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POSTEC 10426 No. of Pages 8
ARTICLE IN PRESS

Postharvest Biology and Technology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Antimicrobial efficacy of liposomes containing D-limonene and its effect on the storage life of blueberries

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ARTICLE INFO

Article history:
Received 26 November 2016
Received in revised form 9 February 2017
Accepted 16 February 2017
Available online xxx

Keywords:
Blueberries
Edible coatings
Limonene
Liposomes
Antimicrobial coating
Postharvest decay

ABSTRACT

The purpose of this study was to investigate the antimicrobial activity of unilamellar nano vesicles (liposome) containing D-limonene against selected fruit rotting fungi (*Botrytis cinerea* and *Penicillium chrysogenum*) and food borne illness causing bacteria (*Escherichia coli* and *Listeria monocytogenes*). Furthermore, the research evaluated the extended shelf life and enhanced food safety of blueberries treated with D-limonene and liposomes. Liposomal nanoparticles were created by thin lipid film hydration followed by sonication. Mean liposome radius was 100.2 ± 3.1 nm. The antibacterial activity against *E. coli* showed 0.99 and 1.6 $\log_{10}$ reductions in CFU mL^{-1} at 10 μM and 50 μM, respectively, within 48 h. The $\log_{10}$ reduction was 1.6 at 10 μM and 3.4 at 50 μM for *L. monocytogenes*.

Germination of *B. cinerea* conidia was completely suppressed over 48 h by a 50 μM concentration of limonene. Liposomes exhibited 2.2 and 2.8 $\log_{10}$ reductions for *P. chrysogenum*, whereas 3.7 and 4.6 $\log_{10}$ reductions were observed for *B. cinerea* with 10 μM and 50 μM limonene, respectively. The *in vivo* study of liposome coatings on blueberries also revealed protection against microbial growth even after nine weeks of storage at 4 °C with liposomes reducing blueberry spoilage by more than 60% at the end of nine weeks. The results of this study can benefit the produce industry through both enhancement of food safety and extending the shelf life of blueberries, further highlighting the commercial applications of liposomes.

1. Introduction

Blueberries are considered to be a healthy “super” food with various forms of bioactive, beneficial compounds and are the second most consumed berry in the United States of America (Kang et al., 2015). While demand for the crop is stable year-round, the short growing season, high susceptible to fungal spoilage, and short shelf life represent major challenges to the blueberry industry. The common postharvest fruit rots of blueberries based on severity of occurrence include: Botrytis or graymold (*Botrytis cinerea*), *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium*, and yeasts (Mehra et al., 2013; Tournas and Katsoudas, 2005). While the low pH of blueberries reduces the impact of bacterial spoilage, cross contamination with food borne illness causing bacteria can occur during agronomic practices and post-harvest handing.

Edible coatings for berries have been used to minimize microbial damage, which helps to extend product shelf life (Abugoch et al., 2016; Arnon et al., 2015). Edible coatings are based on natural, biodegradable ingredients in response to customer demands for safe and healthy foods and research on edible coatings has increased in recent years with reports describing reduced fungal spoilage and extended storability of some fresh berries (Abugoch et al., 2016; Chiabrando and Giacalone, 2015). There are several types of edible coatings made of lipids, proteins, or polysaccharides. A number of chitosan-based edible coating have been explored for blueberries. Abugoch et al. (2016) assessed the properties and effectiveness of a quinoa protein, chitosan, and sunflower oil mixture as a coating and found a delay in fruit ripening and controlled growth of molds over 32 days of storage. In a separate study, both a chitosan coating and

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http://dx.doi.org/10.1016/j.postharvbio.2017.02.007
0925-5214/

Please cite this article in press as: A.L. Umagiliyage, et al., Antimicrobial efficacy of liposomes containing D-limonene and its effect on the storage life of blueberries, Postharvest Biol. Technol. (2017), http://dx.doi.org/10.1016/j.postharvbio.2017.02.007

an alginate coating preserved anthocyanin content, phenolic content, and antioxidant capacity, while also lessening firmness downfall and weight loss over a 45 days storage period at 0 °C (Chiabrando and Giacalone, 2015). Finally, a coating with a blend of chitosan, glycerol, Tween 80 and *Aloe vera* extract showed effective control of fungal spoilage, enhanced antioxidant capacity, and prolong the shelf life of blueberries for five days compared to untreated berries (Vieira et al., 2016). While each coating or layering has advantages as well as weaknesses, these studies demonstrate the great potential for exploration of developing an extended release type edible coatings that deliver natural antimicrobials to both enhance shelf life and improve food safety.

Limonene (4-isopropenyl-1-methylcyclohexene) is a monoterpene present in oranges, mandarins, lemons, limes, grapefruit and many other fruit of the citrus family (Espina et al., 2011). Currently, it is used in cleaning liquids, cosmetics, flavorings, and many other consumer products. In the food industry, limonene is considered as generally regarded as safe (GRAS) material, so under the regulations it is used as a food perseverative as well as a popular food additive to provide a citrus flavor. Limonene has also been studied as an antimicrobial agent, for use in chemotherapy against cancers (Vigushin et al., 1998), and as an antioxidant (Singh et al., 2010). The main challenges in using limonene as a coating include degradation under oxidative conditions and its hydrophobicity, which negatively impacts dispersion in water (Li and Chiang, 2012). Regarding the latter point, the low concentrations required to achieve dispersal in water reduces its antimicrobial efficacy. So while limonene shows great potential as a protective food agent, its chemistry limits applicability as a coating using published approaches.

To overcome this drawback, a novel approach that uses a liposome-based, nano-encapsulated limonene was devised to address both the natural oxidation and hydrophobicity/concentration problems. Liposomes are self-assembled vesicular structures consisting of one or several bilayer membranes of phospholipids encapsulating a volume of aqueous media. Recently, we have encapsulated curcumin (Dogra et al., 2015) and cinnamaldehyde (Makwana et al., 2014) in liposomes that proved highly antimicrobial against foodborne pathogens. Makwana et al. (2014) reported that antibacterial activity of cinnamaldehyde was enhanced by encapsulation into liposome and showed above five log (>5 $\log_{10}$ CFU ml^{-1}) reduction for the population of *E. coli* W1485 and *B. cereus*. Liposome has been explored as nano-carriers as an alternative to encapsulate essential oils for enhanced delivery (Sebaaly et al., 2015). Further, recent studies have shown the capability of liposomes to protect activity of entrapped

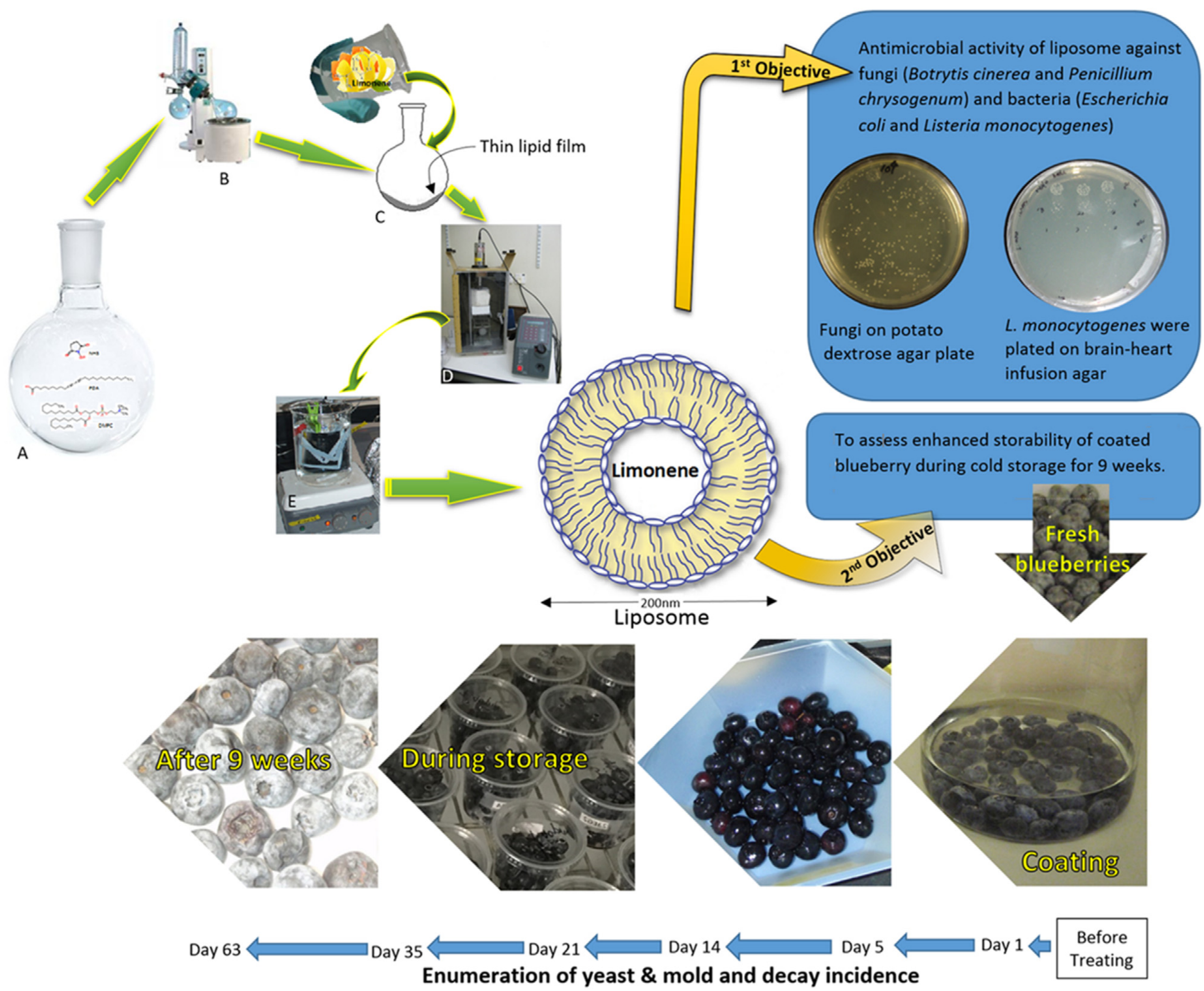

Fig. 1. A pictorial summary of the methodology.