Journal of Food Engineering 141 (2014) 37–43

Contents lists available at ScienceDirect

Journal of Food Engineering

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

# Predictive microbiology quantification of the antimicrobial effect of carvacrol

Clara Miracle Belda-Galbis [a], Anders Leufvén [b], Antonio Martínez [a], Dolores Rodrigo [a,*]

[a] Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC), Carrer del Catedràtic Agustín Escardino Benlloch 7, 46980, Paterna, València, Spain
[b] Nofima AS, P.O. Box 210, 1431, Ås, Norway

A R T I C L E I N F O

*Article history:*
Received 3 March 2014
Received in revised form 5 May 2014
Accepted 11 May 2014
Available online 20 May 2014

*Keywords:*
Food safety
Carvacrol
*Escherichia coli*
*Listeria innocua*
Growth kinetics
Microbial hurdle

A B S T R A C T

A study was carried out to evaluate quantitatively the effect of carvacrol on *Escherichia coli* K12 and *Listeria innocua* growth at different incubation temperatures (37, 30, 15 and 8 °C), from a kinetic point of view. Although the value of the minimum inhibitory concentration depended on microorganism and temperature, *L. innocua* was always more carvacrol-resistant than *E. coli* K12. The lag time and the maximum specific growth rate achieved at different carvacrol concentrations and temperatures were calculated at non-inhibitory doses. The lower the temperature or the higher the carvacrol concentration, the greater the lag time and the smaller the growth rate. These results indicate that carvacrol can inhibit or slow *E. coli* K12 and *L. innocua* growth, especially at low temperatures, because synergism was observed between the two factors. Consequently, carvacrol could be an effective hurdle when temperature or other factors compromise the microbial safety of minimally processed ready-to-eat foods.
© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

Reducing and controlling the concentration and growth of microorganisms in foods is fundamental to prevent their spoilage and guarantee their safety. Foods are naturally contaminated and must undergo treatments making them microbiologically stable and safe. However, some treatments penalize the sensorial and nutritional qualities of foods. Consequently, the use of natural preservatives in combination with refrigerated storage is a suitable approach to avoid the proliferation of bacteria that resist the treatments applied. It is especially important to impede the multiplication of pathogens, such as *Escherichia coli* O157:H7 and *Listeria monocytogenes*, because illnesses transmitted by foods have a wide socioeconomic impact worldwide (FAO and WHO, 2003).

In view of consumer demand for fresh, minimally processed products, without chemical additives but with a long shelf life, the application of mild thermal treatments and the use of non-thermal technologies are seen as promising because conventional processing at high temperatures modifies the flavour, odour, colour, texture and nutritional value of foods. Furthermore, for some years the use of natural substances with bacteriostatic and/or bactericidal properties has been promoted in order to obtain safe foods, maintaining or improving product characteristics and the effectiveness of the treatments applied (Pina-Pérez et al., 2009a,b). Notable among these natural preservatives are the essential oils (EOs) derived from herbs and spices, traditionally used as flavourings, because some of their components can inhibit or control the growth of pathogenic and/or spoiling bacteria (Debbarma et al., 2013; Ferrer et al., 2009; Nowak et al., 2013). EOs are complex aromatic mixtures of secondary metabolites, which have a broad commercial interest because they possess antimicrobial (López et al., 2005), antiviral (Saddi et al., 2007), antimycotic (Chaieb et al., 2007), antiparasitic (Pandey et al., 2000), antitoxigenic (Juglal et al., 2002), insecticidal (Rajkumar and Jebanesan, 2007), antioxidant (Gachkar et al., 2007), analgesic (Martínez et al., 2009), anti-inflammatory (Sousa et al., 2008) and anti-cancer properties (Ravizza et al., 2008). EOs antimicrobial activity has been attributed mainly to phenolic compounds, such as carvacrol. Carvacrol is the major component of the EOs obtained from oregano and thyme (Arrebola et al., 1994; Burdock, 2002). It is a food additive generally recognized as safe (GRAS) and it is used in baked goods, frozen dairy foods, chewing gum, soft sweets, gelatines, puddings, sauces and beverages (Burdock, 2002).

Many studies based on the minimal inhibitory concentration (MIC) or the minimal bactericidal concentration (MBC) have shown that carvacrol is biostatic and/or biocidal against bacteria and fungi (Ait-Ouazzou et al., 2011; Chami et al., 2004; Kim et al., 1995; Ultee et al., 1998), but few data exist about its effects on growth kinetics at non-inhibitory doses, more readily accepted by the consumer,

* Corresponding author. Tel.: +34 963 900 022x2218; fax: +34 963 636 301.
*E-mail address:* lolesra@iata.csic.es (D. Rodrigo).

http://dx.doi.org/10.1016/j.jfoodeng.2014.05.013
0260-8774/© 2014 Elsevier Ltd. All rights reserved.

given its marked flavour and its pungent, warm aroma (Burdock, 2002). From a practical point of view, however, these kind of studies are very interesting because mathematical models allow the prediction of bacterial behaviour in foods over time as a function of various factors, as well as constructing a matrix of responses to a broad range of specific storage conditions (Ross and McMeekin, 1991; Scott et al., 2005; Whiting, 1995), which is the first step for a complete risk assessment and for the planning of an appropriate hazard analysis and critical control point (HACCP) system in the industry. Kinetic studies allow food processors to know whether a product is safe or not, regardless of production, distribution and storage conditions, from farm to fork. Furthermore, they allow taking advantage of economically interesting minimal effective doses. The MIC and the MBC depend on factors such as temperature, so, its establishment to a practical application, avoiding over-processing, is more than complex due to foods are never kept under well-controlled conditions. For this purpose, sensorially unacceptable high doses would be needed, especially because EOs efficacy in foods is reduced. Consequently, the main goal of this work was to evaluate and model the effect of carvacrol and storage temperature on the growth kinetics of *E. coli* K12 and *Listeria innocua*, as non-pathogenic surrogates of *E. coli* O157:H7 and *L. monocytogenes* (Jadhav et al., 2013; Kim et al., 2013), taking into account that these last are pathogens commonly found in minimally processed ready-to-eat foods. For this purpose, the modified Gompertz model was selected because all of its parameters have a biological meaning (Corbo et al., 2009).

## 2. Material and methods

### 2.1. Microorganisms

*E. coli* K12 (CECT 433) and *L. innocua* (CECT 910) in stationary phase were prepared from freeze-dried pure cultures provided by the Spanish Type Culture Collection, following methods previously described (Pina-Pérez et al., 2010; Saucedo-Reyes et al., 2009).

The average cell concentration of stocks obtained was established by viable plate count from 4 samples. The media used and the incubation time at 37 °C were the followings: Nutrient Agar (NA; Scharlau Chemie, SA, Barcelona, Spain), 24 h, for *E. coli* K12, and Tryptic Soy Agar (TSA; Scharlau Chemie, SA, Barcelona, Spain), 48 h, for *L. innocua*.

### 2.2. Antimicrobial assays

Flasks with 10 mL of sterile Nutrient Broth (NB; Scharlau Chemie, SA, Barcelona, Spain) for *E. coli* K12 and Tryptic Soy Broth (TSB; Scharlau Chemie, SA, Barcelona, Spain) for *L. innocua* were prepared. Carvacrol ($\geqslant$98%; Sigma–Aldrich® Chemie GmbH, Steinheim, Germany) freshly diluted in dimethyl sulfoxide (DMSO ACS reagent $\geqslant$ 99.9%; Sigma–Aldrich® Chemie GmbH, Steinheim, Germany) was added to the culture media to obtain flasks containing different concentrations of carvacrol (ranging from 0.02 to 2.00 μL/mL; Table 1). All were determined from preliminary experiments establishing the MIC as the lowest concentration inhibiting visible growth of the microorganisms studied at the temperatures considered (37, 30, 15 and 8 °C), in presence of which no absorbance increase was observed after 300 h of incubation (12.5 d). The mixtures were inoculated with $1 \times 10^7$ cfu/mL *E. coli* K12 or *L. innocua* by diluting stocks in buffered peptone water (Scharlau Chemie, SA, Barcelona, Spain), if necessary. Sterile polystyrene microtiter plates (Deltalab, SL, Barcelona, Spain) were filled with inoculated samples (250 μL) with up to 9 carvacrol concentrations above the MIC (minimum number: 5). In addition, un-inoculated samples with and without carvacrol and DMSO were included in all plates as negative controls for each assay.

Regardless of temperature, the plates were incubated with double orbital shaking (500 rpm) and culture absorbance was measured at 600 nm. The readings were taken at regular intervals, after 20 s of vigorous agitation and until the stationary phase was reached, for a maximum period of 300 h (12.5 d). At 30 and 37 °C the culture absorbance was measured every 30 min. At 15 and 8 °C it was only recorded every 10 and 24 h, respectively. For this purpose, an automated microtiter plate reader (POLARstar Omega plate reader, BMG LABTECH GmbH, Offenburg, Germany) was used. To ensure result reproducibility, at least 3 repetitions of each of the combinations studied were carried out, with a minimum of 4 replicates per repetition.

### 2.3. Modelling microbial growth and determination of kinetic parameters

When growth was observed, optical density (OD) data were transformed to counts ($\log_{10}$cfu/mL). The average absorbance of each of the negative controls was subtracted from the absorbance of the inoculated samples before being transformed (Sampath et al., 2011; Valero et al., 2006). The transformation was carried out by means of calibration curves previously obtained in reference media for each of the microorganisms and test temperatures (Gupta et al., 2012; Valero et al., 2006), taking into account that a significant linear relation ($r > 0.90$) should exist between the OD data and the counts obtained. The agreement between the observed values and the ones obtained from the curves was evaluated by means of the accuracy factor (*Af*) (Ross, 1996). Once transformed, the data obtained were fitted to the modified Gompertz model (Gibson et al., 1988), whose mathematical expression is as follows (Eq. (1)):

$$\log_{10} N_t = A + C \times e^{-e^{-B\times(t-M)}} \tag{1}$$

In this equation, $N_t$ represents the number of microorganisms at time $t$ (cfu/mL); $A$, the $\log_{10}$ of the initial count ($N_0$; $\log_{10}$cfu/mL); $C$, the difference between the final count ($N_{max}$; $\log_{10}$cfu/mL) and $N_0$ ($\log_{10}$cfu/mL); $B$, the relative growth rate when $t = M$

**Table 1**
Carvacrol concentrations (μL/mL) tested, according to microorganism and incubation temperature.

| Microorganism | Temperature (°C) | Concentrations tested (μL/mL) |
|---|---|---|
| *Escherichia coli* K12 | 37 | 0; 0.04; 0.06; 0.08; 0.10; 0.12; 0.14; 0.15; 0.16 |
| | 30 | 0; 0.04; 0.06; 0.08; 0.10; 0.12; 0.14; 0.15; 0.16 |
| | 15 | 0; 0.02; 0.04; 0.06; 0.08; 0.10; 0.15; 0.20; 0.40; 0.80; 1.60; 2.00 |
| | 8 | 0; 0.02; 0.04; 0.06; 0.08; 0.10; 0.15; 0.20; 0.40; 0.80; 1.60; 2.00 |
| *Listeria innocua* | 37 | 0; 0.050; 0.100; 0.125; 0.150; 0.175; 0.200; 0.250 |
| | 30 | 0; 0.050; 0.100; 0.125; 0.150; 0.175; 0.200; 0.250 |
| | 15 | 0; 0.050; 0.075; 0.100; 0.125; 0.150; 0.175; 0.200; 0.250; 0.500; 1.000; 1.250; 1.500; 1.750; 2.000 |
| | 8 | 0; 0.050; 0.075; 0.100; 0.125; 0.150; 0.175; 0.200; 0.250; 0.500; 1.000; 1.250; 1.500; 1.750; 2.000 |

Download English Version:

https://daneshyari.com/en/article/6665719

Download Persian Version:

https://daneshyari.com/article/6665719

Daneshyari.com