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Development and optimization of antifungal packaging for sliced pan loaf based on garlic as active agent and bread aroma as aroma corrector

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ABSTRACT

The aim of the present work was the development of antimicrobial films containing garlic extract to be applied as active packaging for preservative-free sliced pan loaf, with the goal of extending its shelf-life. First, the antimicrobial capacity of garlic extract, a compound used as active agent, was tested against *Penicillium expansum* by the disc diffusion method. The extract showed high antimicrobial activity, 0.1 μL per Petri dish being the minimum inhibitory amount, and 0.25 μL the minimum fungicidal amount. Bread aroma was also used to mask the pungent odour of garlic and it was confirmed to have no antimicrobial activity. Subsequently, polyethylene (PE) aqueous emulsion and ethylene-vinyl alcohol copolymer (EVOH) and zein hydroalcoholic solutions containing 0.25 and 0.5% (w/w per dry polymer) of garlic extract and bread aroma were used to coat PE films, producing PE/PE, PE/EVOH and PE/zein active films. The antimicrobial capacity of the films was studied *in vitro* against *Penicillium expansum*, and *in vivo* with natural sliced bread. The results showed that all the films presented some antimicrobial activity, PE film coated with zein containing 0.5% of garlic extract and bread aroma being the film presenting the best results, maintaining bread free of mould infection for 30 days. Sensory tests showed that the addition of 1% of bread aroma improved the sensory experience of consumers and also revealed good purchase intention.

1. Introduction

Bread is an essential food product in the traditional diet in Europe, the Middle East, India, America and Oceania. According to the Codex Alimentarius, bread is the product resulting from baking dough obtained by mixing flour and water, with or without addition of edible salt, fermented by baker's yeast, *Saccharomyces cerevisiae*. Bread slowly deteriorates after baking, owing to a combination of chemical and physical processes called staling and owing to microbiological spoilage.

Pan loaf is a type of bread baked in a pan, with a soft texture and high water activity ($0.94 < a_w < 0.97$) (Legan, 1993), and commonly sold sliced. To delay staling and dehydration, the sliced bread is packaged in bags, keeping a humid headspace and, consequently, great conditions for fungal spoilage.

Fungal spores are very resistant to thermal treatments employed by the food industry, including baking, which explains their prevalence in a wide range of products. Under favourable conditions they develop and disperse the fungi, colonizing not only the bread slice surface but also the inside of the crumb. This phenomenon causes deterioration of the sensory properties of the product, discoloration, decomposition and generation of mycotoxins, which can pose a health risk (Filtenborg et al., 1996). Mould growth causes great economic losses to the industry.

Aspergillus, Eurotium and *Penicillium* species in general are the main fungi implicated in the deterioration of bakery products and pan loaf (Dagnas et al., 2014). *Penicillium expansum* is a widespread fungal pathogen in many foods, which causes great economic losses and also health issues, since it produces toxic secondary metabolites (Andersen et al., 2004; Sadok et al., 2018; Tannous et al., 2017). To reduce fungal growth and increase shelf-life, the bakery industry adds low amounts of authorized preservatives to the dough, mainly sodium propionate (E 281), sorbic acid (E 200), sodium sorbate (E 201), potassium sorbate (E 202), calcium acetate (E 263) and ascorbic acid (E 300) (Guynot et al., 2005). Owing to the inclusion of these chemicals, and the slicing and packaging, sliced pan loaves are associated with artificial bread (compared to freshly baked baguettes).

Consumers are concerned about the presence of chemical additives and preservatives in food, and are demanding their elimination and the

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provision of more natural and healthy products. Consequently, companies are looking for alternative ways to inhibit microbiological growth and maintain both sensory and nutritional quality of the product, including the development of active packaging incorporating antimicrobial agents.

Active packaging is a food/packaging/environment system that acts in a coordinated way to improve food safety and quality and increase its shelf-life. The purpose of antimicrobial packaging is to impede or inhibit the growth of microorganisms in food. The great advantage of antimicrobial packaging over the direct addition of preservatives is that the active agent is gradually released on the food surface during storage and distribution, producing an inhibitory or lethal effect against pathogens that affect food products (Balaguer et al., 2013). The current demand for natural products also affects the selection of antimicrobial agents, and there is a preference for the incorporation of natural compounds in packaging materials, such as herbs, spices and, especially, essential oils (Ribeiro-Santos et al., 2017). Numerous studies have demonstrated the inhibitory activity of various essential oils and extracts from plants such as tea, cloves, mustard, oregano, anise, cinnamon or garlic against various microorganisms in food (Giteru et al., 2017; Higueras et al., 2014; Johnson et al., 2016; Ju et al., 2018; Matan et al., 2012; Teixeira et al., 2014).

In this work, garlic (*Allium sativum*) extract was selected as a natural antimicrobial agent against *Penicillium expansum*, and it was used to develop active packaging for preservative-free sliced pan loaf. In addition to having a great antioxidant capacity (Fratianni et al., 2016), garlic extract is a broad-spectrum antimicrobial agent, fungi being highly susceptible to its action (Ross et al., 2001). The antimicrobial capacity of garlic is mainly due to the presence of sulfur compounds, also responsible for its characteristic flavour and aroma, as well as its therapeutic properties (Agarwal, 1996; Chen et al., 1998; Fratianni et al., 2016).

Allicin is an organosulfur compound containing the thiosulfinate group generated by the enzymatic conversion of alliin, the active substance responsible for the antimicrobial activity in garlic (Borlinghaus et al., 2014; Kyung, 2012). Allicin decomposes when garlic is processed at high pressures and temperatures, producing volatile sulfur compounds that include diallyl sulfide (DAS) and diallyl disulfide (DADS), which maintain the biological activity (Corzo-Martínez et al., 2007).

A limiting factor in the use of an extract or essential oil as antimicrobial packaging is the potential organoleptic impact on the packaged product. Owing to the pungent aroma of garlic extract, in this work we also incorporated an industrial essence of bread aroma which could hide the sensory impact of garlic. One of the compounds in the bread crust used is 2-acetyl-1-pyrroline, responsible for the smell of freshly baked bread (Cho and Peterson, 2010).

Most common packaging systems for sliced pan loaf are low-density polyethylene (LDPE) bags. LDPE is a plastic polymer that is converted by extrusion into flexible, transparent films with a good barrier to water vapour but highly permeable to oxygen and apolar compounds, and with easy heat sealability (Catala and Gavara, 2001). In this work, these LDPE bags are used as the substrate for the development of the active packaging system because they provide excellent functional properties (mechanical, barrier, optical, thermal, …) at low cost. The activity is incorporated by adding an internal coating with the active agents. As potential polymers for the coatings, polyethylene, ethylene-vinyl alcohol copolymer (EVOH) with a molar ethylene content of 29% (EVOH-29) and zein were selected.

EVOH is a hydrophilic material that provides a good barrier to gases and aromas at low relative humidity, but the barrier declines severely in wet conditions (Cerisuelo et al., 2014; Muriel-Galet et al., 2014). EVOH has been used in previous works for the development of active coatings (Catala et al., 2015).

Zein is the protein fraction from corn, a biopolymer insoluble in water but soluble in alcohol, owing to its high content of proteins rich in hydrophobic amino acids, glutamic acid, leucine, alanine and prolamin (Bisharat et al., 2018; Shukla and Cheryan, 2001). Zein has good film formability, although these films are rather fragile, so the use of plasticizing agents to impart flexibility and facilitate handling is necessary (Liang et al., 2015). Zein films have been used previously in the preparation of active packaging (Kashiri et al., 2016, 2017).

The aim of the present work was to develop active antifungal packaging by incorporating garlic extract and bread aroma in a polymeric coating on a PE bag for the preservation of natural sliced pan loaf without chemical preservatives.

2. Materials and methods

2.1. Chemicals and reagents

Linear low-density polyethylene (LLDPE) aqueous emulsion, AquasealTM 2200, was kindly provided by Paramelt (the Netherlands). A poly(ethylene-*co*-vinyl alcohol) with 29% ethylene molar content, Soarnol 2908, was kindly provided by The Nippon Synthetic Chemical Company (Osaka, Japan). Odourless zein was obtained from the Kobayashi Perfumery Co., Ltd. (Japan). 1-Propanol and ethanol 100% were acquired from Sigma (Madrid, Spain). Garlic extract (GE) was kindly provided by DOMCA (Granada, Spain) and bread aroma containing 2-acetyl-1-pyrroline by Dulcesol (Gandia, Spain). Glycerol was supplied by WVR International S.A.S. (France). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France).

2.2. Preparation of fungal cultures

Penicillium expansum, previously isolated and identified from natural sliced bread, was grown on potato dextrose agar (PDA) in polystyrene Petri dishes for 7 days at 28 °C. The inoculum was collected by flooding the surface of the plates with sterile peptone water with Tween 80 (0.05% v/v) and then scraping the surface with a spatula. A 5 mL sample of the mould culture suspension was transferred to sterile polypropylene tubes and shaken to obtain a homogeneous suspension. Several dilutions were made to obtain 10^6 spores/mL. The spore count was determined using the improved Neubauer method (Bright-Line Hemacytometer, Hausser Scientific, Horsham, PA).

2.3. Garlic extract and bread aroma inhibition assay

The antimicrobial activity of garlic extract (GE) and bread aroma (BA) was analysed by determining the minimum inhibitory amount and the minimum fungicidal amount (volume) against *P. expansum*. Samples of 3 μL of the mould culture suspension with 10^6 spores/mL were inoculated at three equidistant points on PDA. Different concentrations of each compound (GE and BA) were added to 25 mm sterile blank filter discs and placed on top of a Petri dish. The plates were sealed with Parafilm® to prevent leakage of the volatile agent. Blanks were also prepared. The plates were incubated at 28 °C for 7 days. The minimum amount of agent spread in the paper disk, is considered the smallest amount that causes a reduction in fungal growth (compared to the control sample). The minimum fungicidal amount is the smallest amount that yields a no-growth effect.

2.4. Film preparation

A polyethylene (PE) dispersion was stirred for 15 min using a magnetic stirrer. Ethylene-vinyl alcohol copolymer (EVOH) was dissolved in a 1:1 (v:v) 1-propanol:Milli-Q water mixture at 75 °C. The solution was stirred for 30 min using a magnetic stirrer hotplate. Zein was dissolved in an 80:20 (v/v) of ethanol:Milli-Q water mixture at 80 °C. The solution was stirred for 60 min using a magnetic stirrer hotplate. After complete dissolution, the solution was cooled down to 37 °C and the plasticizer (15% of glycerol with respect to polymer content) was added and stirred for 5 min. Once the film solutions had