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Effect of peppermint oil on the shelf-life of dragon fruit during storage

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ABSTRACT

Dragon fruit (*Hylocereus undatus*) is a short shelf-life, non-climacteric fruit which can be easily destroyed by mold growth during storage time. This study investigated the use of peppermint oil as an alternative method to inhibit surface mould and prolong the shelf-life of dragon fruit during storage. Peppermint oil adsorbed activated carbon at different concentrations (100–1000 μL L^{-1}) was placed with the dragon fruit in the storage box (1 L) at 25 ± 2 °C and 75 ± 5%RH for 21 days. The effect of peppermint oil adsorbed activated carbon on antifungal activity and quality of dragon fruit were evaluated. It was found that peppermint oil adsorbed activated carbon at 700 μL L^{-1} could provide 100% inhibition of surface mould and decay fungi for more than 14 days of storage (control start decayed at day 7). In addition, essential oil vapour maintained a more firm fruit, greenness of the bract, titratable acid value and total phenolic content after 21 days in comparison to the control. The possible mode of action was demonstrated by gas chromatography-mass spectrometry (GC-MS) analysis to involve the release of menthol from peppermint oil activated carbon, which then interacts with other compounds to exhibit antifungal activity.

1. Introduction

Recently, the consumption of dragon fruit (*Hylocereus undatus*) as a popular non-climacteric fruit with high nutritive properties has increased rapidly. However, the dragon fruit has a limited shelf-life, so exposure to ambient temperatures during transportation and storage can lead to an undesirable senescent appearance, such as rapid shrivelling and loss of bract's greenness. Moreover, it is also susceptible to fungal contamination by species such as *Rhizopus, Fusarium*, *Botryosphaeria* and *Colletotrichum* (Ali et al., 2013; Ma et al., 2009; Valencia-Botín, Sandoval-Islas, Cárdenas-Soriano, Michailides, & Rendón-Sánchez, 2003; Zahid, Ali, Siddiqui, & Maqbool, 2013), which can cause deterioration of the fruit skin and leaves such as apple and lemon (Castillo et al., 2014; Pieczywek et al., 2018). Furthermore, surface mould contamination is a well-known problem for fruit storage and transportation to the market, often making the fruit unsuitable for consumption. Spores of moulds such as *Aspergillus* spp. (Pisani, Nguyen, & Gubler, 2015) and *Penicillium* spp. (De Corato, Salimbeni, De Pretis, Avella, & Patruno, 2017) can survive in soil, where they infect the surface of fruit post-harvest. The fruit transportation/storage conditions are favourable for the growth of moulds and also expose fruit to the risk of mycotoxin contamination (Pitt, Taniwaki, & Cole, 2013). Post-harvest loses caused by the mold infection made the industry lose their profit, therefore, it is important to develop a novel method to inhibit microbial growth, thereby improving the shelf-life of dragon fruit.

Natural preservatives such as natural extracts (Matan, Puangjinda, Phothisuwan, & Nisoa, 2015) and essential oils (Palou, Ali, Fallik, & Romanazzi, 2016) have been used to inhibit the growth of moulds on fresh fruit. One such essential oil is peppermint, a natural bioactive compound derived from plants that exhibits mild to strong antifungal activity depending on its main components. The application of peppermint oil to crops is growing in popularity due to its well-recognised low influence on sensory properties and it is highly effective against several types of post-harvest fungi (Servili, Feliziani, & Romanazzi, 2017). However, the application of essential oil vapour in post-harvest storage is limited due to the exposure concentration, the vapour is rapidly dispersed and lost to the atmosphere. Accordingly, it needs an adsorbent to control its release into the storage system.

Activated carbon (AC) is a high capacity adsorbent that is widely available and safe for contact with food. It has been used in many fields from food processing, post-harvest, medical, energy storage to environmental application since it can efficiently adsorb various substances under a wide range of temperature and humidity

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(Cermakova, Kopecka, Pivokonsky, Pivokonska, & Janda, 2017). Moreover, its use as a carrier of antimicrobial agents such as nanoparticles and ethanol are growing rapidly (Altintig, Arabaci, & Altundag, 2016; Biswas & Bandyopadhyaya, 2016). The objective of this research was to develop activated carbon as a carrier to adsorb and control the release of peppermint oil and its main components (menthol and menthone) to prevent fungal growth and prolong the shelf-life of dragon fruit during storage.

2. Materials and methods

2.1. Mould strains

All strains (*Aspergillus flavus, Aspergillus niger, Penicillium* spp. and *Rhizopus* spp.) were provided by the Innovation of Essential Oil for Food Safety and Packaging Laboratory of Walailak University in Nakhon Si Thammarat, Thailand. The moulds were cultured on the Malt Extract Agar (MEA) at 25 °C for 7 days before the preparation of spore suspensions (10^7spores ml^{-1}) by flooding the surface of mould.

2.2. Dragon fruit

Fresh dragon fruits (300 ± 100 g) were obtained from local supermarket at Thasala distict, Nakhon Si Thammarat province, Thailand, and fruit with uniform age, size, shape, weight (300 ± 100 g) and free from mechanical and biological defects were selected for the experiment. This fruit was harvested in June 2017. After cleaning and removal of excess soil, the experiments were performed as soon as possible in the laboratory conditioned at 25 °C and an average relative humidity of 70% RH.

2.3. Peppermint oil, menthol, and menthone

Peppermint oil (*Mentha piperita* L.) was supplied by the Thai China Flavors and Fragrances Industry Co., Ltd. of Bangkok, Thailand. Menthol and menthone were purchased from Sigma-Aldrich.

Main components of peppermint oil was extracted from the dragon fruit (n = 3, 5 g) using ethyl acetate following a method adapted from Friedman, Kozukue, and Harden (2000) for GC analysis. The extract was transferred to vials with 4 mL of ethyl acetate. The vials were sealed with a Teflon-lined cap and mixed gently by shaking and the sample allowed to stand for 30 min before being extracted twice more with ethyl acetate. The combined ethyl acetate extracts were then reduced to dryness under a stream of nitrogen at room temperature. Finally, the residue of each was dissolved in 1 mL ethyl acetate, before 1 μl aliquots of each solution was subjected to GC analysis.

Peppermint oil was analysed using GC/MS (Thermo Scientific Inc., USA) with an Agilent DB-5 column (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature programme was initiated at 60 °C, held for 1 min, then raised up to 200 °C at a rate of 5 °C min^{-1}, and maintained for 5 min. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The detector temperature was 250 °C and the split ratio was set at 200:1. The compounds of the oil were identified by comparison of their retention time (RT) and mass spectra fragmentation with those stored in the NIST 0.8 L (database/ChemStation data system) (Modified from Matan, Woraprayote, Saengkrajang, Sirisombat, and Matan (2009) and Suhem, Matan, Matan, Danworaphong, and Aewsiri (2017).).

2.4. Granular activated carbon

Commercial food grade coconut shell granular activated carbon (GAC) was purchased from the Mazuma Co., Ltd, Thailand. Prior to the experiment, the GAC was dried at 100 °C in a hot air oven for 4–5 h to remove adsorbed gases. Then, the GAC was kept inside an airtight box in the desiccator until use.

2.5. Determination of minimum inhibitory concentration (MIC)

The peppermint oil and its main components (menthol and menthone) adsorbed activated carbon were prepared by adsorption of peppermint oil at 100–1000 μL onto 2.5 g activated carbon at 25 °C for 10 min. The treated activated carbon was then placed in a 1 L airtight glass jar. In order to determine the MIC of peppermint oil adsorbed activated carbon on moulds after 7 days of incubation at 25 °C, 1 mL of spore suspensions were sprayed on the surface of dragon fruit before adding into the glass jar. MICs were recorded as the lowest concentration of the peppermint oil adsorbed activated carbon which could visually inhibit fungal growth. All jars were then incubated at 25 °C for 10 days.

2.6. Shelf-life extension of dragon fruit using peppermint oil adsorbed activated carbon

Dragon fruits (n = 5) were prepared and placed into small plastic box (1 L) containing saturated NaCl solution to adjust the relative humidity of the system to 75 ± 5%RH. Then, 700μLof peppermint oil adsorbed activated carbon and control (only activated carbon) was placed into the box. All boxes were stored at 25 °C for 21 days. The quality of the dragon fruit was assessed every week according to Ali et al. (2013). Surface mould and fungal decay counting was done according to Suhem et al. (2017) as the following analytical methods.

2.6.1. Surface mould and fungal decay

The dragon fruits were divided into 2 groups, those which were inoculated with spores of *Aspergillus flavus, Aspergillus niger, Penicillium* spp. and *Rhizopus* spp., and those which were not inoculated with spores.

For evaluation of mould surface and fungal decay of dragon fruit, the visible fungal growth on the dragon fruit surface was rated using a scale of 0–5 [0 = no growth; 1 = slight (0–20%); 2 = moderate (20–40%); 3 = moderately severe (40–60%); 4 = severe (60–80%); 5 = extremely severe (80–100%)](Suhem et al., 2017). The fungal inhibition as a percentage of the control was calculated as in equation (1):

$$\text{The percentage fungal inhibition} = (A - B)/A \times 100 \quad (1)$$

Where A is the total score for the control. B is the total score for each treatment.

2.6.2. Determination of weight loss

The weight loss of each dragon fruit was determined using a digital balance (model STX2202, Ohaus Corp., USA) and expressed as a percentage of the initial weight loss using equation (2):

$$\text{Weight loss (\%)} = (W_1\text{-}W_2/W_1) \times 100 \quad (2)$$

Where W_1= Fruit weight in the beginning. W_2=Fruit weight at the week of sampling.

2.6.3. Determination of firmness

The fruit firmness was measured using a texture analyser (model LR5K, Lloyd instrument) in compression mode. The test was performed using a 5-mm diameter stainless steel probe to penetrate 25 mm into three random positions of the fruit with a speed