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Holding time effect on microwave inactivation of *Escherichia coli* K12: Experimental and numerical investigations

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ABSTRACT

Non-uniform microwave heating is the main drawback in assuring the microbiological safety of food products. The focus of this study is to compare the efficiency of the inactivation of *Escherichia coli* K12, entrapped within calcium alginate gel, during a microwave and a water bath processing with a holding phase at set points of 55 °C and 57 °C. Microbial thermal inactivation equations coupled with heat transfer and Maxwell's equations are integrated and solved numerically via a finite element method to interpret the experimental results. The water bath microbial inactivation parameters are estimated by using inverse techniques and then applied to the microwave treatment. The simulated values are in good agreement with the experimental measurements. The simulation shows uneven temperature distribution during microwave heating which leads to a lower inactivation efficiency of microwave treatment. In this study, the holding phase did not help to homogenize the temperature distribution within the sample. This model can be used to improve the design of microwaves systems and to develop this process as a reliable pasteurization method.

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1. Introduction

Due to the different ways the energy is delivered, microwave heating technology has several advantages compared to conventional methods (Salazar-González et al., 2012). The various applications of microwave heating in the food industry, such as drying, thawing, tempering, baking and pasteurization, have been widely reviewed (Chandrasekaran et al., 2013; Salazar-González et al., 2012; Vadivambal and Jayas, 2007; Venkatesh and Raghavan, 2004).

Under specific operating conditions, microwaves have been successfully used as a microbial inactivation method for food products (Ahmed and Ramaswamy, 2007; Cañumir et al., 2002; Kindle et al., 1996; Laguerre et al., 2011; Valsechi et al., 2004; Yilmaz et al., 2005) resulting in higher efficiency and better quality products compared with conventional heating processes (Coronel et al., 2008; Huang et al., 2007; Kumar et al., 2008; Zhu et al., 2012).

The mechanisms of microwave inactivation are extensively discussed in the literatures (Banik et al., 2003; Hong et al., 2004; Shamis et al., 2012). Several studies have proved that this technology is effective against a wide range of microorganisms (Fujikawa et al., 1992; Gedikli et al., 2008; Giuliani et al., 2010; Guan et al., 2003; Zhou et al., 2010). Although some publications suggest that microwave irradiation could cause non-thermal effects on microorganisms (Barnabas et al., 2010; Bohr and Bohr, 2000; Hong et al., 2004; Kozempel et al., 1998), it seems more realistic to consider that microwaves inactivate microorganisms exclusively by heat (Heddleson and Doores, 1994; Shazman et al., 2007). The difficulty lies in the precise monitoring of the operating conditions during microwave processing.

The problem with microwave heating is the large number of factors that affect the absorption of microwave energy and thus the heating behavior (Campanone and Zaritzky, 2005; Hossan and Dutta, 2012; Vilayannur et al., 1998), resulting in a non-uniform temperature distribution within the heated products (Goksoy et al., 2000, 1999; Ramaswamy and Pilletwill, 1992; Vadivambal and Jayas, 2010). This is one of the major drawbacks associated with microwave heating and can lead to the survival of pathogens in the less heated zones (Apostolou et al., 2005; Farber et al., 1998; Heddleson et al., 1996).

Temperature distribution during microwave processing has been studied, both numerically and experimentally, for a large variety of food products (Fakhouri and Ramaswamy, 1993; Gunasekaran and Yang, 2007a; Manickavasagan et al., 2006; Mullin and Bows, 1993; Oliveira and Franca, 2002; Ramaswamy and Pilletwill, 1992; Yang and Gunasekaran, 2001). Modeling of microwave heating has been developed in order to understand

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Nomenclature

a, b dimensions of the wave guide (m)
AsymD_{ref} asymptotic decimal reduction time (min)
C_c critical component representing physiological state of cells (–)
C_p apparent specific heat ($J\ kg^{-1}\ K^{-1}$)
E electric field intensity ($V\ m^{-1}$)
E_0 amplitude of the electric field ($V\ m^{-1}$)
E_{local} electric field intensity at every point ($V\ m^{-1}$)
h convective heat transfer coefficient ($W\ m^{-2}\ K^{-1}$)
H magnetic field intensity ($A\ m^{-1}$)
k thermal conductivity ($W\ m^{-1}\ K^{-1}$)
k_{max} specific inactivation rate (min^{-1})
L sample length (m)
N microbial population (CFU/g)
N_0 initial microbial population (CFU/g)
N^{simul} simulated microbial population (CFU/g)
N^{exp} experimental microbial population (CFU/g)
Q_{abs} volumetric heat generation term ($W\ m^{-3}$)
r radial distance (m)
R radius (m)
S_l shoulder length of the inactivation curve (min)
T temperature (°C)
T_0 initial temperature of product (°C)
$T\infty$ ambient temperature (°C)
T_{ref} microbial inactivation reference temperature (57 °C)
x, y, z spatial coordinates in the three dimensions (m)
z value thermal resistance constant (°C)

Greek letters
ε complex permittivity ($F\ m^{-1}$)
ε_0 permittivity of vacuum ($8.85 \times 10^{-12}\ F\ m^{-1}$)
ε'_r relative dielectric constant
ε''_r relative dielectric loss factor
μ magnetic permeability of the material ($H\ m^{-1}$)
μ_0 magnetic permeability of vacuum ($1.256 \times 10^{-6}\ H\ m^{-1}$)
ρ density ($kg\ m^{-3}$)
σ electrical conductivity ($S\ m^{-1}$)
ω pulsation of the microwave radiation ($rad\ s^{-1}$)

better and thus optimize the process (Chatterjee et al., 2007; Chen et al., 2008; Gunasekaran and Yang, 2007b; Knoerzer et al., 2008; Pitchai et al., 2012; Salvi et al., 2011). During microwave heating, electromagnetic propagation equations and the heat transfer equation must be combined to predict the microwave power absorption as well as the temperature distribution inside the product (Ayappa et al., 1991; Curet et al., 2008).

To avoid an undesirable non-uniform temperature distribution during microwave heating, several methods have been proposed and investigated in the literature; for example, combining conventional and microwave heating (Datta et al., 2005; Datta and Rakesh, 2013), controlling the product geometry (Bhattacharya and Basak, 2006; Cha-um et al., 2009; Hossan et al., 2010; Romano et al., 2005), using 915 MHz microwaves (Lau and Tang, 2002), applying a holding time at a reference temperature (Ohlsson and Bengtsson, 2001), using pulsed microwave heating (Basak and Rao, 2010, 2011; Gunasekaran and Yang, 2007b; Yang and Gunasekaran, 2004), using specific packaging (Albert et al., 2012), or keeping the product in constant rotation or movement (Chatterjee et al., 2007; Feng and Tang, 1998; Geedipalli et al., 2007; Koskiniemi et al., 2011). Furthermore, some of these experimental techniques have been modeled (Chen et al., 2008; Geedipalli et al., 2007; Kopyt and Celuch, 2003; Pedreno-Molina et al., 2007; Plaza-Gonzalez et al., 2005; Wäppling-Raaholt et al., 2006). Nevertheless, due to technical considerations, the suggested solutions are restricted to specific designs and devices and thus cannot be generalized (Vadivambal and Jayas, 2010).

To our knowledge, there is a lack of studies highlighting both experimental and numerical data concerning the microwave microbial inactivation process. Recently, a study by Hamoud-Agha et al. (2013) proposed a multi-physics approach to understand the microwave inactivation of *Escherichia coli* K12 under dynamic heating conditions. A 3D finite element coupled model was used to interpret the microwave inactivation efficiency compared to a water bath treatment under similar heating conditions. This study clearly demonstrated that the differences between microwave and conventional inactivation treatments are due to temperature heterogeneities. A reliable microwave pasteurization process requires a full monitoring of temperature patterns and the inactivation kinetics of microorganisms at each location of the product (Hamoud-Agha et al., 2013).

The objective of the present study is to investigate, numerically and experimentally, a microwave microbial inactivation process with a holding period at a reference temperature. This could be a potential solution to improve the temperature field uniformity and, as a result, the microwave inactivation efficiency compared to a conventional water bath treatment under similar operating conditions.

2. Materials and methods

2.1. Bacterial culture and sample preparation

E. coli K12 CIP 54.117 (Pasteur Institute, Paris, France) was used in this study. The stock culture was stored frozen at −80 °C in a Brain Heart Infusion (BHI) broth (Fluka-53286 Sigma–Aldrich, 38297 St. Quentin Fallavier, France) supplemented with 30% (v/v) glycerin (104094 Merck, Darmstadt, Germany). A pre-culture was aerobically prepared in 10 mL of Tryptic Soy Broth (TSB) (9100461, 30 g/L Biokar, Grosseron, 44819 St. Herblain, France) at 30 °C for 24 h with constant shaking at 175 rpm (Excella E24, New Brunswick Scientific, Edison, New Jersey, USA). Then, 1 mL of the pre-culture was subcultured into 100 mL of fresh TSB in a 250-mL Erlenmeyer flask at the same conditions for 16 h in order to obtain cells in the early stationary phase of growth. The cultures were centrifuged for 10 min at 8720×g at 4 °C (Jouan, GR 20.22, Thermo Scientific, 91963 Villebon sur Yvette, France) and resuspended in 20 mL of sterile Phosphate Buffered Saline (PBS) to give a final approximately concentration of 1.5×10^{10} CFU/mL.

One volume of the cell suspension was mixed with two volumes of sterile 3% (w/w) sodium alginate solution (Algogel 3021, Cargill, 78100 St. Germain en Laye, France). By using a 3 mm-diameter cut-ended micropipette, 0.5 mL of this mixture was then flowed gently into a sterile 1% (w/w) $CaCl_2$ solution. The resulting almost cylindrical gels were hardened in this solution for 15 min then washed with sterile PBS to remove excess calcium ions and untrapped cells. The gels were then dried for 10 min inside the laboratory fume hood. One sample of gel (8 mm in diameter, 10 mm in length) of 0.53 ± 0.2 g with a cell concentration of 10^9 CFU/g was placed in a sterile sealed Eppendorf® tube (11 mm in diameter, 40 mm in length) (Fig. 1).

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