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Increasing strawberry shelf-life with carvacrol and methyl cinnamate antimicrobial vapors released from edible films

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ABSTRACT

The effect of carvacrol and methyl cinnamate vapors incorporated into strawberry puree edible films on the postharvest quality of strawberry fruit (*Fragaria* × *ananassa*) was investigated. Fresh strawberries were packed in clamshells and kept at 10 °C for 10 days with 90% RH. Strawberry puree edible films, applied in the clamshell, served as carriers for the controlled release of natural antimicrobial compounds without direct contact with the fruit. Changes in weight loss, visible decay, firmness, surface color, total soluble solids content, total soluble phenolics content and antioxidant capacity of strawberries during storage were evaluated. A significant delay and reduction in the severity of visible decay was observed in fruit exposed to antimicrobial vapors. Carvacrol and methyl cinnamate vapors released from the films helped to maintain firmness and brightness of strawberries as compare to the untreated strawberries. The natural antimicrobial vapors also increased the total soluble phenolics content and antioxidant activity of fruit at the end of the storage period.

1. Introduction

Strawberries are a perishable fruit characterized by high respiration and metabolic rates that limit shelf-life (Atress et al., 2010). Rapid deterioration and postharvest losses are mainly caused by inappropriate storage temperatures and microbial spoilage. Fungi are ubiquitous microorganisms with a great capacity to colonize many kinds of substrates and to proliferate under common environmental storage conditions, such as low temperature (El-Shiekh et al., 2012). Among them, gray mold caused by *Botrytis cinerea* is considered the most common disease affecting strawberries (Wang, 2003). Therefore, reducing microbial spoilage plays a key role in prolonging the shelf-life of fresh strawberries as well as preserving their quality attributes during storage. Even though rapid cooling after harvest and low storage temperatures are usually applied because of their effects to reduce the rates of biological reactions and microbial growth (Kader and Saltveit, 2002), other techniques must be combined with refrigeration in order to maintain quality and delay strawberry decay. A wide range of different approaches, mainly based on the application of modify atmosphere packaging (MAP) has been developed. However, traditional MAP is not enough to ensure final product quality and safety (Serrano et al., 2008). Among the various alternatives, edible films made from fruit and vegetables may be considered a valid and effective way to preserve quality of fresh fruit and vegetables since they act as a selective barrier to moisture transfer, limiting therefore water loss, and protecting fresh fruit to external hazards. Edible films may also retard loss of volatile compounds, reduce respiration rate, and delay changes in fruit physical properties. The ability of edible films to extend the shelf-life of fresh food products may be further improved by including antimicrobial plant essential oils (EOs) for controlling pathogenic microorganisms.

Methyl cinnamate is a methyl ester of cinnamic acid and is one of the major volatile components of strawberry aroma produced and released during fruit maturation (Lunkenbein et al., 2006). Because of the sweet fruity flavor, methyl cinnamate is commonly used in many applications as an ingredient in decorative cosmetics and fragrances (Ali et al., 2010), and since it is generally recognized as safe (GRAS), it may also be potentially used as a food additive (Huang et al., 2009). Methyl cinnamate has been proven to have antifungal activity against phytopathogenic fungi under in vitro conditions (Vaughn et al., 1993; Rahmani et al., 2010); nevertheless its utilization in shelf-life studies of fresh products has never been investigated. In this study, it was used in combination with carvacrol (the major component of essential oils from oregano and thyme) because of its well known powerful antimicrobial properties (Lambert et al., 2001; Burt and Reinders, 2003).

Essential oils, and their aromatic volatile components, have been largely investigated for their antimicrobial properties in vitro as vapors (Du et al., 2009; Avila-Sosa et al., 2012; Kloucek et al., 2012)

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as well as in direct contact with the food product (Hammer et al., 1999; Friedman et al., 2002; Burt, 2004; Holley and Patel, 2004). Few studies have reported the beneficial effects of essential oil treatments on strawberry quality (Bhaskara Reddy et al., 1999; Tzortzakis, 2007; Wang et al., 2007). To the best of our knowledge, this is the first study to investigate the effect of essential oil vapors released from edible films on shelf-life and quality of strawberries without direct contact with the fruit. Furthermore, since most of the volatiles are characterized by a strong flavor that clashes with the natural characteristics of the fruit, the selection ofa strawberry puree edible film and methyl cinnamate was made on the basis of organoleptic compatibility with strawberry fruit.

Considering the potential use of volatile compounds as fumigants for the storage of fresh products, the aim of this study was to determine the effectiveness of a new approach based on the use of strawberry puree edible films for the controlled release of antimicrobial carvacrol and methyl cinnamate vapors during storage on strawberry shelf-life and overall quality.

2. Materials and methods

2.1. Materials

Seedless strawberry puree (Sabroso Co., Medford, OR, USA) was used as primary ingredient in strawberry puree edible films. High methoxyl pectin 1400 (TIC Gums, Belcamp, MD, USA) was added to increase film strength, create a semi-permeable film, and facilitate the release from the cast surface. Vegetable glycerine (Starwest Botanicals Inc., Rancho Cordova, CA, USA) was used as a plasticizer agent. Carvacrol and methyl cinnamate were the active compounds tested and, along with Folin-Ciocalteau phenol reagent, sodium carbonate anhydrous, 1-diphenyl-2-picrylhydrazyl (DPPH), and Trolox, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Gallic acid monohydrate and methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA) and ethyl alcohol from Pharmco-Aaper (Oakland, CA, USA).

2.2. Preparation of strawberry puree edible film

Strawberry puree solution was obtained by combining 490 g of strawberry puree (49% w/w) with 500 g of 3% w/w pectin solution and 10 g (1% w/w) of glycerine in a mixer bowl at slow speed for 30 min according to McHugh and Senesi (2000). Carvacrol and methyl cinnamate were then incorporated at 0.75% (w/w) and homogenized for 15 min at 20,000 rpm using a Polytron 3000 homogenizer (Kinematica, Luzern, Switzerland). Methyl cinnamate, because of its insolubility in water, was previously dissolved in ethanol (50% w/w) for 15 min at 200 rpm on a stirring plate. The solutions were degassed under vacuum for almost 1 h to remove bubbles, and then used for film casting. The concentration of volatile compounds was chosen based on the results obtained from preliminary studies (data not shown) in which different concentrations of carvacrol and methyl cinnamate were tested on visual appearance, decay, and weight loss of strawberries. Incorporation of both carvacrol and methyl cinnamate in the strawberry puree film solution at 0.75% (w/w) showed the most promising results, and therefore they were used for this experiment.

2.3. Film casting and application

Films were cast on 29 cm × 29 cm glass plates covered with polyester film to facilitate the removal of dry films after ∼15 h at room temperature. A 35 mil (1 mil = 0.0254 mm) gap draw down stainless steel bar was used to spread 55 g of strawberry puree solution on each plate. The quantity of the solution poured on the plate was chosen based on previous experiments in order to obtain a constant thickness of the film in the whole surface. Films were then cut into 14.5 cm × 8 cm patches and used for the treatments. Some of the films were stored on layers of aluminum foil in zip plastic bags at 4 °C and 65% RH until physical–chemical and mechanical properties were evaluated. Two film patches were then taped on the top and bottom of PET clamshells. The clamshells were previously modified by taping the holes and placing a second PET layer (with 14 holes; 0.6 cm in diameter) inside the clamshell, 2 cm from the bottom. This arrangement was made to allow the release of vapors from the films without touching the fruit.

2.4. Film thickness

Film thickness was measured with a micrometer IP 65 (Mituoto Manufacturing, Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in.) at five random positions around the film. Mean values were used to calculate water vapor permeability (WVP) and tensile strength.

2.5. Water vapor permeability (WVP) of films

The gravimetric modified cup method (McHugh et al., 1993) based on standard method E96-80 (ASTM, 1989) was used to determine WVP. A cabinet with a variable speed fan was used to test film WVP. Cabinet temperature of 25 ± 1 °C was maintained in a Forma Scientific reach-in incubator (Thermo Electron Corp., Waltham, MA). Fan speeds were set to achieve air velocities of 152 m/min to ensure uniform relative humidity throughout the cabinets. Cabinets were pre-equilibrated to 0% relative humidity (RH) using anhydrous calcium sulphate (W.A. Hammond Drierite, Xenia, OH). Circular test cups made from polymethylmethacrylate (Plexiglas TM) were used. A film was sealed to the cup base with a ring containing a 19.6 cm^2 opening using 4 screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant. Distilled water (6 mL) was placed in the bottom of the test cups to expose the film to a high percentage RH inside the test cups. Average stagnant air gap heights between the water surface and the film were measured. Test cups holding films were then inserted into the pre-equilibrated 0% RH desiccator cabinets. Steady state of water vapor transmission rate was achieved within 2 h. Each cup was weighed 8 times at 2 h intervals. Eight replicates of each film were tested. Relative humidity at the film undersides and WVPs were calculated using the WVP correction method (McHugh et al., 1993). The WVP of the films was calculated by multiplying the steady state water vapor transmission rate by the average film thickness determined as described above and dividing by the water vapor partial pressure difference across the films: $WVP = (WVTR \times thickness)/(p_{A1} - p_{A2})$, where WVTR is water vapor transmission rate, p_{A1} and p_{A2} are water vapor partial pressure inside and outside the cup, respectively. Units for WVP were g mm/kPa h m^2.

2.6. Tensile properties of films

Mechanical properties of strawberry puree edible films incorporated with volatile compounds were tested and compared with strawberry puree film to evaluate the effects carvacrol and methyl cinnamate may have on physical–mechanical properties of films. Standard method D882-97 (ASTM, 1997) was used to measure tensile properties of films. Films were cut into strips with a test dimension of 165 mm × 19 mm according to standard method D638-02a (ASTM, 2002). All films were conditioned for 48 h at 23 ± 2 °C and 50 ± 2% RH before testing, in a saturated salt solution of magnesium nitrate (Fisher Scientific, Fair Lawn, NJ, USA). The ends of the equilibrated strips were mounted and clamped with