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Short communication

Synergistic bactericidal effect of carvacrol, cinnamaldehyde or thymol and refrigeration to inhibit *Bacillus cereus* in carrot broth

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Abstract

Possible use of three different essential oil components as natural food preservatives was studied by examining their influence in the kinetics of growth from activated spores of four *Bacillus cereus* strains in tyndallized carrot broth over the temperature range 5–16 °C. Selected low concentrations of carvacrol, cinnamaldehyde, or thymol showed a clear antibacterial activity against *B. cereus* in the vegetable substrate. The addition of 2 μl cinnamaldehyde or 20 mg thymol to 100 ml of broth in combination with refrigeration temperatures (⩽8 °C) was able to inhibit the outgrowth from activated spores of the psychrotrophic strain INRA TZ415 for at least 60 days, but only cinnamaldehyde did it even at the mild abuse temperature of 12 °C. Five microliters of carvacrol per 100 ml of inoculated carrot broth, however, were unable to inhibit bacterial growth at 8 °C.

Keywords: *Bacillus cereus*; Refrigerated minimally processed foods; Hurdle technology; Food preservatives; Essential oil components

1. Introduction

The development of food preservation processes has been driven by the need to extend the shelf-life of foods. Several food preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of food poisoning, however, these techniques frequently have associated adverse changes in organoleptic characteristics and loss of nutrients. Besides, consumer demand of natural, fresh, chemical-additive free and safe food products is increasing at present in Europe (Gould, 1996). Therefore, there is interest recently in the development of novel combinations of natural antimicrobial systems in conjunction with reduced levels of traditional physical and chemical food preservation processes to improve the quality and safety of agroindustrial products. Natural antimicrobial compounds available for use in food processing and shelf-life extension include enzymes (lactoperoxidase, lactoferrin, avidin, lysozyme), microbial preservatives produced from starter cultures (nisin, lactacin/lactococin/lacticin, natamycin, variacin), and plant sources (herbs, spices, extracts, essential oils and their isolated components) (Davidson, 1997).

Due to its ubiquitous nature and the heat resistance of its spores, associated with psychrotrophic properties, *Bacillus cereus* is a potential contaminant of Refrigerated Minimally Processed Foods of Extended Durability, RMPFEDs (also known as cooked chilled foods), based on vegetables. In fact, it was isolated from 80% to 100% of samples of cooked, pasteurized and chilled vegetable purées of leek, zucchini, broccoli, split pea, carrot and potato stored at 10 °C (Carlin et al., 2000). Lower percentages of samples containing *B. cereus* were found by us in different Spanish RMP foods containing vegetables (Valero et al., 2002). The vegetable purées

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which carry the bacterium were the cause of a severe *B. cereus* outbreak in a French nursing home for elderly persons (Lund et al., 2000).

Inhibitory effects of carvacrol, an antimicrobial compound present in the essential oil fraction of oregano and thyme, on growth and diarrheal enterotoxin production by *B. cereus* inoculated in brain heart infusion medium (BHI), cooked rice and mushroom soup have been published (Ultee et al., 2000; Ultee and Smid, 2001; Pol et al., 2001). Observations that higher concentrations were needed to achieve the same effect in food as in laboratory medium showed that carvacrol was less effective in a food matrix, most likely as a result of interaction with food components (Ultee and Smid, 2001; Pol et al., 2001). These studies pointed out, nevertheless, the potential use of carvacrol for preservation of foods, increasing the safety of the products.

Bactericidal effects of cinnamaldehyde and thymol against *B. cereus* (Demo et al., 2001; Kwon et al., 2003; Valero and Giner, 2005), as well as the development of synergistic effects between carvacrol or thymol and nisin have been also reported (Pol and Smid, 1999; Periago and Moezelaar, 2001; Periago et al., 2001). The objective of this study was to verify the influence of these three essential oil components in the kinetics of growth from activated spores of four *B. cereus* strains in carrot broth (a RMP food made with carrots and subjected to moderate heat treatment) over the temperature range 5–16 °C.

2. Materials and methods

2.1. Bacterial strains and sporulation

For this study four strains of *B. cereus* were used: the psychrotrophic strain INRA TZ415 and the mesophilic strains INRA L2104, EPSO-15CA and EPSO-25TO. Strains INRA TZ415 and INRA L2104, isolated from RMPFEDs containing vegetables, were provided by Dr. Frédéric Carlin (Institut National de la Recherche Agronomique, Centre de Recherches d'Avignon—Station de Technologie des Produits Végétaux, Domaine Saint-Paul-Site Agroparc, Avignon, France). The EPSO-15CA and EPSO-25TO strains isolated from samples of carrots (CA) and tomatoes (TO), respectively, were characterized by our laboratory (Valero et al., 2002). All the strains are enterotoxigenic, except EPSO-15CA.

Spores of the four *B. cereus* strains were developed on fortified nutrient agar (FNA; Mazas et al., 1995) at 30 °C for 4 days, harvested, centrifuged, resuspended, and stored in distilled water at 4 °C as previously detailed by us (Valero et al., 2000). The viability of heat-shocked (80 °C for 10 min) spores in water-suspension was estimated by the spread-plate technique on plate count agar (PCA, Scharlau Chemie, S.A., Barcelona, Spain) at 30 °C for 24 h.

2.2. Preparation of carrot broth

Carrots (*Daucus carota* L. var. Sativa) of the Nantesa type were bought at a local supermarket. Samples containing 500 g of washed and peeled vegetable material were mashed with 850 ml of distilled water using a Tecator 1094 Homogenizer (Foss Tecator, Höganäs, Sweden), and filtered through a nylon mesh 0.5 mm in diameter. Carrot extract was dispensed in 100 ml volumes into sterile 250 ml Erlenmeyer flasks and heated at 80 °C for 1 h on three consecutive days and stored at room temperature among heatings (Valero et al., 2003). Samples were taken and there was a very low level of natural microflora (0–1 cfu ml^{-1}) present in the carrot substrate. Polymyxin-mannitol-egg yolk-phenol red agar (PMYPA; Valero et al., 2002) and PCA were used for the detection of *B. cereus* and counting total mesophilic aerobes at 30 °C.

2.3. Essential oil components

Purified carvacrol, cinnamaldehyde and thymol were obtained from Destilerías Muñoz Gálvez, S.A. (Murcia, Spain). Five microliters, 2 μl, or 20 mg of carvacrol, cinnamaldehyde or thymol, respectively, were added to 250 ml Erlenmeyer flasks containing 100 ml of tyndallized carrot broth stored at 5, 8, 12, and 16 °C. The flasks were further shaken by hand for approximately 10 s.

2.4. B. cereus growth in carrot broth with components

For inoculation, initial spore suspensions were diluted with distilled water to give 10^4 spores ml^{-1}. Each broth with components was inoculated with 1 ml of the selected strain dilution to give a final concentration of 10^2 cfu ml^{-1}. The broths were incubated without shaking at 5, 8, 12, and 16 °C (±1 °C) during the course of the experiments. These temperatures were chosen because they represented those corresponding to both refrigeration and abusive (mild and moderate) storage conditions of RMP foods (Advisory Committee on the Microbiological Safety of Food, 1992; European Chilled Food Federation, 1996). An initial sample was taken at 0 h and samples (1 ml) were removed at selected intervals, using a sterile sampling system similar to that described by Sutherland et al. (1996). Dilutions were made if necessary in peptone water (Scharlau Chemie, S.A.) and *B. cereus* counts were performed on PCA at 30 °C for 24 h. The $\log_{10}$ cfu ml^{-1} was plotted against the incubation interval time. Unsupplemented controls also were sampled and enumerated at selected incubation conditions. Duplicate growth curves under different