Food Control 50 (2015) 435–440

Contents lists available at ScienceDirect

Food Control

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

Short communication

Antimicrobial activity of cauliflower (*Brassica oleracea* var. Botrytis) by-product against *Listeria monocytogenes*

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ARTICLE INFO

Article history:
Received 9 April 2014
Received in revised form
11 September 2014
Accepted 21 September 2014
Available online 28 September 2014

Keywords:
Listeria monocytogenes
Natural antimicrobials
Vegetable by-products
Cauliflower

ABSTRACT

The antimicrobial potential of cauliflower by-product was assessed against *Listeria monocytogenes* at different concentrations [0–15]% (w/v) and incubation temperatures [5–22] °C, in reference medium. Survival curves under cauliflower by-product exposure versus time were obtained. The bactericidal effect of the cauliflower by-product was shown at concentration levels ≥5% (w/v) at all temperatures studied. Both temperature and cauliflower by-product concentration significantly ($p \leq 0.05$) influenced the reduction levels achieved in the initial *L. monocytogenes* contamination. Growth/inactivation kinetics of *L. monocytogenes* under cauliflower by-product exposure were fitted to a modified Gompertz equation for each of the conditions studied (concentration–temperature combinations), and maximum inactivation rate (μ_{max}) and lag phase duration (t_{lag}) parameters were obtained. It was observed that the higher the incubation temperature and the cauliflower by-product concentration added to the reference medium, the higher the μ_{max} and the lower t_{lag}. In spite of this, the maximum inactivation level achieved at stationary phase was 2.25 $\log_{10}$ cycles after 20 days of exposure to a 15% (w/v) concentration of cauliflower added to reference medium. Both conclusions indicate the effective control that cauliflower by-product could provide as an additional preservation measure during shelf-life of refrigerated RTE products, specifically when there is an accidental rise in storage temperature, e.g. in cold chain breakdown situations.

1. Introduction

Listeria monocytogenes is an opportunistic psychrotrophic microorganism with a reported capability of multiplying itself at temperatures down to a few degrees below 0 °C, persisting in refrigerated industrial settings. The incidence of listeriosis mainly affects young and elderly people (over 65 years), pregnant women and immune-compromised people (Adzitey et al., 2010; Gambarin et al., 2012), with high morbidity and mortality rates associated with *L. monocytogenes* (about 30%). Nowadays, *L. monocytogenes* is one of the most worrying foodborne pathogens, with one of the highest hospitalization rates (91%) and long-term sequels in affected patients (Denny & McLauchlin, 2008). Despite the fact that a wide variety of foods may be contaminated with *L. monocytogenes*, outbreaks and sporadic cases of listeriosis are predominately associated with ready-to-eat (RTE) foods – a large, heterogeneous category of foodstuffs that can be subdivided in many different ways and vary from country to country according to local eating habits, availability and integrity of the chill chain, and regulations specifying, for example, the maximum temperature at retail level.

Recent sporadic cases of listeriosis have been described in Europe (from 2006 to 2010) (Cairns & Payne, 2009; Goulet, Hedberg, Le Monnier, & de Valk, 2008; Kvistholm et al., 2010). A large outbreak was recorded in Canada in 2008 (PHAC, 2008), and there has been an increasing number of *L. monocytogenes* food isolates in the USA and Canada in recent years. RTE products are likely to act as vehicles for transmission of *L. monocytogenes*, mainly because they do not require additional preparation or cooking before consumption. RTE products (e.g. pasteurized milk, ice cream, fermented meat and cold smoked fish) can be contaminated by *L. monocytogenes* during post-processing steps, and then it can proliferate during storage at refrigeration temperature because of the psychrotrophic nature of the microorganism (Cobo et al., 2009; Zhu, Du, Cordray, & Uk Ahn, 2005). In order to prevent *L. monocytogenes* contamination in RTE products, some natural bioactive substances with antimicrobial capability are added to control pathogenic bacteria in food systems (Lianou & Sofos, 2007). Among possible added natural antimicrobials, increasing interest is focussing on vegetable by-products, as rich natural sources of fibre, vitamins, minerals, secondary plant metabolites and antioxidants.

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http://dx.doi.org/10.1016/j.foodcont.2014.09.031
0956-7135/

These vegetable residues from the food industry that are mainly destined to landfill or incineration, causing important economic and environmental problems, can be re-evaluated as supplements for animal feed or, in novel approaches, as food additives with bioactive properties, specifically antioxidant and antimicrobial, to be added in the formulation of new food products for human consumption (Fernández-López, Zhi, Aleson-Carbonell, Pérez-Álvarez, & Kuri, 2005; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2007). Compounds such as polyphenols, flavonoids and glucosinolates have been reported as being responsible for the bioactive properties attributed to vegetables. These bioactive compounds are mainly retained in cellular tissues, leaves and roots of vegetables (Ayaz et al., 2008; Hu et al., 2004). Consequently, assessment of the potential antimicrobial capability of vegetable by-products is a novel approach for food technologists and scientists to work on.

Among these by-products, one of most the important groups consists of members of the Brassicaceae family, which are among the most extended food crops in many countries (Cabello-Hurtado, Gicquel, & Esnault, 2012). Cauliflower (*Brassica oleracea* var. Botrytis) is one of the main Brassicaceae crops, with edible parts, such as leaves and stems, which have been widely described as sources of fibre and antioxidant substances. These bioactive properties give them the healthy, nutritious quality so extensively documented currently (Brandi, Amagliani, Schiavano, De Santi, & Sisti, 2006; Köksal et al., 2007; Stojceska, Ainsworth, Plunkett, Ibanoglu, & Ibanoglu, 2008; Volden, Bengtsson, & Wicklund, 2009).

In this context, the main objective of the present study is to evaluate the antimicrobial activity of cauliflower by-product against *L. monocytogenes* at several temperatures and cauliflower by-product concentrations.

2. Material and methods

2.1. Microbiology

A pure culture of *L. monocytogenes* (CECT 4032), which has a food origin and has been associated with meningitis after eating soft cheese, was provided freeze-dried by the Spanish Type Culture Collection and was rehydrated with 10 mL of tryptic soy broth (TSB) (Scharlab Chemie, Barcelona, Spain). After 20 min, the rehydrated culture was transferred to 500 mL of TSB and incubated at 37 °C, with continuous shaking at 200 rpm for 14 h to obtain cells in a stationary growth stage. Growth curves were obtained by plate count (colony forming units per mL (CFU/mL)). The cells were centrifuged twice at 4000 × *g* at 4 °C for 15 min and then resuspended in TSB. After the second centrifugation, the cells were resuspended in 50 mL of TSB with 20% glycerol, and then dispensed in 2 mL vials to a final concentration of 10^8 colony forming units per millilitre (CFU/mL). The 2 mL samples were immediately frozen and stored at −80 °C until needed for the kinetic inactivation studies.

2.2. Antimicrobial substances

Cauliflower by-product was provided as leaf residue from primary production and was tested to screen its bacteriological quality. The bacteriological analysis determined the presence/absence of microbial contamination and was carried out according to Aycicek, Oguz, and Karci (2006) procedures. Cauliflower by-product samples presented positive contamination with *L. monocytogenes* and *Bacillus cereus* (Gram-positives), mostly below 5 CFU/g. No samples were contaminated by *Escherichia coli* O157:H7 or *S.* Typhimurium (Gram-negatives).

The raw by-product was washed in sterile water to eliminate contaminating substances, and then dried, triturated and homogenized using a laboratory grinder to obtain a powder with a particle size of 40 μm, which was used to perform the experiments.

2.3. Total phenolic compounds

The total phenol content of the cauliflower by-product was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). Gallic acid calibration standards with concentrations of 0, 100, 200, 300, 400, 500, 600, 700, 800 and 1000 ppm were prepared. Three mL of sodium carbonate solution (2% (w/v)) (Sigma–Aldrich Co. LLC, USA) and 100 μL of Folin-Ciocalteu reagent (1:1 (v/v)) (Sigma–Aldrich Co. LLC, USA) were added to an aliquot of 100 μL from each gallic acid standard (Sigma–Aldrich Co. LLC, USA) or sample tube. The mixture was vortexed and allowed to stand at room temperature in the dark for 1 h. Absorbance was measured at 750 nm using a Lan Optics Model PG1800 spectrophotometer (Labolan, Spain), and the results were expressed as mg of gallic acid equivalents (GAE)/L.

2.4. Substrate and inoculation

Buffered peptone water (Scharlab Chemie, Barcelona, Spain) (1‰ (w/v)) was used as the reference substrate, in accordance with previous antimicrobial capability determination studies (Lin, Sheu, Hsu, & Tsai, 2010; O'Bryan at al., 2008). Then, 1 mL from a vial of stock culture was added to reference medium to a final concentration of 10^7 CFU/mL. The inoculated medium was supplemented with natural cauliflower by-product. The antimicrobial potential of the cauliflower by-product was tested against *L. monocytogenes* over a wide concentration range, [0–15]% (w/v), and the influence of incubation temperature on the antimicrobial potential of the vegetable by-product was assessed at 5 °C, 10 °C and 22 °C. Inactivation curves were prolonged to achieve a stationary point. The plates were incubated at 37 °C for 48 h in TSA (Scharlab Chemie, Barcelona, Spain).

2.5. Viable microorganisms count

At regular time intervals (hours), the cell suspension was evaluated for each sample by plate count after serial dilution with 1‰ (w/v) buffered peptone water. Each dilution was plated and the plates were incubated. The plate counts were used for (CFU)/mL enumeration.

2.6. Mathematical modelling of microbial inactivation

Microbial behaviour was fitted to a modified Gompertz equation to mathematically describe the bacterial inactivation kinetics under the intervention of cauliflower by-product at different by-product concentrations and temperatures (Linton, Carter, Pierson, & Hackney, 1995):

$$Log_{10}\left(\frac{N}{N_0}\right) = Ce^{-e^{-BM}} - Ce^{-e^{-B(t-M)}} \quad (1)$$

where N is the cell concentration at time t (CFU/mL); N_0 is the initial cell concentration (CFU/mL); C is the difference between the upper and lower values of the asymptote; B is the relative death rate at time M, M being the time at which the absolute death rate is maximal. A minus sign before C means microbial inactivation.

Subsequently, with the B, C and M values obtained, the maximum death rate (μ_{max}) and the lag phase duration (t_{lag}) were calculated as follows.