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Antimicrobial and antioxidant properties of pullulan film containing sweet basil extract and an evaluation of coating effectiveness in the prolongation of the shelf life of apples stored in refrigeration conditions

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ABSTRACT

The antimicrobial and antioxidant activities of pullulan films containing sweet basil extract (SBE) at varying concentrations were examined in the paper. Additionally, the content of bioactives in SBE was determined. A strong correlation was noted between SBE concentrations in the film and the obtained film thickness. Pullulan film containing SBE was characterized by a smooth surface as well as a uniform and compact internal structure. The study involved an examination of the effectiveness of pullulan coatings containing 24 mg SBE/cm^2 on the prolongation of the shelf life of "Jonagored" apples. Pullulan coating with SBE was found to offer low antibacterial activity against mesophilic bacteria and good antifungal protection against *Rhizopus arrhizus* on apple surfaces. This coating also contributed to a reduction in weight losses and lower changes in the color and soluble solids of fruits during storage. Apples with pullulan enriched SBE coating presented better overall preference parameters. *Industrial relevance:* The consumers express an increasing aversion and objections towards an application of chemical means in order to prolong the durability of food products. This prompts food producers to search for new methods of preventing raw materials against harmful microbial activity. Plant extracts, due to their chemical variability, ensure unlimited possibilities of microorganism growth control. An application of natural plant extracts combined with edible coatings for fruit and vegetable durability prolongation may find an acceptance among the consumers. Incorporation of plant extract to edible coating ensures slow release of bioactives on food surface, i.e. at the site of the highest microbiological food changes. Pullulan is a natural polysaccharide, a carrier of small energy amounts, forming glossy, colorless and flavorless films, resistant to oils and impermeable for oxygen. The authors decided to elaborate the procedure concerning an application of edible pullulan coating combined with sweet basil extract for an elongation of apple durability, which will improve the quality of fresh fruits during their storage and in the trade.

1. Introduction

There is a current interest in the application of biomaterial for the manufacturing of edible coatings in order to prolong the shelf-life of fresh-like foods, e.g. minimally processed fruits and vegetables (Diab, Biliaderis, Gerasopoulos, & Sfakiotakis, 2001; Kraśniewska & Gniewosz, 2012; Qi, Hu, Jiang, & Li, 2011). Edible coatings can be used as semi-permeable barriers allowing the limiting of moisture and solute migration, gas exchange, respiration and maturation of fruits and vegetables (Qi et al., 2011; Rojas-Graü, Tapia, Rodriguez, Carmona, & Martin-Belloso, 2007). Moreover, edible coatings can be also applied as food additive carriers, e.g. antibrowning agents, dyes, aromas and antimicrobial agents (Gniewosz & Synowiec, 2011; Pramoto, Salokhe, & Rakshit, 2005; Rojas-Graü et al., 2007; Wu & Chen, 2013).

Pullulan, a natural microbial polysaccharide, is capable of forming edible films. Pullulan films exhibit a range of profitable features, which makes it an interesting biomaterial and has led to an increase in its application in the food packaging industry. In addition, pullulan films are tasteless, transparent and elastic, and also highly impermeable to oil, soluble in water, heat sealable, and demonstrate good barrier properties towards oxygen (Yuen, 1974). Pullulan films themselves do not demonstrate antimicrobial activity.

Therefore, bioactive substances, such as lysozyme, bacteriocin-sakacin A, thymol or caraway oil, have been incorporated into pullulan films in order to increase their functionality. Studies have demonstrated an inhibiting activity of the films against pathogenic bacteria, such as *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, or epidemic clones of *L. monocytogenes*. The results of the study show that pullulan

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films may be used in the improvement of the microbiological stability and safety of food products (Gniewosz, Kraśniewska, Woreta, & Kosakowska, 2013; Gniewosz & Synowiec, 2011; Kandemir et al., 2005; Trinetta, Floros, & Cutter, 2010).

Application of natural antimicrobials, such as plant extracts, may be a suitable alternative for food safety assurance. Plant extracts contain a variety of substances called phytochemicals that are naturally occurring components present in plants. These phytochemical compounds are known to retard the growth of or kill food-borne pathogens (Negi, 2012). Sweet basil (*Ocimum basilicum* L.) is one of the most popular culinary herbs all over the world. The antimicrobial activity of the essential oil of basil has been thoroughly studied (López, Sánchez, Batlle and Nerín, 2005). Several studies have demonstrated the significant effectiveness of Gram-positive and Gram-negative bacteria inhibition by various extracts of sweet basil, and to a lesser extent towards selected fungi (Adigüzel et al., 2005; Shan, Cai, Brooks, & Corke, 2007).

Thus far, not many researchers have focused on the influence of pullulan-based edible coatings by incorporating antimicrobial agents for the prolongation shelf-life of fresh or minimally processed fruits and vegetables (Gniewosz, Kraśniewska, Woreta, & Kosakowska, 2013; Gniewosz & Synowiec, 2011; Wu & Chen, 2013).

The present study focused on elaborating the procedure concerning the application of a pullulan-based edible coating combined with sweet basil extract (SBE) in apple durability prolongation. The study involved an examination of bioactive compounds in SBE, antimicrobial activity of this extract and pullulan film with incorporated SBE. Then, the influence of SBE including pullulan coating on microorganism growth, weight losses, changes in the content of soluble solids and the color of the apples of "Jonagored" cultivar was studied.

2. Materials and methods

2.1. Plant materials

Sweet basil (*O. basilicum* L.) and apples (*Malus domestica*) cv. Jonagored were obtained from experimental fields and orchards of the Department of Pomology Warsaw University of Life Sciences (WULS-SGGW, Warsaw, Poland).

2.2. Reagents and growth medium

Ethanol (96%, v/v), phosphoric acid, acetonitrile, glycerol, NaCl and sodium hypochlorite were purchased from POCH S.A. (Gliwice, Poland). Nutrient Agar (NA), Sabouraud Agar (SA), Sabouraud Broth (SB), Plate Count Agar (PCA) were obtained from BTL (Łódź, Poland). Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) were purchased from Merck (Darmstadt, Germany). The phenolic compound standards were purchased from ChromaDex (CA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Hamburg, Germany).

2.3. Tested microorganisms

Bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *S. enteritidis* (ATCC 13076), *E. coli* (ATCC 25922) and mold and yeast strains: *Penicillium expansum* (ATCC 7861), *Rhizopus arrhizus* (ATCC 11145), *Aspergillus niger* (ATCC 9142), *Saccharomycopsis fibuliger* ZBMZ 2 and *Saccharomyces cerevisiae* IPF5 were obtained from the pure cultures collection of the Department of Biotechnology and Food Microbiology (WULS-SGGW, Warsaw, Poland). Bacterial strains and fungi were cultivated on NA and SA, respectively, and stored at 4 °C. Bacteria were cultivated at 37 ºC for 24 h, yeasts at 28 ºC for 24 h and molds at 25 ºC up to the moment of spore formation (about 14 days).

2.4. Pullulan preparation

Pullulan was obtained from the culture of white mutant *Aureobasidium pullulans* B-1 following the protocol described in an earlier work (Gniewosz & Duszkiewicz-Reinhard, 2008; Gniewosz, Kraśniewska, & Synowiec, 2013). *A. pullulans* B-1 was cultured in a BioFlo 3000 bioreactor (New Brunswick Scientific, NJ, USA). After the culture, the biomass was centrifuged at 18,800 ×*g* (Eppendorf, Hamburg, Germany). To precipitate pullulan, 96% ethanol (v/v) was added to the supernatant (2:1, v/v). The precipitated crude preparation of pullulan was centrifuged at 18,800 ×*g* and dried to a constant weight. Pullulan content in the raw preparation was 72% and this was determined according to the procedure described by Gőksungur, Dağbağli, Uçan, and Güvenç (2005).

2.5. SBE preparation

Sweet basil extract (SBE) was prepared by using a pilot plant scale, custom made, universal 3EU01 apparatus for herbal raw material extraction and distillation (OBR Pleszew, Poland). The device was designed for research purposes to conduct studies on large batches of raw material (0.5–5.0 kg). It consisted of a stainless steel extraction chamber (vol. approx. 12 L, with a steam heating jacket), which was closed and sealed by a lid with a stirrer, a water-cooled condenser, a manometer, and a safety valve. Ethanol solution (40%, v/v) was used as a solvent. The ratio of raw material to solvent was 1:10. The raw material was extracted while maintaining the temperature at 70 °C for 2 h at a pressure of 0.03 MPa. The obtained raw extract was filtered through Whatman no. 2 filter paper (Whatman International, Ltd., Maidstone, England), and subsequently condensed in a rotary evaporator (Rotovaporator R-215, Büchi, Flawil, Switzerland). Liquid water–ethanol extract of a density of 0.35 g d.w./ml was obtained.

2.6. Characterization of SBE

2.6.1. HPLC analysis of phenolic acid and flavonoids

The analyses were performed by using a Shimadzu liquid chromatograph (Kyoto, Japan) equipped with an SIL-20 auto sampler, an SPD-M10A VP PDA photodiode array detector and Class VP 7.3 chromatography software. A C18 reversed phase column (Phenomenex Kinetex® 2.6 μm, 100A, 100 × 4.60 mm i.d.) was used as the solid phase. Binary gradient of mobile phase A (deionised water adjusted to pH 3 with phosphoric acid) and B (acetonitrile adjusted to pH 3 with phosphoric acid) was used. The following conditions were applied: flow rate 1.3 ml/min, oven temperature 32 °C, total time of analysis 10 min. Sample injection volume was 1 μl. The obtained extract was filtered with a Supelco Iso-DiscTM Syringe Tip Filter Unit, with a PTFE membrane, diameter 25 mm, pore size 0.20 μm after injection. UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with appropriate parameters of standards. The content of the determined compounds was calculated in mg/100 g dry matter of raw material and SBE.

2.6.2. Disc-diffusion method

The susceptibility of tested microorganisms to SBE concentrations was verified by using the disc-diffusion method (Clinical Laboratory Standards Institute, 2006). Sterile cellulose discs (12 mm diameter, and area c.a. 1 cm^2) were impregnated with 9–52 μl SBE (density 350 mg/ml). Equal SBE contents were obtained, and these were 3, 6, 9, 12 and 18 mg d.w./disc. Inocula of bacteria were prepared by suspending indicatory cultures, originating from overnight cultures, in MHB with the quantity corresponding to 0.5 McFarland (10^8 CFU/ml). The suspensions of yeasts and mold spores at the concentration of 10^5 CFU/ml were prepared in physiological saline (0.9% NaCl).

The suspensions of tested bacteria were spread evenly on the surface of solidified MHA medium, and yeasts or mold spore suspensions on SA