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A new technique to prevent the main post harvest diseases in berries during storage: Inclusion complexes β-cyclodextrin-hexanal

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Abstract

Natural occurring volatiles such as hexanal have a well know antifungal capacity but limited post harvest use due to their volatility. Taking this into consideration, hexanal was inserted into β-cyclodextrins (β-CD) to develop a controlled release mechanism and then evaluated *in vitro* against *Colletotrichum acutatum*, *Alternaria alternata* and *Botrytis cinerea*, the three main causes of post harvest diseases in berries. Different concentrations of both pure volatile hexanal and its inclusion complexes (IC) were analyzed for their fungistatic and fungicidal effects for 7 days at 23 °C. Hexanal has fungistatic effect on all fungi tested, however, fungicidal activity was only observed on *C. acutatum*. Results showed that hexanal's effectiveness was greater against *C. acutatum* than *A. alternata* and *B. cinerea.* Concentrations of 1.1, 2.3 and 1.3 μL hexanal/L air respectively were necessary to prevent *C. acutatum*, *A. alternata* and *B. cinerea* growth. Lower concentrations reduced fungal growth depending on the included amount and type of fungus. Same amount of hexanal released from β-cyclodextrin had a lower antifungal effect on *C. acutatum*. Thus, ICs β-cyclodextrin-hexanal can be used to reduce or avoid post harvest berry diseases because of their capacity to provide an antifungal volatile during storage, distribution, and consumer purchasing.

Keywords: *Colletotrichum acutatum*; *Alternaria alternata*; *Botrytis cinerea*; Hexanal; β-cyclodextrins; Post harvest

1. Introduction

The taste and flavor of berries are appreciated worldwide in fresh and manufactured foods. Nowadays, these fruits are used for making juice, desserts, jam, syrup and wine, and market opportunities continue to grow. Berries have also been shown to have diverse health benefits such as prevention of certain cancers, heart disease and aging because of the vitamins, minerals, dietary fiber and the phytochemicals that they contain (Beattie et al., 2005; Juranic and Zizak, 2005). However, berries are very perishable fruits with a short shelf life. High sugar content and other nutrients along with a high water activity and low pH provide an ideal environment for fungal growth. Thus, post harvest losses during berry harvesting and marketing are mainly caused by fungal diseases. Fungi such as *Colletotrichum acutatum*, *Alternaria alternata* and *Botrytis cinerea* are the most common causes of berry decay (Smith, 1990; Smith et al., 1996; Polashock et al., 2005; Tournas and Katsoudas, 2005). The control of these fungi is very difficult, even with the application of pre-harvest fungicides.

Means for reducing or avoiding fungi in fresh products are being studied. The addition to the fresh product surroundings of natural occurring plant volatiles is known to have antifungal effectiveness (Gardini et al., 2001; Utama et al., 2002; Almenar et al., 2004). Several natural substances have been identified and numerous studies on their antifungal activity have been reported. Aroma compounds such as hexanal have been shown to have antimicrobial activity against spoilage micro-organisms *in vitro* and in real systems (Caccioni et al., 1997; Lanciotti et al., 1999; Corbo et al., 2000; Neri et al., 2006). This volatile has been shown to be a metabolizable fungicide, having adequate volatility and the capability of enhancing the fruit's aroma production by its conversion into other aroma volatiles. Furthermore, hexanal is commercially available, is approved as a food additive by the US Food and Drug Administration and has an ORL-MAM LD_{50} of 3700 mg kg^{-1}, (Song et al., 1996; Guerzoni et al., 1997; EAFUS, 2006). The main disadvantage of using this aldehyde is its highly volatile nature and the resulting quick release from the application point.

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Cyclodextrins are naturally occurring molecules (produced enzymatically from starch) composed of glucose units arranged in a bucket shape with a central cavity (Fig. 1). These oligosaccharides are composed of six, seven and eight anhydroglucose units, namely α, β and γ, respectively. All have a hydrophilic exterior and a hydrophobic cavity, which enables them to form inclusion complexes (IC) with a variety of hydrophobic molecules. The number of glucose molecules is associated with the size of the cavity. The various cavity sizes allow for great application flexibility since ingredients with different molecular sizes can be effectively complexed with cyclodextrin (α: 4.7–5.3 Å, β: 6.0–6.5 Å and γ: 7.5–8.3 Å) (Martin del Valle, 2004). Cyclodextrins were chosen for the encapsulation of hexanal because of their ability to prevent the quick release of this volatile and thus allow its slow diffusion over a long period of time. These complexes could be used to retard or inhibit microbial activity and so prolong berry shelf-life. This has potential application during fumigation in cold storage, insertion into packaging materials (active packages) or the creation of special atmospheres in greenhouses. According to Martin del Valle (2004), after the complex is formed and dried, the displacement of the complexed guest by water molecules is due to an equilibrium between the guest and water, with the guest being replaced by water molecules entering the cyclodextrins. Since berries are stored under high humidity conditions, the water content in the atmosphere would be high enough to achieve the replacement of the volatile by water molecules Fig. 2 (a).

The aims of this research were to develop and evaluate an alternative fungicide method for berries which would be effective against their main fungal diseases, environmentally responsible (hexanal and CD are natural occurring compounds from plants), and that would be active during long periods of time in storage and transport.

2. Materials and methods

2.1. Materials

α, β and γ-cyclodextrin (purity > 99%) were provided by Wacker Chemical Corporation (Adrian, MI, US). The volatile compound hexanal (purity > 98%) was purchased from Sigma–Aldrich Corp. (Saint Louis, MO, USA). Axenic cultures of *C. acutatum*, *A. alternata* and *B. cinerea*, originally isolated from blueberries were provided by the Department of Plant Pathology, MSU, East Lansing, MI, US.

2.2. Preparation of complexes

β-cyclodextrin (5.7 g) was added into a beaker containing 57 mL of hot distilled water and then stirred using a hot plate stirrer (Thermolyne® Mirak™ hot plate/stirrer; Sigma–Aldrich Corp. (Saint Louis, MO, USA)). After the β-cyclodextrin had dissolved, 307, 610, 1230 or 1845 µl of hexanal were slowly released into the solution, and stirred for 2 h at 100 °C at 264 rpm. The beaker was then placed on another stirrer plate (Thermolyne Nuova II stir plate, Barnstead International, Testware, Sparks, NV, US) for 20 min at room temperature. Finally, the sample was centrifuged at 1600 rpm for 40 min, and the paste obtained was dried at 60 °C for 24 h. All samples were evaluated in triplicate and stored in hermetically sealed flasks at 23 °C.

2.3. Emission of hexanal from β-cyclodextrin complexes

A simple desorption system was used to evaluate the efficacy of IC. Glass vials of 40-mL were filled with 1 mL of distilled water and on the bottom of this a 2-mL glass vial containing 0.1 g of IC was positioned (Fig. 2 (b)), and immediately closed with Mininert® valves (Supelco, Bellefonte, PA, US). After 1, 2, 3, 5 and 7 days, the hexanal concentration released from the IC to the vial headspace was measured using a 65-µm PDMS/DVB SPME fiber (Supelco, Bellefonte, PA, US) and gas chromatography. The fiber was exposed to the vial headspace for 10 min. The volatile trapped in the SPME was quantified by desorbing the volatile (for 5 min) at the splitless injection port of a GC Hewlett–Packard 6890 series esc (Agilent Technology, Palo Alto, CA, US) equipped with FID and a HP-5 column (30 m × 0.32 mm × 0.25 µm, Hewlett–Packard, Agilent Technology, Palo Alto, CA, US). The oven temperature was initially 40 °C for 5 min. It was then increased to 220 °C at 5 °C/min and maintained for 10 min. The injector and detector temperatures were 230 °C. Quantification of hexanal in the headspace was

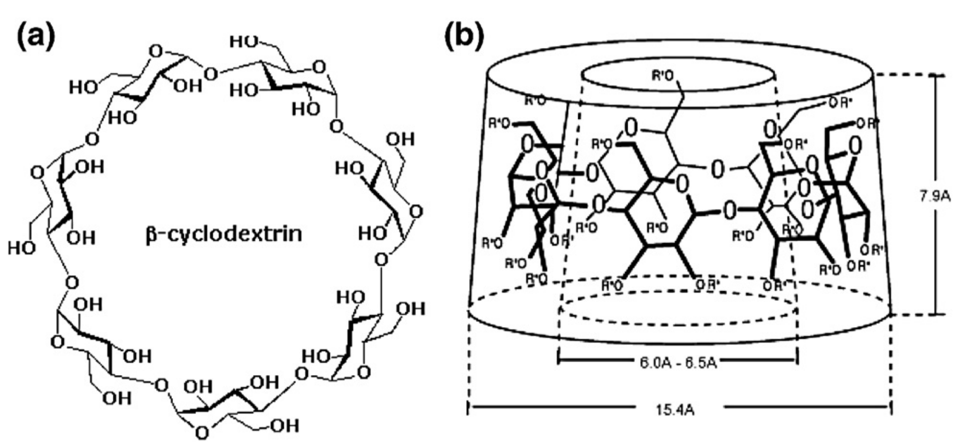

Fig. 1. Chemical structure of β-cyclodextrin: (a) top view (www.cem.msu.edu/…/Images3/b-cydext.gif access date 05/19/06) and (b) front view (www.cyclodex.com/images/figure2.gif access date 05/15/06).