

EVALUATION OF GROWTH OF *Listeria monocytogenes* WITH CINNAMON OIL AND POWDER UNDER DIFFERENT TEMPERATURES BY INDIRECT CONDUCTIMETRY

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Abstract

Cinnamon oil and cinnamon powder apply as a flavouring agent and to suppress the growth of acid tolerant food borne pathogens. The attempt was taken to evaluate the growth of *L. monocytogenes* under two temperature regimes by adding cinnamon oil and powder and to select the form of cinnamon having antibacterial properties. The growth rates of *L. monocytogenes* in tryptone soy broth plus yeast extract (TSBYE) were estimated using indirect conductimetry by Don Whitley RABIT system under 25oC and 37oC. Concentrations 0.05%, 0.10% and 0.15% (v/v) cinnamon oil and 0.01%, 0.03% and 0.05% (w/v) cinnamon ground were added to TSBYE as treatments. Treated samples with inoculated *L. monocytogenes* were incubated for 48hrs using Don Whitley RABIT system at 25oC and 37oC. Detection solution (KOH + 0.1% Agar) with conductivity more than 6000 µS was used throughout the investigation. Conductivity measurements of the detection solution were taken for every 6 mins over the period of 48 hrs and recorded by detection software. Time to detection (TTD) was recorded as 15hrs for 25oC and 14hr and 6 mins for 37oC in the cinnamon oil treated samples. Results indicate cinnamon oil exerted significant effect on TSBYE media against *L. monocytogenes* in both 25oC and 37oC. Cinnamon oil has the ability to suppress the growth of *L. monocytogenes*. Growth suppression of *L. monocytogenes* was not observed from ground cinnamon in TSBYE at 25oC and 37oC. Therefore, cinnamon oil can be used as flavoring agent with antimicrobial to suppress the growth of *L. monocytogenes*.

Keywords: *L. monocytogenes*, indirect conductimetry, cinnamon oil, cinnamon powder.

Introduction

Food-borne disease associated with *Listeria monocytogenes* is considered as lethal diseases in the world. Stricter controls in food manufacturing processes are essential to decrease the incidence of food infections caused by *L. monocytogenes*. Antimicrobials have been tested by many scientists to inactivate *L. monocytogenes* in food products without alteration of fresh sensory quality and nutrient content of it. Essential oils from plants used as antimicrobials and have also been investigated due to the demand for food

products free from synthetic preservatives. Cinnamon oil and powder both have shown to be of value in this regard and cinnamon alone or in combination with other antimicrobial treatments or agents has been documented [1, 11]. *L. monocytogenes* was found to be more sensitive to cinnamon bark oil than *E. coli* and *Salmonella enteritidis* [6]. Gill and Holley [4, 7] observed cinnamon oil and cinnamaldehyde cause a decrease in the intracellular ATP by ATPase activity without obvious changes to the cell membrane of *L. monocytogenes*.

The antibacterial activity of cinnamon depends on the intrinsic factors and extrinsic factors of the product. Storage temperature is an important extrinsic factor that influences the antibacterial activity of cinnamon. Temperature is affecting the efficacy of essential oils because; the permeability of microbial cell membrane increased at high temperatures and could explain as an increased cellular diffusion of antimicrobial substances [2]. Yuste and Fung [11] reported a 4 to 6 log CFU/ml reduction of *L. monocytogenes* inoculated in pasteurized apple juice with 0.1%, 0.2%, and 0.3% (w/v) of cinnamon after 1 hr of incubation at 5 and 20 °C. Moreover they found no growth of any microorganism during 7 days of storage. An immediate 2 log CFU/ml reduction of *E. coli* in unpasteurized apple cider maintained at 42 °C by adding 2% (w/v) cinnamon powder was observed [5]. Hence the optimum temperature for effective antimicrobial activity of cinnamon oil/ powder against the pathogenic microorganisms may be of interest to industry.

However, the effects of these factors on the antimicrobial activity of cinnamon oil and powder have been assessed using classical plate count methods. Determinations of quantitative data on microbial growth rates, under a wide range of conditions, are essential for mathematical modeling studies, which are seen as the basis for improved food safety measures [11]. The conventional cultural methods for the detection of *L. monocytogenes* are slow, typically 3–5 days and labour intensive. The conductimetric technique is now a well-established method and has been legally accepted in the UK for the detection of *Salmonella* in processed animal protein for some time [1].

In the indirect conductimetric technique, the electrodes are immersed in a separate solution (usually a potassium hydroxide solution) instead of the inoculated growth medium. The indirect conductance method of growth detection for bacteria relies on the production of carbon dioxide (CO₂) by the bacteria as they metabolise carbohydrates and amino acids. The gas dissolves in the aqueous phase of the growth medium and can then diffuse into the headspace from where it can be absorbed into a solution, or gel, of potassium hydroxide (KOH). Electrodes placed in the KOH

measure the electrical changes caused as the CO₂ produced by the bacteria reacts with the KOH to form bicarbonate ions whose conductivity is less than that of the original solution. Impedance systems measure the relative or absolute changes in conductance, capacitance or impedance at regular time intervals during growth of bacteria at a given temperature. The measured electrical signals are then graphically plotted on the ordinate against the incubation times on the abscissa, producing impedance growth curves. In a typical impedance growth curve, the first region has quite a stable impedance value and then starts decreasing. The time corresponding to a point at which the decrease in impedance value exceeds a threshold value is called as the detection time (td). The detection time does not appear until the bacterial number reaches 10⁶–10⁷ cfu/ml. The impedance value finally levels off when the bacteria have reached a concentration of 10⁸cfu/ml or more, and all the nutrients in the medium have been metabolized to end products [10].

This investigation sought to determine growth rates for *L. monocytogenes* using indirect conductimetry with the treatments of cinnamon oil and cinnamon powder under temperatures of 25°C and 37°C which can be used as a alternative method to determine the effects of antimicrobial compounds under wide range of conditions and to select the form of cinnamon which can be used to suppress the growth of *L.monocytogenes*.

Materials and Methods

Microorganisms and culture conditions

Listeria monocytogenes (NCTC 10357) were obtained from National Collection of Type Cultures (NCTC), Health Protection Agency, United Kingdom and used throughout the investigation.

Stock cultures of *L. monocytogenes* were grown on Tryptone Soy Broth (TSB) (Oxoid, CM1065) with 0.6% (w/v) Yeast Extract (YE) (Oxoid, CM0019). Cultures were incubated at 37 °C for 24 hrs to obtain cells in early stationary growth phase. Maximum growth (10⁹) of bacteria was expected.

Universal containers each containing 9 ml of Maximum Recovery Diluent (MRD) were taken and labelled as 10-1 to 10-6 to prepare dilution series. 1 ml aliquots of bacterial suspension of each stock culture were transferred in to the 10-1labelled container under aseptic conditions and shook well. 1 ml from 10-1 labelled container was then transferred to 10-2 labeled container. Process was repeated for others to obtain the required densities of cells of pathogen. A 10-5 dilution of the stock culture was prepared and 1 ml of this dilution was inoculated into 100 ml pre-warmed TSBYE to give an initial count of approximately 10³ CFU ml⁻¹; this is used as the working culture. At the same time 1 ml aliquots of bacterial suspension of each stock culture were poured on Nutrient agar plate and incubated for 24 hrs at 37oC. Colony counts were taken in each plate to make sure the initial count is 10³ CFU ml⁻¹.

Sample preparation

A volume of 0.025ml, 0.050ml and 0.075ml of cinnamon bark oil was added to 300 ml of TSBYE in individually bottled TSBYE containing sterilized screw cap bottles to obtain 0.05% (v/v), 0.1% (v/v) and 0.15% (v/v) concentrations respectively under horizontal laminar flow air cabinet. 0.050g, 0.15g and 0.25g of cinnamon ground will be mixed with 300 ml of TSBYE samples in individually bottled sterilized screw cap bottles in order to obtain 0.1% (w/v), 0.3% (w/v) and 0.5% (w/v) concentrations respectively under horizontal laminar flow air cabinet. 300 ml TSBYE samples in sterilized screw cap bottles without adding cinnamon bark oil (0% (v/v) and cinnamon ground 0% (w/v) served as controls. 2 replicates of samples were prepared from each concentration of cinnamon bark oil and cinnamon ground.

Determination of antimicrobial activity of cinnamon bark oil and cinnamon powder at 25oC and 37oC through indirect conductimetry

A Don Whitley Scientific RABIT system (Don Whitley Scientific, Shipley, UK) was used for determination of the antimicrobial activity of cinnamon powder and

cinnamon oil. 0.35 g of KOH was dissolved in 50 ml of deionised water. 1g of Bacteriological Agar was dispersed in 100 ml sterilized screw cap bottle and dissolved by boiling. At 70oC temperature of Agar, cold KOH solution was added and mixed thoroughly. 700µl of equal volumes of KOH bacteriological Agar was dispensed to the clean sterile RABIT tubes, allow solidifying for about 15 mins and were tightly stoppered and allowed to stabilized for 2 days and tubes were chosen for experiment only if their conductance was over 6000µS at 30oC.

The growth medium (Cinnamon oil / cinnamon ground) with TSBYE (4.5 ml) was contained in sterile glass test tubes (12×75 mm) and inoculated (100 µl) with the working culture then placed within the RABIT tubes, which were resealed and loaded into the conductimetric incubator block. Throughout the investigation, readings were taken every 6 min over a 24 h period and a detection criterion of -20 µS was chosen to ensure this value was reached only when cultures were showing abundant growth. The value is the difference between successive conductance measurements and was entered into the RABIT growth detection software. When this criterion was reached for three successive readings, the time taken to detect this threshold was automatically recorded by the RABIT software and this is referred to as the time to detection (TTD).

Results And Discussion

Effect of cinnamon oil on growth of L .monocytogenes in TSBYE

Effect of cinnamon oil and ground cinnamon on the growth of *L. monocytogenes* was examined in TSBYE media. The growth of food borne pathogen was examined through indirect conductrimetry under 25oC and 37oC temperatures. Using conductimetry, the growth of *L.monocytogenes* was detected when the culture had grown enough to produce sufficient CO₂ to cause a detectable fall in the conductance (µS) of the detecting solution.

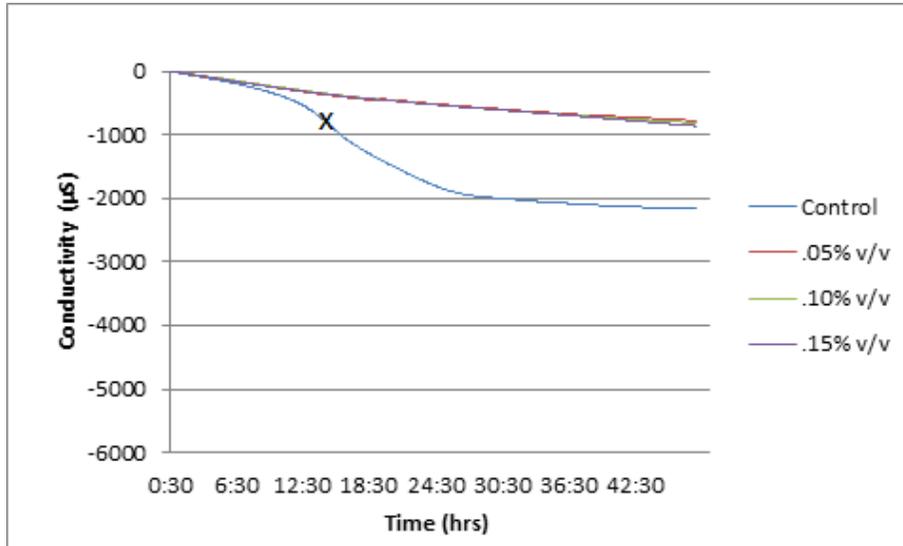


Figure 1. Fall of conductivity in detection solution for TSBYE containing cinnamon oil against *L. monocytogenes* at 25°C. Key: (X) TTD

A major fall in conductance corresponds to late log/early stationary phase (Fig. 1), when the highest numbers of metabolically active cells are present. According to the graph after 15hrs of incubation *L. monocytogenes* showed detectable conductivity fall in the control, which indicates that *L. monocytogenes* had

grown in TSBYE without cinnamon oil at 25 °C. But no observed growth of *L. monocytogenes* was detected in 0.05% (v/v), 0.10% (v/v) and 0.15% (v/v) cinnamon oil treated TSBYE at 25°C after 15 hrs. Therefore no conductivity fall observed in treated samples with compared to control after 15 hrs of incubation.

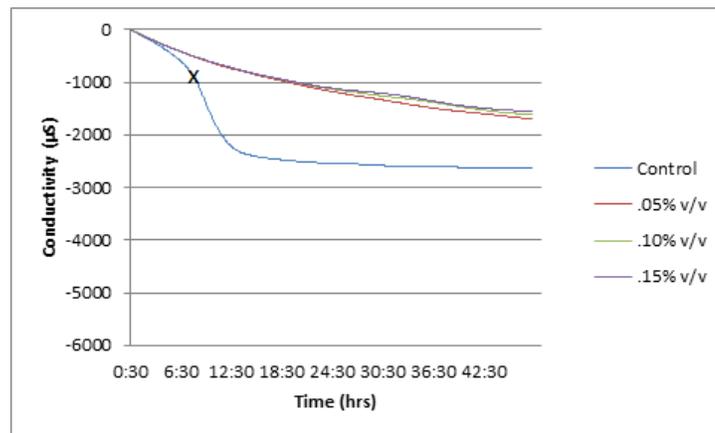


Figure 2. Fall of conductivity in detection solution for TSBYE containing cinnamon oil against *L. monocytogenes* at 37°C. Key: (X) time to detection (TTD)

Results reported in Figure 2 show a TTD value of 7hrs 36mins in the control, which indicates *L. monocytogenes* had grown in TSBYE at 37°C. Growth of *L. monocytogenes* was not detected in 0.05% (v/v),

0.1% (v/v) and 0.15% (v/v) cinnamon oil treated TSBYE samples.

Results from the study indicate that growth of *L. monocytogenes* occurred in TSBYE at 25°C and 37°C with no cinnamon oil. But, samples treated with 0.05%

(v/v), 0.1% (v/v) and 0.15% (v/v) concentrations of cinnamon oil shows promising inhibitory activity against pathogen. Similar results have been made by Prabuseenivasan [9]. This result let us to believe that cinnamon oil might be involved in cell death occurring. Paparella [8] reported further that at 37oC *L. monocytogenes* showed growth suppression in treated with 0.05% (v/v) and 0.02% (v/v) cinnamon oil in apple juice. Therefore, result reported in experiment points out 0.05% (v/v), 0.10% (v/v) and 0.15% (v/v) cinnamon

oil had a similar effect on suppressing growth of *L. monocytogenes*. Minimum concentration of cinnamon oil used (0.05% (v/v) in experiment exerted a antimicrobial effect *L. monocytogenes* shows there would be a potential to assessed cinnamon oil as an antimicrobial agent.

Effect of cinnamon ground on growth of L. monocytogenes in TSBYE

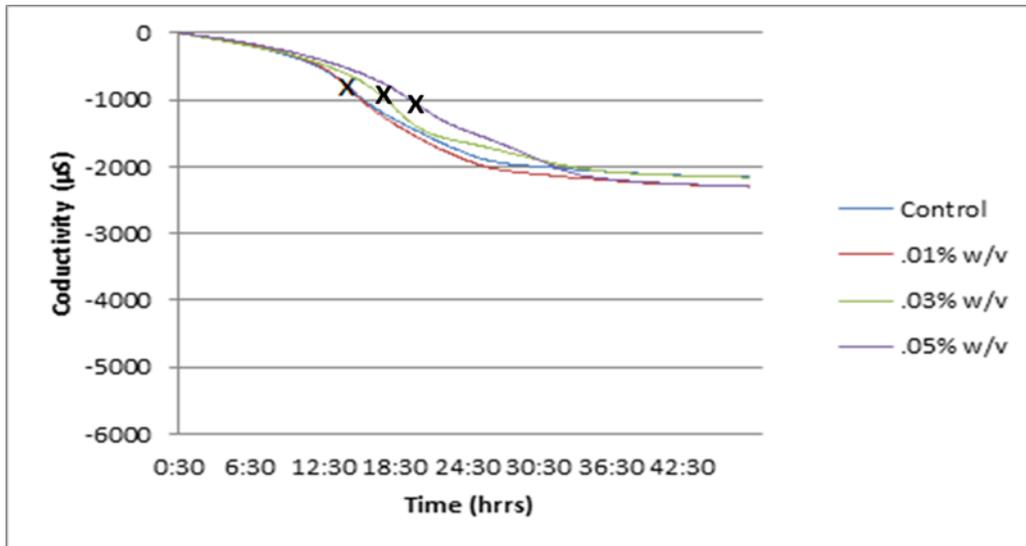


Figure 3. Fall of conductivity in detection solution for TSBYE containing with cinnamon ground against *L. monocytogenes* at 25oC Key: (X) TTD

The TTD value was observed as 14 hrs 6 mins in control which indicating *L. monocytogenes* had shown growth in TSBYE (Figure 3). TTD values for 0.01(w/v) cinnamon ground treated sample was observed at the

same time. Growth of *L. monocytogenes* were detected in 0.03% and 0.05% ground cinnamon treated samples of TSBYE. Therefore TTD is recorded.

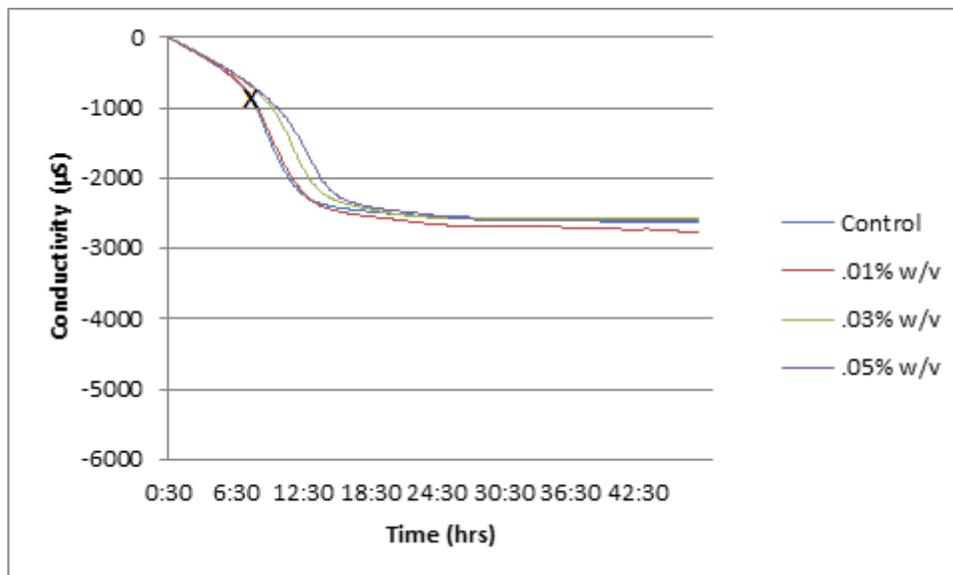


Figure.4. Fall of conductivity in detection solution for TSBYE containing ground cinnamon against *L.monocytogenes* at 37 oC Key: (X) TTD

TTD value was observed as 7hrs 36 mins in control which indicating *L.monocytogenes* has been shown growth in TSBYE (Figure 4). Growth of *L. monocytogenes* was detected in 0.01%, 0.03% and 0.05% ground cinnamon treated samples of TSBYE.

Results obtained from the experiment shows there was no antibacterial activity of ground cinnamon against *L. monocytogenes* at 25oC and 37oC in TSBYE. Yuste and Fung [11] showed that ground cinnamon in apple juice reduces populations of *L. monocytogenes* [1, 5,11]. Thus, the experiment had not given the same results. According to the results of the investigation, cinnamon oil has ability to suppress *L. monocytogenes* growth at both 25oC and 37oC perhaps cinnamon ground did not show suppression in *L. monocytogenes* both 25oC and 37oC. Therefore, it can conclude cinnamon oil can be used as an antimicrobial and as a flavoring agent to suppress the growth of *L. monocytogenes* and cinnamon ground can only used as a flavoring agent.

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