could have advantages towards consumer perception and sustainability. A modelsystem for a new oxygen scavenging poly(ethylene terephthalate) (PET) bottle is proposed using an endospore-forming bacteria genus *Bacillus amyloliquefaciens* as the active ingredient. Spores were incorporated in poly(ethylene terephthalate, 1,4-cyclohexane dimethanol) (PETG), an amorphous PET copolymer having a considerable lower processing temperature and higher moisture absorption compared to PET. To asses spore viability after incorporation, a method was optimized to extract spores from PETG using a chloroform/water mixture. Samples were also analyzed using a *Live/Dead Bac*Light Bacterial Viability kit. It was shown that endospores were able to survive incorporation in PETG at 210 °C. Incorporated spores could actively consume oxygen for minimum 15 days, after an activation period of 1–2 days at 30 °C under high humidity conditions.

Industrial relevance: The study describes a modelsystem for the use of incorporated spores genus *Bacillus amyloliquefaciens* as an active oxygen scavenger in PET multilayer bottles using PETG as the middle layer material. Industrially, oxygen scavengers using incorporated viable spores as the active compound could have advantages towards consumer perception, recyclability, safety, material compatibility, production costs, … compared to currently available chemical oxygen scavengers.

1. Introduction

During storage of many packed food products, oxygen is often unwanted because it promotes food deterioration, alteration of color and flavor and spoilage by aerobic micro-organisms (Ozdemir & Floros, 2004). For this reason, packaging of oxygen sensitive products is mainly done in high oxygen barrier materials, often in combination with Modified Atmosphere Packaging (MAP). The weaker gas barrier properties of plastic packaging materials, such as polyethylene terephthalate (PET), compared to glass and metal, restricts their use as a container for oxygen sensitive products (Robertson, 2005). However, even with high barrier packaging materials, the use of MAP or vacuum packaging cannot always completely remove oxygen (Brandon, Beggan, Allen, & Butler, 2009; Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999). The incorporation of active oxygen scavengers (OS) in the polymeric matrix can improve the gas barrier properties of the package and simultaneously remove residual oxygen. Many chemical OS, based on different absorption or reaction systems, are commercially available. Several of these systems consist of an active oxygen scavenging compound incorporated in the packaging material (Kerry, O'Grady, & Hogan, 2006; Ozdemir & Floros, 2004).

In recent years, alternative natural and biological approaches have been proposed (Altieri et al., 2004; Andersson, Andersson, Adlercreutz, Nielsen, & Hornsten, 2002; Doran & Bailey, 1986; Edens, Farin, Ligtvoet, & Van Der Plaat, 1991; Fernandez, Cava, Ocio, & Lagaron, 2008; Gosmann & Rehm, 1986, 1988; Nezat, 1985; Tramper, Luyben, & Vandentweel, 1983). OS based on natural and biological

* Corresponding author: Tel.: +32 9 264 6164; fax: +32 9 225 5510.
E-mail addresses: Tom.Anthierens@ugent.be (T. Anthierens), Peter.Ragaert@ugent.be (P. Ragaert), Sam.Verbrugghe@ugent.be (S. Verbrugghe), Bruno.Degeest@ugent.be (B.G. De Geest), Bert.Noseda@ugent.be (B. Noseda), Johan.Mertens@ugent.be (J. Mertens), Lynda.Beladjal@ugent.be (L. Beladjal), Dirk.De.Cuyper@resilux.com (D. De Cuyper), William.Dierickx@resilux.com (W. Dierickx), Filip.Duprez@ugent.be (F. Du Prez), Frank.Devlieghere@Ugent.be (F. Devlieghere).

1466-8564/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ifset.2011.06.008

components could have advantages towards the consumer such as perception, recyclability, safety, material compatibility and production costs compared to currently available chemical OS. These include biocatalytic-based systems making use of glucose oxidase (Fernandez et al., 2008) or a mixture of glucose oxidase and catalase (Andersson et al., 2002) or the use of entrapped aerobic microorganisms, capable of consuming oxygen (Altieri et al., 2004; Doran & Bailey, 1986; Edens et al., 1991; Gosmann & Rehm, 1986, 1988; Nezat, 1985; Tramper et al., 1983).

A significant restriction for these systems is the heat lability of incorporated biological compounds during the high temperature and pressure production process of plastic containers, such as during the production of PET-bottles (i.e. 300 °C). For this reason, currently available biological systems are incorporated in water soluble polymers (Altieri et al., 2004), gels (Gosmann & Rehm, 1986, 1988; Tramper et al., 1983) or in a low melting wax or paraffin (Edens et al., 1991), which limits their application in food packaging.

The aim of this work was to evaluate the applicability of heat resistant endospore-forming aerobic micro-organisms (genus *Bacillus amyloliquefaciens*) as an active OS in multilayer PET materials (Fig. 1.).

Bacterial endospores (spores) are generally known to have a high resistance to dry heat (Bond & Favero, 1977; Molin, 1977; Molin & Ostlund, 1975; Rank & Pflug, 1977; Setlow, 2006) and pressure, (Gould, 2006; Nicholson, Munakata, Horneck, Melosh, & Setlow, 2000) which makes them suitable to be directly incorporated in a plastic material in the same way as a current chemical OS. Germination of these spores is initiated when water, originating from the packaged food product, is absorbed in the PET matrix (Setlow, 2003). Although the principals of this work have been patented by the authors for industrial application (Dierickx et al., 2006; Mertens et al., 2010), several fundamental aspects such as the survival rate and condition of incorporated spores and the oxygen scavenging efficiency of the system remained unclear. In the current study, a model system was therefore developed on which the oxygen absorption rate and the viability and condition of incorporated spores were evaluated.

2. Materials and methods

2.1. Organism and spore preparation

Spores of *Bacillus amyloliquefaciens* ID9698 were used in this study. The strain was maintained on nutrient agar (NA, Oxoid, Hamsphire, United Kingdom) slants in the culture collection of the Laboratory of Food Microbiology and Food Preservation, Ghent University.

Dry spore powder was produced according to the following procedure: growth medium consisting out of 20 g l^{-1} peptone (Oxoid, Hamsphire, United Kingdom), 20 g l^{-1} sucrose (Fluka, Steinheim, Germany), 10 g l^{-1} yeast extract (Oxoid, Hamsphire, United Kingdom) and 20 g l^{-1} agar (Oxoid, Hamsphire, United Kingdom) was prepared, autoclaved and poured sterile into petri dishes. Nutrient broth (NB, Oxoid, Hamsphire, United Kingdom) was inoculated with a small amount of the strain and incubated for 24 h at 30 °C. Subsequently, plates containing growth medium were inoculated with 1 ml NB and incubated for 5 days at 37 °C. Spores were harvested by scraping the surface with sterile spatulas. Scrapings were collected in a sterile petri dish and dried for 2 weeks at 37 °C after which they were grinded. Purity and spore concentration of the powder was evaluated by dissolving a known amount of powder in sterile physiological peptone solution [PPS: 8.5 g l^{-1} NaCl and 1 g l^{-1} bacteriological peptone (Oxoid, Hamsphire, United Kingdom)]. The appropriate serial tenfold dilutions were performed in PPS and plated out before and after pasteurization (10 min 80 °C) using spread plating on NA. Plates were incubated for 48 h at 30 °C after which they were counted.

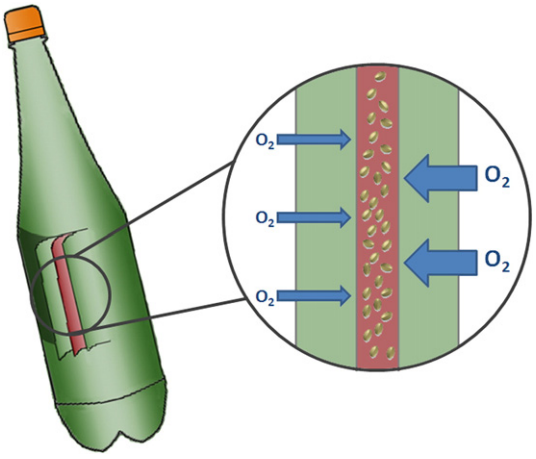

Fig. 1. Schematic representation of a multilayer PET bottle consisting of a PETG middle layer containing bacterial spores surrounded by two outer PET layers. The inside of the bottle is in contact with the product, allowing moisture uptake of the bottle needed for spore germination. The system allows scavenging of residual oxygen from the in-bottle environment and scavenging from atmospheric oxygen permeating through the bottle wall.

2.2. Polymeric matrix

2.2.1. Incorporation procedure

Spores were incorporated in Poly(ethylene terephthalate, 1,4-cyclohexane dimethanol) (PETG, Tradename Eastar Copolyester 6763, Eastman Chemical Company, Kingsport, USA), an amorphous copolymer of terephtalic acid with ethylene glycol and cyclohexane dimethanol. It was reported to have an Mn ≈ 2.6 10^4 g mol^{-1}, and to consist of cyclohexane dimethanol, ethylene glycol and terephthalic acid in a molar ratio of approximately 1:2:3 (Papadopoulou & Kalfoglou, 1997). PETG resins were pressed into round samples with a diameter of 16 mm and a thickness of 50 μm with a mold and a table press (TP400, Fontijne, The Netherlands) at a temperature of 210 °C and a total process time of 3 min (2 min with no added pressure, 1 min under 30 bar). After pressing, the mold was placed in a water cooled stage under a pressure of 30 bar and subsequently cooled to room temperature. Spores were incorporated by placing spore powder in a 4% w/w (for 100 g PETG, 4 g dry spore powder was used) between two PETG plates. A new plate was pressed with a diameter of 20 mm and a thickness of 50 μm using the same press conditions.

2.2.2. Moisture absorption

Moisture absorption, expressed as % w/w, was determined by placing 30 g resins of PET (Eastman Chemical Company, Kingsport, USA), PETG and PETG with 4% spores in a closed recipient with 100% R. H at 30 °C. Samples were measured gravimetrically every day for 5 days.

2.3. Evaluation of incorporated spores viability

2.3.1. Spore extraction

Processed plates were cut into homogeneous parts of 5 mm × 5 mm with sterile scissors and placed in a falcon tube containing 25 ml chloroform (VWR International, Fontenay-sous-Bois, France) and 10 ml sterile demineralized water. Falcon tubes were placed for 20 min in an ultrasonic bath until PETG was completely dissolved and the spores were migrated to the water phase. After phase separation 1 ml of the water phase and 1 ml of the chloroform phase were serially diluted in PPS. Counts were performed before and after pasteurization on NA using the spread plating technique. Plates were incubated for 48 h at 30 °C after which they were counted.