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Carvacrol-loaded chitosan nanoparticles maintain quality of fresh-cut carrots

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ABSTRACT

The effects of carvacrol-loaded chitosan-tripolyphosphate nanoparticles (Np-EO) on the physicochemical, sensory and microbial quality of fresh-cut (FC) carrot slices stored up to 13 days at 5 °C were studied. Np-EO was compared to samples treated by NaOCl (100 mg L^{-1}), Np (chitosan-tripolyphosphate nanoparticles without carvacrol) or individual chitosan (0.5%) and carvacrol (0.5%) solutions. Np-EO achieved the best sensory scores also avoiding carvacrol-related off-flavours found with the carvacrol solution. Furthermore, whitening of FC carrot slices was highly reduced in Np-EO samples. Np-EO reduced microbial levels in FC carrot slices by 0.6–3.0 log units on processing day compared to untreated (control) samples. Np-EO allowed to reduce the microbial growth in FC carrot slices during the first 9 days of storage similarly to carvacrol solution. Furthermore, Np-EO highly controlled microbial loads at the end of storage showing 2.3 (lactic acid bacteria), 6.1 (yeasts and moulds) and 5.1–5.4 (mesophiles, psychrophiles and *Enterobacteriaceae*) lower log CFU g^{-1} units compared to control samples. Conclusively, Np-EO highly maintained microbial (2–6 lower log CFU g^{-1} units compared to control), sensory (up to 2.5 better scores than control) and physicochemical quality of FC carrot slices than control for 13 days at 5 °C.

Industrial relevance: Natural essential oils industrially extracted from plants are potential alternative substances with high antimicrobial properties when tested *in vitro*. However, their microbicidal efficacy is greatly reduced due to their low solubility in washing solutions of fresh-cut products. Accordingly, chitosan-tripolyphosphate nanoencapsulation of essential oils such as carvacrol is a great opportunity to increase the antimicrobial properties of carvacrol to be used in fresh-cut fruit and vegetables alternatively to conventional NaOCl sanitation.

1. Introduction

Carrot is a vegetable that occupies an important segment in the fresh-cut (FC) industry due to their versatility of use, pleasant flavour and nutritional and health-promoting benefits. However, minimal processing of FC carrots such as slicing induces tissue damage, which triggers non-microbial and microbial spoilage during storage and a subsequent negative impact on sensory quality (Martínez-Hernández, Amodio, & Colelli, 2016). Washing with 50–150 mg L^{-1} chlorinated water (NaOCl) is a method widely used in the FC fruits and vegetables industry, thus reducing their initial microbial loads and ensure the food safety of these products (Martínez-Hernández et al., 2015). However, NaOCl may be potentially harmful for humans and the environment (Hrudey, 2009). Thus, several natural antimicrobials such as plant essential oils (EOs) are being studied as alternatives to NaOCl (Sivakumar & Bautista-Baños, 2014).

Generally, EOs possessing the strongest antibacterial properties are those that contain phenolic compounds such as carvacrol, eugenol, and thymol (Hirasa & Takemasa, 1998; Rota, Carraminana, Burillo, & Herrera, 2004). Carvacrol is a major component of the EOs derived from oregano, thyme, marjoram and summer savoury, and is generally recognized as a safe food-grade additive. The mechanism of action of carvacrol and EOs in general against microorganisms involves the interaction of phenolic compounds with the proteins (porins) in the cytoplasmic membrane that can precipitate and lead to leakage of ions and other cell contents causing cell lysis (Nychas, Skandamis, & Tassou, 2000). Carvacrol is reported to have strong bactericidal action (Lambert, Skandamis, Coote, & Nychas, 2001). Accordingly, good antimicrobial effects have been reported in different FC fruits and vegetables, such as lettuce, kiwifruit, apples and melons, treated with carvacrol-containing washing solutions (Bagamboula, Uyttendaele, & Debevere, 2004; Onursal et al., 2014; Roller & Seedhar, 2002). EOs have a strong antibacterial activity according to *in vitro* studies although it has generally been found that a greater concentration of EOs is needed to achieve the same effect in foods (Burt, 2004). However, high concentrations of EOs may transfer strong off-flavours perceived as non-

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characteristic of the food product to FC fruits and vegetables. Furthermore, due to their lipophilic nature, it is difficult to dissolve EOs in aqueous solutions used as sanitising washing treatments for FC fruits and vegetables. Moreover, carvacrol is a volatile compound which easily evaporates and/or decomposes (oxidation) during food processing owing to direct exposure to light or oxygen. Chitosan has received great interest in the encapsulation of bioactive compounds due to its biocompatibility, low toxicity and biodegradability (Muzzarelli, 2010). The chitosan–tripolyphosphate (TPP) nanoparticles, composed of food-safe ingredients, have shown their capacity for the encapsulation and delivery of carvacrol and oregano oil allowing to retain the functional properties of these EOs (Hosseini, Zandi, Rezaei, & Farahmandghavi, 2013; Keawchaoon & Yoksan, 2011). Furthermore, encapsulation of carvacrol may reduce the characteristic off-flavours occurred in carvacrol crude solutions (occurring for the high concentrations needed for a good sanitising effect) due to the controlled release of the encapsulated EO. In this way, sanitising solutions containing encapsulated carvacrol may provide an excellent sanitising washing treatment with similar or higher antimicrobial activity compared to NaOCl without the problems related to dissolution in water of carvacrol and their characteristics off-flavours. To our knowledge there has been no prior work regarding the use of carvacrol nanoparticles in FC products so the study of its potential as a substitute of NaOCl is relevant for this sector. Among the variety of methods developed to prepare chitosan nanoparticles, ionic gelation technique has attracted considerable attention as this process is non-toxic, organic, solvent-free, convenient and controllable (Agnihotri, Mallikarjuna, & Aminabhavi, 2004). The ionic gelation technique is based on the electrostatic interaction between the positively charged primary amino groups of chitosan and the negatively charged groups of polyanions such as TPP. Present safety and risk assessment methods are based on knowledge gathered for conventional chemicals. Accordingly, knowledge on the toxicity of nanoparticles has been limited although it is rapidly growing (Bouwmeester et al., 2009). The aim of this study was to investigate the effects of a sanitising solution containing chitosan–TPP nanoparticles loaded with carvacrol (Np-EO) on the microbial and physiochemical quality of FC carrots throughout storage. Such treatment may be a potential substitute of NaOCl in the FC industry.

2. Material and methods

2.1. Plant material

Carrots (*Daucus carota* L.) were obtained from a local market (Foggia, Italy) and stored at 5 °C and 90–95% RH until the next day, when they were processed. Minimal processing was accomplished in a disinfected cold room at 10 °C. Plant material was inspected; carrots were free from defects and with similar visual appearance. The carrots were washed (2 min) with NaOCl (100 mg L^{-1}; 5 °C; pH 6.5 ± 0.1) with a ratio of 300 g of plant material to 5 L of disinfected water (w/v), rinsed (1 min) with tap water (5 °C) and drained in a perforated basket. The carrots were peeled using a manual peeler and cut into slices of 8-mm thickness using a manual slicer. The peeler and slicer were regularly disinfected with 70% ethanol during preparation. Slices corresponding to the central part of the carrots ranging from 14 to 18 mm in diameter were selected for the treatments.

2.2. Preparation of washing solution containing carvacrol-loaded Ch-TPP nanoparticles

Np-EO were prepared according to Keawchaoon and Yoksan (2011). Chitosan solution (1% w/v) was prepared by dissolving chitosan flakes in aqueous acetic acid solution (1% v/v) at ambient temperature overnight. Tween 80 (HLB 15.9, 2.25 g) was then added to the solution and stirred at 60 °C for 2 h to obtain a homogeneous mixture. Carvacrol (2.5 g) was dissolved separately in ethanol (20 mL) and then this oil phase was gradually added drop-by-drop into the aqueous chitosan solution (200 mL) during homogenization (Ultra-Turrax T25 basic, IKA, Germany) at a speed of 13,500 × *g* for 10 min under a cold water bath (containing ice) to obtain an oil-in-water emulsion. Subsequently, a TPP solution (0.5% w/v, 200 mL) was slowly added into the oil-in-water emulsion drop-by-drop with stirring. Agitation was continuously done for 30 min at 13,500 × g. The particles were collected by centrifugation at 10,500 × *g* for 10 min at 25 °C and washed with aqueous Tween 80 solution (1% v/v) and distilled water several times to remove free carvacrol. The obtained wet particles were dispersed in aqueous acetic acid solution (0.48% v/v; 420 mL; pH = 4.3). A weight ratio of chitosan to carvacrol 1:1.25 was used for the present study. The carvacrol was successfully loaded into chitosan-TPP nanoparticles as confirmed by UV–vis spectrophotometry (Shimadzu UV1700, Kyoto, Japan) using a standard curve with different carvacrol concentrations (diluted with ethanol). Np-EO were immersed in ethanol for 1 h and maximum absorption peak at 275 nm, corresponding to carvacrol, was registered while it was not observed Np samples (Fig. 1).

2.3. Washing treatments

Plant material slices were submerged in the washing treatments (5 °C) for 4 min with a ratio of 300 g of plant material to 5 L of sanitising washing (w/v). The washing treatments are detailed below.

- Control: water.
- NaOCl: 100 mg L^{-1} (acidified with citric acid to pH 6.5 ± 0.1). The product was rinsed after washing treatments for 1 min with cold tap water.
- Chitosan solution: prepared at 0.5% as described in the previous section. pH of final solution was 4.36 ± 0.02.
- Carvacrol solution: carvacrol was previously dissolved in ethanol (98%) and subsequently in water to final concentration of 0.5%. pH of final solution was 4.35 ± 0.03.
- Solution with Ch-TPP nanoparticles (Np). Nanoparticles were prepared as previously described using 2.5 mL of distilled water instead of carvacrol. pH of final solution was 4.44 ± 0.02.
- Solution containing carvacrol-loaded Ch-TPP nanoparticles (Np-EO). Nanoparticles were prepared as previously described pH of final solution was 4.33 ± 0.02.

After treatments, product was drained in a perforated basket. Samples (150 g) were placed in 1-L rigid polypropylene (PP) clamshells (12 × 17 × 5 cm). Three clamshells (analytical replicates) per treatment were prepared for this experiment (one experiment replicate). Subsequently, the samples were stored at 5 °C (90–95% RH) up to 13 days. A synergism between reduced oxygen atmospheres and the antimicrobial effects of EOs may occur (Burt, 2004; Galvez, Abriouel, Lopez, & Ben Omar, 2007). Accordingly, samples were stored under aerobic conditions, using individual polyethylene bags to prevent water loss, to better understand the simple effect of the treatment during storage independently of the atmosphere conditions. Analyses were conducted on the processing day and after 3, 6, 9 and 13 days of storage.

2.4. Microbial analysis

Standard enumeration methods were used to determine the microbial growth according to Rinaldi et al. (2013) but with slight modifications. A 10-g sample of carrots was mixed with 90 mL of sterile saline solution (8.5 g NaCl L^{-1}; Sigma Aldrich, Germany) for 1 min with a stomacher (Colwort Stomacher 400 Lab, Seward Medical, London, UK). One millilitre of the appropriate sample dilution was pour-plated on (1) plate count agar (PCA, Oxoid, Basingstoke, United Kingdom) incubated at 30 °C/24–48 h and 5 °C/7 days for total aerobic mesophilic and psychrophilic bacteria, respectively; (2) violet red bile dextrose agar (VRBD, Oxoid, Basingstoke, United Kingdom) incubated at 37 °C/