LWT - Food Science and Technology 84 (2017) 353–358

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Microbial inactivations with hydrolysed lactoferrin and other natural antimicrobials in fresh-cut fennel

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ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form
15 May 2017
Accepted 31 May 2017
Available online 1 June 2017

Keywords:
Minimal processing
Bacteriocins
Lactoferrin
Essential oils
Quality

ABSTRACT

Natural antimicrobials (nisin, lactoferrin, thymol and citrus extract) were studied as sustainable alternatives to conventionally used NaOCl in fresh-cut (FC) fennel. *Enterobacteriaceae* was the most sensitive microbial group to studied sanitizers according to δ values, while psychrophiles were the most resistant. Based on inactivation modelling and sensory scores, nisin (N-250; 0.250 g L^{-1}), lactoferrin (L-50; 50 g L^{-1}), together with hydrolysed L-50 (LFH), were selected and studied comparing to water-washed (CTRL) and NaOCl (150 mg NaOCl L^{-1}) on FC fennel. LFH achieved the highest psychrophilic reduction of 2.5 log units. Although NaOCl achieved the highest mesophilic, *Enterobacteriaceae*, lactobacilli and yeasts and moulds reductions of 1.7, 1.0, <1.7 and 1.6, respectively, N-250 still achieved 0.50–1.0 log unit reductions compared to CTRL. Conclusively, LFH and N-250 are natural antimicrobial treatments with good microbial inactivation rates which may be a good alternative to conventional NaOCl used by the FC industry.

1. Introduction

The minimal processing steps required in fresh-cut (FC) products result in microbial proliferation which may highly reduce their shelf life. Chlorine, mainly sodium hypochlorite (NaOCl), may be potentially harmful for humans and the environment (Hrudey, 2009). Thus, several natural antimicrobials such as bacteriocins, lactoferrin (LF) and essential oils (EOs) seem to be useful alternatives to preserve quality of horticultural products (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009).

Nisin is a food-grade bacteriocin, permitted as a food ingredient that is widely used in the food industry. Many studies have demonstrated the improvement of its effect when it is combined with metal-chelating agents like ethylene diaminotetracetic acid (EDTA) (Alakomi, Saarela, & Helander, 2003; Hancock & Rozek, 2002). The solubility of bacteriocins may also increase at lower pH, facilitating diffusion of bacteriocin molecules (Galvez, Abriouel, Lopez, & Ben Omar, 2007).

EOs are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). The antimicrobial properties of EOs tested *in vitro* need to be incremented to a higher concentration to achieve the same effect in foods and several interactions with food components may occur (Burt, 2004). Accordingly, its effectiveness between different food products may be studied. Physical conditions that improve the action of EOs are low pH, low temperature and low oxygen levels (Burt, 2004).

The ability of bovine LF to control the growth of pathogen microorganisms is known since the seventies (Jenssen & Hancock, 2009). Some authors have demonstrated that the peptide lactoferricin B (LfcinB) released by bovine LF pepsin digestion, is mainly responsible for its antibacterial activity against pathogens and that the antimicrobial potency of this peptide is much higher than that of an equimolar amount of intact bovine LF (Quintieri et al., 2012). Accordingly, a direct evidence of the ability of bovine LF hydrolysed by pepsin (LFH), containing LfcinB, to delay the growth of *Pseudomonas* spp. and coliforms contaminating commercial high moisture Mozzarella cheese under cold storage condition has been recently reported (Caputo et al., 2015; Quintieri et al., 2012). However, the antimicrobial effects of LFH have been not studied in FC fruit and vegetables yet.

Fennel is a plant which edible bulb has high potential for the FC industry in order to supply to consumers a new ready-to-eat product with high organoleptic and health-promoting properties

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http://dx.doi.org/10.1016/j.lwt.2017.05.079
0023-6438/

(Rinaldi, Amodio, & Colelli, 2007). However, fennel morphology and types of tissues may highly promote microbial growth in the FC product when compared to other vegetables and fruit. Conversely, there are no previous studies on such natural antimicrobials on FC fennel. Accordingly, the aim of this work was to study natural sanitising treatments alternative to conventionally used NaOCl and select the best concentration according to microbial quality and sensory acceptance.

2. Materials and methods

2.1. Plant material

Fennel (*Foeniculum vulgare*) was obtained from a local producer (Foggia, Italy) and stored at 5 °C and 90–95% relative humidity until the next day, when it was processed. Minimal processing was accomplished in a disinfected cold room at 10 °C. The two outer bulb bracts of fennel were removed and the obtained bulbs were prewashed with cold (5 °C) tap water for 1 min. Subsequently, the bulb was sliced using a sharp knife to 7 mm thickness. The knife was regularly disinfected with 70% ethanol during preparation.

2.2. Sanitising treatments

Two different experiments were conducted. In the first experiment, microbial inactivations kinetics of the natural antimicrobials nisin, LF, thymol and citrus extract treatments, at three concentrations levels (based on previous studies), were studied on FC fennel being selected the most effective treatments with adequate sensory quality. In the second experiment, the best LF concentration was selected to study microbial inactivations with a LFH treatment prepared at such concentration compared to conventional NaOCl and nisin concentration (thymol and citrus extract were discarded due to sensory rejection as shown later).

2.2.1. First experiment: microbial inactivation kinetics after sanitising treatments with different concentrations

FC fennel slices were submerged in the following washing treatments (5 °C) for 4 min with a ratio of 300 g of plant material to 5 L of sanitising washing (weight/volume; w/v):

- Nisin (N): three different nisin (Sigma-Aldrich, St Louis, MO, US) concentrations of 0.025 (hereinafter described as N-25), 0.100 (N-100) and 0.250 g L^{-1} (N-250) were used in combination with 0.10 g L^{-1} EDTA and 2.0 g L^{-1} citric acid. pH of final solution was 4.1 ± 0.2.
- Bovine LF (LF): three different bovine LF (Farmalabor, Canosa di Puglia, Italy) concentrations of 10 (LF-10), 20 (LF-20) and 50 g L^{-1}(LF-50) were used. pH of final solution was 7.0 ± 0.1.
- Thymol (T): three different thymol (SAFC, St. Louis MA, USA) concentrations of 0.2 (T-200), 0.3 (T-300) and 0.4 g L^{-1} (T-400) were used. The thymol was previously dissolved in ethanol (98%) and subsequently in water with a final ethanol concentration of the washing solution <1%. pH of final solution was 7.0 ± 0.1.
- Citrus (C) extract: three different concentrations of 0.5 (C-500), 1.0 (C-1000) and 1.5 g L^{-1} (C-1500) of citrus (grapefruit, mandarin, bergamot orange and sweet orange) extract (Biocitro®, Probena, Zaragoza, Spain) were used. pH of final solution was 7.0 ± 0.1.

2.2.2. Second experiment: microbial inactivations with hydrolysed lactoferrin

FC fennel slices were submerged in the following washing treatments (5 °C) for 4 min with a ratio of 300 g of plant material to 5 L of sanitising washing (w/v). Furthermore, based on the best LF treatment concentration, hydrolysed LF (LFH) treatment was also studied.

- Control (CTRL): water
- NaOCl: 150 mg NaOCl L^{-1} with pH 6.5 ± 0.1 (adjusted with citric acid).
- Nisin at 0.250 g L^{-1} (N-250): nisin at concentration of 0.250 g L^{-1} was used, according to previous experiment, in combination with 0.10 g L^{-1} EDTA and 2.0 g L^{-1} citric acid. pH of final solution was 4.1 ± 0.2.
- Bovine LF at g L^{-1} (LF-50): a bovine LF at concentration of 50 g L^{-1} was used according to previous experiment. pH of final solution was 7.0 ± 0.1.
- Bovine LF hydrolysed by pepsin (LFH): Lactoferrin was hydrolysed according to Bellamy et al. (1992). Briefly, a 5% (w/v) bovine LF water solution, acidified to pH 2.5 with HCl, was digested at 37 °C with 30.0 g L^{-1} (w/v) of pepsin from porcine gastric mucosa (250 units mg^{-1} solid; Sigma-Aldrich, St Louis, MO, US) for 120 min. Digestion was stopped heating the solution at 80 °C for 15 min. When it was cooled down at room temperature the pH was neutralized by adding l M NaOH and centrifuged at 17,000 × g at 4 °C for 20 min pH of final solution was 7.1 ± 0.1.

In both experiments, the product was rinsed after sanitising treatments for 1 min with cold (5 °C) tap water and drained in a perforated basket. Three replicates per treatment and sampling day were prepared.

2.3. Microbiological analyses

Standard enumeration methods were used to determine the mesophilic, psychrophilic, *Enterobacteriaceae*, *Pseudomonas* spp., lactic acid bacteria (LAB) and yeasts and moulds (Y + M) as previously described (Martínez-Hernández, Amodio, & Colelli, 2016; Rinaldi et al., 2013). All microbial counts were reported as log colony-forming units (CFU) per gram (log CFU g^{-1}). Each of the three replicates per treatment was analysed in duplicate.

2.4. Sensory evaluation

Sensory analyses were performed according to international standards (ASTM, 1986). The panel consisted of eight assessors (four women/four men, aged 24–40 years) screened for sensory ability (colour, odour detection, firmness and basic taste). A 5-point scale of damage incidence and severity was scored for off-flavours, off-odours, surface dryness (dehydration) and bulb/apical browning (5: none; 4: slight; 3: moderate, limit of usability, LU; 2: severe; 1: extreme). General appearance, flavour, odour, firmness and overall quality were assessed using another 5-point hedonic scale of acceptation (5: excellent, 4: good, 3: fair, LU, 2: poor; 1: extremely bad).

2.5. Data analysis

Inactivation curves after different concentrations of antimicrobial treatments were obtained in the first experiment by plotting the logarithm of the survival fractions (N/N_0) (where N is the number of CFU g^{-1} after the sanitising treatment and N_0 the initial number of CFU g^{-1}) *versus* the treatment doses, expressed in g L^{-1}.

A power law equation was used as described in Equation (1).