International Journal of Food Microbiology 220 (2016) 73–90

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Review

Active packaging with antifungal activities

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ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form 2 December 2015
Accepted 3 January 2016
Available online 7 January 2016

Keywords:
Essential oils
Preservatives
Natural products
Nanoparticles
Chitosan
Food
Packaging
Mould

ABSTRACT

There have been many reviews concerned with antimicrobial food packaging, and with the use of antifungal compounds, but none provided an exhaustive picture of the applications of active packaging to control fungal spoilage. Very recently, many studies have been done in these fields, therefore it is timely to review this topic. This article examines the effects of essential oils, preservatives, natural products, chemical fungicides, nanoparticles coated to different films, and chitosan *in vitro* on the growth of moulds, but also *in vivo* on the mould free shelf-life of bread, cheese, and fresh fruits and vegetables. A short section is also dedicated to yeasts. All the applications are described from a microbiological point of view, and these were sorted depending on the name of the species. Methods and results obtained are discussed. Essential oils and preservatives were ranked by increased efficacy on mould growth. For all the tested molecules, *Penicillium* species were shown more sensitive than *Aspergillus* species. However, comparison between the results was difficult because it appeared that the efficiency of active packaging depended greatly on the environmental factors of food such as water activity, pH, temperature, NaCl concentration, the nature, the size, and the mode of application of the films, in addition to the fact that the amount of released antifungal compounds was not constant with time.

Contents

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.01.001
0168-1605/

1. Introduction

According to the USDA Economic Research Service estimates, about 96 billion pounds of food, or 27% of the 356 billion pounds of the edible food available for human consumption in the United States, were lost to human use at three marketing stages, retail, foodservice and consumers, in 1995. Fresh fruits and vegetables (19.6%), fluid milk (18.1%), grain products (15.2%), and sweeteners (12.4%), mostly sugar and high-fructose corn syrup, accounted for two-thirds of these losses (Scott Kantor et al., 1997). Fruits are usually quite acid and hence quite resistant to invasion by bacteria. Therefore spoilage of fruits and fruit products is almost always caused by fungi (Pitt and Hocking, 1999).

In the fruit industry, postharvest losses amount to 5–10% when postharvest fungicides are used (Cappellini and Ceponis, 1984), but without fungicides, losses of 50% or higher have occurred in some years. For example, in a 1993 test to assess the decay potential of stone fruit, an average of 52.8% (range 15–100%) of the fruit decayed during the ripening of eight collections not previously treated with postharvest fungicides (Margosan et al., 1997). In the fruit juice industry these losses, due to heat-resistant ascospores, varied greatly depending on season, type of product and processing method. A rough estimate of these losses would be less than 1% of packages in a lot (Sant'Ana et al., 2010).

In the baking industry, these losses varied between 1% and 3% depending on season, type of product and method of processing (Malkki and Rauha, 1978). Another estimate from one bakery in the US was 5% losses (Killian and Krueger, 1983). Even assuming only 1% losses, moulds could be spoiling over 23,000 tons of bread worth nearly £20 million in the UK every year. Throughout Western Europe the annual losses could be around 225,000 tons of bread worth £242 million (Legan, 1993). Nowadays, it is very difficult to get an accurate picture of the real contamination of food products from the industry, as this information is rarely published and more recent references are not available. However, contamination by yeasts and moulds is still of major concern for the food industry. Contamination in the baking industry is infrequent, but when it occurs, the percentage of spoiled products can be up to 50% (Dantigny, personal data).

Traditionally, antimicrobial agents are directly mixed into the initial food formulations. Direct addition may result in excessive amounts of the antimicrobial agent which may change the taste of the food (Uz and Altinkaya, 2011). Direct application techniques, such as dipping, spraying or brushing, are used to deposit antimicrobial substances on the food surface to limit the undesirable microorganisms. However, direct application of antimicrobial substances may result in the inactivation or evaporation of active agents and rapid migration into the bulk of the foods (Quintavalla and Vicini, 2002). Therefore, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration. The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods where a large portion of spoilage and contamination occurs. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. The gradual release of an antimicrobial from a packaging film to the food surface may have an advantage over dipping and spraying. Many antimicrobials are incorporated at 0.1–5% *w/w* of the packaging material, particularly films. Antimicrobial packaging materials must contact the surface of the food if they are non-volatile, so the antimicrobial agents can diffuse to the surface, therefore, surface characteristics and diffusion kinetics become crucial (Appendini and Hotchkiss, 2002).

The development of active materials with properties for enhancing the shelf-life and safety of packaged food is nowadays one of the most challenging research activities (Gutiérrez et al., 2009). The use of antifungal packaging is a possible solution to control the growth of phytopathogens in fruits during postharvest shelf life (Junqueira-Gonçalves et al., 2013), and to extend the safety and shelf-life of ready-to-eat foods (Moditsi et al., 2014). The use of antimicrobial packaging can be effective during the storage period, handling or transport, and once the package is opened, the antimicrobial film will still be active (Gutierrez et al., 2011). The antimicrobials embedded in films can also be transferred to the food surface for further action, and relatively low amounts are required to achieve a target shelf-life (Min and Krochta, 2005).

2. Assessment of antifungal properties

2.1. Growth

Bacteria divide to form single cells that can be easily enumerated, especially in liquid broth, as CFU/ml or CFU/g. Unlike bacteria, fungi do not grow as single cells, but as hyphal filaments that cannot be quantified by the enumeration technique (Dantigny and Bensoussan, 2013). Fungal hyphae can penetrate solid substrates, such as food, making their extraction difficult. In addition, fungi differentiate to produce spores, resulting in large increases in viable counts often with little relationship to biomass (Pitt, 1984). Despite these limitations, the CFU method which can provide only an evaluation of the effect of an active substance as compared to the control, has been used by many authors. For example, Valverde et al., 2005; Kechichian et al., 2010; Azarakhsh et al., 2014; Lopes et al., 2014; Mehyar et al., 2014; Otoni et al., 2014, have used this method for the enumeration of yeasts and moulds in food products. In these studies, samples of food (10 or 25 g) were diluted, and serial dilutions were plated on relevant media such as Dichloran Rose-Bengal Chloramphenicol Agar or Petrifilms.

In the agar plate test, antimicrobial film is placed on a solid agar medium containing the test microorganism. The concentration of the antimicrobial compound decreases from the film, usually a disc placed at the centre of the dish, to the edge of the Petri dish, (Kuorwel et al., 2014). At the same time, the concentration of the antimicrobial compound in the disc decreases according to first order kinetics (Mascheroni et al., 2011). The agar plates are incubated until growth is visible. A clear zone surrounding the film indicates antimicrobial diffusion from the film and subsequent growth inhibition. The antifungal index, fungistatic inhibition, or percentage inhibition is calculated according to the following equation (Guo et al., 2006):

$$\text{Antifungal index}\ (\%) = [(D_b - D_a)/D_b] \times 100 \quad (1)$$

where D_a is the diameter (or the radius) of the growth zone in the test plate, and D_b is the growth zone in the control plate.

Lack of growth under a film may indicate inhibition, but appropriate controls must be included because this inhibition may be due to simple restriction to oxygen. The agar plate test method simulates wrapping of foods and may suggest what can happen when films contact contaminated surfaces and the antimicrobial agent migrates from the film to the food. The method can be quantitative if the diameter of the clear zones around the films is measured. In the studies of Avila-Sosa et al. (2010, 2012) the growth rate was estimated by the Gompertz model.

The minimum inhibitory concentration, MIC, of an antimicrobial that inhibits completely the visible growth after incubation was defined by Andrews (2001). Below the MIC value, the concentrations of the antimicrobial compounds are sub-inhibitory. However, in some cases this definition was misused as the MIC was defined as the lowest concentration at which a decrease in the growth rate was detected, not at which growth was absent.

2.2. Shelf-life of food products

Inoculation of food products, especially fruits and vegetables is usually achieved by wounding or puncturing with a sterile cork borer, and a spore inoculum is inserted. An alternative method consists in spraying the spore suspension at the surface of the product. After drying, a period of time during which spores can germinate, the products are coated. The