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Inhibition of pork and fish oxidation by a novel plastic film coated with horseradish extract

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ABSTRACT

An anti-oxidative plastic film coated with microcapsules containing volatile horseradish extract was developed. The oil-in-water-type microcapsule was produced by a modified orifice method to encapsulate the methanol extract of horseradish. The presence of naturally occurring antioxidants in the extract was confirmed by high performance liquid chromatography. Increasing the amount of chitosan during microcapsule formation increased the size of the microcapsules and decreased the rate of release of the horseradish extract, suggesting that the amount of extract released from the film can be modulated by the chitosan content of the microspheres. Covering pork and fish fillets with the anti-oxidative film delayed oxidative discoloration and rancidization. Collectively, these results showed that a horseradish-coated film containing natural antioxidants efficiently enhanced the stability of both pork and fish. This novel film may be a promising tool to prolong the shelf-life of meats.
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1. Introduction

Prolonging the shelf-life of fresh pork and fish is important for both manufacturers and consumers. The shelf-life of fresh meats can be extended by protecting them from discoloration, lipid oxidation, and microbial growth. One of the initial indices used to assess the freshness of meats is color. Oxidation decreases overall meat quality and is a primary cause of changes in meat color, flavor, and texture (Saleemi, Janitha, Wanasundara, & Shahidi, 1993; Sánchez-Escalante, Torrescano, Djenane, Beltrán, & Roncalés, 2003). In particular, seafood products contain high levels of polyunsaturated fatty acids that are easily attacked by oxygen-derived free radicals, resulting in lipid peroxidation and meat rancidization (Huang & Weng, 1998).

Oxidative deterioration of meats such as pork and fish is caused by the degradation of fats and pigments. The surface discoloration of fresh pork cuts largely depends on the oxidative change of oxymyoglobin into metmyoglobin and gives meat an unattractive brown color (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2003; Ranken, 1989; Wood et al., 2003). Furthermore, lipid oxidation causes rancidization of fish, rendering the product unacceptable for human consumption. Although antioxidant application could prevent oxidation processes, their direct application on fresh-cut foods is prohibited in many countries. Nevertheless, consumer interest in safe dietary antioxidants has promoted research on natural products that are known to have antioxidation properties.

Green tea (Choi et al., 2006) and horseradish (Cejpek, Urban, Velíšek, & Hrabcová, 1998; Delaquis, Ward, Holley, Cliff, & Mazza, 1999; Yamaguchi, Kanoo, Ikeda, & Kijima, 1984) have been shown to retard oxidation of meats. Specifically, horseradish is a plant used primarily as a condiment for sushi and seafood dishes in Japanese cuisine. Horseradish contains allyl isothiocyanate and phenyl isothiocyanate as primary components. Allyl isothiocyanate is particularly pungent and volatile and is released from the naturally occurring glucosinolate sinigrin by the action of myrosinase. Previous studies (Manesh & Kuttan, 2003; Kinae, Masuda, Shin, Furugori, & Shimoi, 2000) showed that isothiocyanate and allyl isothiocyanate inhibit lipid oxidation and the food-poisoning bacterial activities of *Escherichia coli, Staphylococcus aureus,* and *Vibrio parahaemolyticus*. These compounds also inhibit the growth of mold and yeast (Kinae et al., 2000) and have been tested for use as food-preservation agents. However, their pungent flavor has dissuaded their use on fresh-cut foods.

This problem can be solved by microencapsulation. Microencapsulation of compounds in carrier matrices can provide protection against degradation, prevent loss of volatile flavors from the

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encapsulated materials themselves, and enhance the stability and flavor of core materials (Kanakdande, Bosale, & Singhal, 2007). The objectives of this study were to develop an anti-oxidative packaging film coated with microencapsulated volatile horseradish extract and to verify the effects of this film on fresh-cut meats for the purpose of prolonging their shelf-life.

2. Materials and methods

2.1. Preparation of volatile horseradish extract

Dried and ground Japanese horseradish (*Wasabia japonica matsum*) was purchased at a local market and extracted with 80 mL/100 mL methanol overnight at room temperature. A portion of the extract was centrifuged at 2700*g* for 20 min at 4 °C, and the supernatant was filtered through a 0.45 μm filter. The filtered supernatant was evaporated using a rotary vacuum evaporator (Rotavapor, R114, Büchi, Flawil, Switzerland) at 40 °C for 15 min to remove methanol and moisture from the supernatant, leave the extract alone as an essential oil form, and measure its antioxidant activity. The resulting extract was sealed and stored at −40 °C for further processes.

2.2. Free radical scavenging assay

A free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH; Wako Chemicals, Ltd., Osaka, Japan) was used to compare the short-term antioxidant potencies of horseradish and green tea extract (Boo, Chon, Kim, & Pyo, 2005). Horseradish and green tea extracts were dissolved in 80 mL/100 mL methanol to concentrations of 5–2000 μg/mL. The extract sample solutions were then added to the wells of 96-well plates containing 100 μL of 200 μmol/L DPPH solution (in 80 mL/100 mL methanol) and were incubated for 30 min at room temperature. The absorbance was measured at 517 nm with a Micro-plate Absorbance Reader (OPSYS MR, Dynex Technologies Inc., USA). Methanol solution and L-ascorbic acid were used as a reference and a positive control, respectively. The radical scavenging activity of DPPH was calculated according to the following equation:

$$\text{Radical scavenging activity}\,(\%) = [(A_{\text{reference}} - A_{\text{sample}})/A_{\text{reference}}] \times 100$$

where A is the absorbance.

2.3. Identification of allyl isothiocyanate extracted from horseradish

Horseradish extract was analyzed with a Hitachi LaChrom Elite high performance liquid chromatograph (Hitachi Co., JAPAN) equipped with a photodiode-array detector using a YMC Pack-Pro C_{18}, 5 μm, 250 × 4.6 mm I.D. column (YMC, Waters, USA). The extract was diluted with methanol (1 mg of extract/1 mL of methanol) and separated using a 50-min linear gradient from 5 mL/100 mL acetonitrile/95 mL/100 mL distilled water to 55 mL/100 mL acetonitrile, followed by 50–55 min of 100 mL/100 mL acetonitrile at a flow rate 1 mL/min. Elution of the metabolites was monitored using a photodiode-array detector. The retention times and UV spectra were compared to authentic standards such as reagent allyl isothiocyanate.

2.4. Preparation of film coated with microcapsules containing horseradish extract

The encapsulation of the horseradish extract was conducted as follows: 2 mL of the extract were blended with 20 mL of solution containing 0.5, 1.0, or 1.5 g/100 mL chitosan, and the mixture was emulsified by stirring for 10 min at 800 rpm using a homogenizer (Ultra Turax T50, IKA). After emulsification, 1 g/100 mL NaOH was added with slow stirring to form microcapsules. The chitosan-microcapsules were washed twice with distilled water and were dried in a vacuum oven at 30 °C overnight. The weight of the microcapsules was then measured and defined as W_m. The release of volatile compounds from the microcapsules was estimated by measuring the weight $W_m(t)$ of the microcapsules that were placed in an Infrared Moisture Determination Balance (AD-4712, AND). The volatile compound release was defined as:

$$\text{Volatile compound release}\,(\%) = [W_m - W_m(t)/W_m - W_0] \times 100$$

where W_0 denotes the *S* weight of the microcapsules after complete evaporation of the volatiles at 120 °C for 3 h, and t is the incubation time. Volumetric size distribution of the microcapsules was determined by light scattering with a 2062-LC particle analyzer (Malvern Instruments) with the mean diameter and standard deviation calculated from the cumulative distribution curve. Five grams of encapsulated microcapsules were coated on ordinary plastic film (ethylene vinyl acetate film (19 g/100 g of vinyl acetate); 50.8 μm of thickness; 97.2 g/m^2/24 h of moisture vapor transmission rate; and 250 × 150 mm of size) using supernatant of boiled aqueous solution (60 g/100 mL) of corn dextrin (Klimets, Grinyuk, & Krul, 2004) as an adhesive and a bar-coating roller (Webster, USA, RDS30) and dried for 2 h at room temperature.

2.5. Determination of the anti-oxidative properties of the developed film

Pork and Japanese Spanish mackerel (*Scomberomorus niphonius*) were purchased at a local market. Fifty grams of pork or fish fillet were placed on petri dishes (9 cm diameter). The petri dishes containing the pork or fish fillet were wrapped with ordinary plastic film or the microcapsule-coated film in the atmospheric condition, avoiding contact of the pork or fish fillet with the film, and stored at 5 °C for 9 days in darkness. During the storage, color changes and lipid oxidation of the pork and fish were observed.

2.5.1. Color measurements

The color characteristics were assessed using a hand-held tristimulus meter (Lloyd Model LRX-2500 N, Lloyd instrument Ltd., UK) to determine the L^* value (lightness or brightness), the a^* value (redness or greenness), and the b^* value (yellowness or blueness) of the pork and fish samples. The colorimeter was warmed up for 10 min and calibrated with a white standard.

2.5.2. Estimation of peroxide value

The lipids were extracted from the pork and fish samples with a mixture of water, methanol, and chloroform (30:50:100). The peroxide values of the extracts were measured using the modified method of Lea (1952). One gram of the extract was dissolved in 25 mL of solvent (2 parts chloroform: 3 parts acetic acid). Saturated potassium iodide (1 mL) was then added, and the solution was kept in the dark for 10 min. After stabilization, 30 mL of distilled water and 1 mL of starch solution (1 g/100 mL) were added to the solution and titrated with 0.01 mol equiv/L $Na_2S_2O_3$ until colorless. Peroxide values (POVs) were calculated as follows:

$$\text{POV (meq/kg)} = (S - B) \times F \times \text{mol equiv/L}(N) \times 1000/W$$

where S, B, F, mol equiv/L(N), and W denote the titration amount of sample, the titration amount of blank, the titer of 0.01 mol equiv/

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