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Synergistic effect of UV, laser and microwave radiation or conventional heating on *E. coli* and on some spoilage and pathogenic bacteria

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ABSTRACT

Although laser sterilisation has been well studied in dentistry and medicine, there have been few studies within the food industry. UV radiation has been used for sterilisation of surfaces and water. The killing effect of microwave radiation has been investigated on many bacteria in food and there has been much controversy over its killing mechanism. In this study, the killing effect of laser, microwave and UV radiation was studied on *E. coli* and on some spoilage and pathogenic bacteria. The bacterial suspensions were exposed to the treatment processes in sequence and viable cell counts were made before and after each treatment. A difference in the reduction in viable counts was apparent when the sequential treatment was compared with the sum of the individual treatments alone. Similar results were obtained when conventional heating was used in place of microwave radiation. It was found that the order of the treatment processes had a significant influence on killing.

Industrial relevance: Spoilage organisms often require treatment greater than the damage threshold of the substrate food, indicating that removal of these organisms with conventional meansmay lead to substrate damage. The present work develops protocols and experiments in liquid to investigate the combination of relatively low level treatments to reduce the effect of one treatment alone but with the added advantage of more severe degradation of the spoilage organisms. This technology is of interest to the foodprocessing or packaging industry where the presence of spoilage or pathogenic organisms are of concern.

1. Introduction

There are limitations in the microbial decontamination of foods by current methods and the development of novel techniques in this area would be advantageous. Chemical treatment of foods, beverages and water is becoming less popular with consumers, with concerns over chemical residues and toxicity. Similarly, the public has rejected gamma radiation treatment of foods. Heat treatment of foods at high temperature can affect texture, flavour and appearance of the product whereas less severe thermal treatment may not achieve adequate decontamination.

There are several physical and optical methods which potentially can be used for decontamination of food. UV radiation is widely used for surface sterilisation. Also, the use of UV radiation for treatment of drinking water is increasing (Amirsardari, Yu, & Willams, 2001; Hijnen, Beerendonk, & Medema, 2006; Murphy, Payne, & Gagnon, 2008). UV treatment has been shown to be effective in reducing bacterial counts prior to packing, and hence prolonging the shelf-life of fish (Huang & Toledo, 1982). The killing effect of UV on selected bacteria on surfaces, in chilled brines, peptone water and on chicken meat and poultry skin was reported (Gailunas et al., 2008; Kim, Silva, & Chen, 2002; Sumner, Wallner-Pendleton, Froning, & Stetson, 1996). UV has also been shown to be effective in reducing bacteria on fresh meat with no deleterious effect on its colour or general appearance (Stermer, Lasatel-Smith, & Brasington, 1987).

Laser sterilisation has been investigated in dentistry and medicine, but there have been few studies within the food industry (Watson & Stewart-Tull, 2000). The effect of laser, microwave, UV and chemical treatment of potatoes and carrots has been investigated with a view to reducing spoilage organisms and extending shelf-life (Watson & Stewart-Tull, 1999). The bactericidal effects of laser radiation have been studied on surfaces and liquids (Ward, Watson, StewartTull, Wardlaw, & Chatwin, 1996; Ward et al., 2000; Watson et al., 2006; Yanagawa, Koike, Yamada, & Hashimoto, 1992; Yeo, Watson, Stewart-Tull, Wardlaw, & Armstrong, 1998) and this treatment potentially can be exploited within the food industry. Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk has been investigated (Smith, Lagunas-Solar, & Cullor, 2002). Also UV excimer lasers have been used to inactivate *Bacillus subtilis* spores to sterilise packaging cartons (Warriner, Kolstad, Rumsby, & Waites, 2002) and packaging surfaces (Warriner et al., 2000) related to the food industry.

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Microwave radiation is also effective in killing bacteria, for example in the inactivation of selected bacteria in milk (Kindle, Busse, Kampa, Meyer-König, & Daschner, 1996; Thompson & Thompson, 1990), chicken portions (Apostolou, Papadopoulou, Levidiotou, & Ioannides, 2005), apple juice (Cañumir, Celis, Bruijn, & Vidal, 2002) and minced beef (Quesada, Arias, & Chaves, 2003). However, microwave use is not without its potential problems. Questions remain as to whether the treatment is thermal or non-thermal and there is concern over potential uneven heating leading to survival of microorganisms in the treated food and greater risk of inducing food-borne diseases (Heddleson & Doores, 1994; Papadopoulou et al., 1995; Vela & Wu, 1979).

A combination of different treatments may prove beneficial if it can be shown to reduce contact time and energy requirements for decontamination and killing of spoilage organisms and pathogens. The killing mechanisms of UV, laser and microwave are different and the purpose of this study was to develop protocols for simple saline systems to investigate possible synergistic effects between these processes. The longer term objective is to develop combined treatment processes that can be used to reduce spoilage bacteria on food without damaging the substrate material. Consequently, it was the relative sizes of the inactivation achieved that was of interest, and not the overall magnitude. The organisms chosen for investigation were *Listeria monocytogenes*, which is a food-borne pathogen, *Shewanella putrefaciens*, *Pseudomonas fragi*, and *Micrococcus luteus* which are marine spoilage organisms and also *E. coli* which is commonly used as an indicator organism.

2. Material and methods

2.1. Bacteria

E. coli (DH5α) was obtained from the culture collection of the Division of Infection and Immunity, University of Glasgow.

L. monocytogenes (strain R479a) was isolated from cold-smoked salmon by Vogel et al. (2001). The bacterium was kindly provided by Dr. L. Gram, Department of Seafood Research, Technical University of Denmark.

S. putrefaciens (NCIMB 1732) and *P. fragi* (NCIMB 1353) were obtained from the National Collection of Industrial and Marine Bacteria (NCIMB) Ltd., Aberdeen, UK.

M. luteus was isolated from chilled and pickled prawns in the Division of Infection and Immunity, University of Glasgow.

2.2. Preparation of bacterial suspensions

A colony from a fresh agar plate culture was inoculated into 50 ml of the appropriate broth (Table 1). The flask was shaken at 180 rpm for 18–20 h at the appropriate temperature. The culture (15 ml) was then placed in a sterile plastic universal bottle and centrifuged at 4000 rpm for 7 min. The pellet was resuspended in 15 ml of sterile physiological saline and further diluted with saline to compare with the MacFarland opacity standard no. 4 and then 100 µl aliquots of the bacterial suspensions were removed for viable counting. It should be noted that *E. coli* (DH5α) is an ampicillin resistant bacterium. To prevent contamination during the experiment this antibiotic was added to the agar.

Table 1
Media and incubation conditions used for each bacterial strain.

Strain	Broth medium	Agar medium	Incubation temperature (°C)
E. coli	Nutrient broth + ampicillin	Nutrient agar + ampicillin[a] TSA[b]	37
S. putrefaciens	Nutrient broth	Nutrient agar	20
P. fragi	Nutrient broth	Nutrient agar	20
L. monocytogenes	TSB[c]	TSA Listeria selective agar[d]	37
M. luteus	Nutrient broth	Nutrient agar	37

[a] *E. coli* (DH5α) is an ampicillin resistance bacterium. To prevent contamination during experiment the antibiotic was added to the agar.
[b] TSA: Trypticase soy agar.
[c] TSB: Trypticase soy broth.
[d] To prevent contamination during experiment Listeria selective agar was used.

2.3. Instruments

UV lamps (3×30 W) operating at band C (180–280 nm) were used. Before use the UV lamps were allowed to warm up for 30 min to stabilise the output. The power of the lamps was measured by a UV meter (MACAM®, Scotland, UK), which was placed at the target sites. Throughout the experiments the applied energy density (J cm^{-2}) was calculated by multiplying the measured power at each distance by the exposure time and dividing by the sensor area.

A pulsed, 400 W, Nd:YAG laser (Lumonics, MS830, Rugby, UK) operating at 1064 nm, with a fibre optic beam delivery system and collimating focusing lens assembly was used. The laser output power at the target was measured with a power/energy meter (FieldMaster, Coherent, UK). The beam diameter was fixed at 2 cm on the surface of the suspension as determined by measuring burn prints produced on photographic paper (Rypma, 1997). Through the experiments, the laser output pulse energy was set at 20 J, delivered over 8 ms and operated at a pulse repetition frequency (PRF) of 15 Hz.

A standard domestic microwave oven (800 W, Sanyo EMS 153, UK) operated at 2450 MHz and a normal laboratory water bath with digital temperature control was used. As a comparison to microwave heating, this treatment was replaced with conventional heating in the water bath.

3. Treatments

The treatments were combined in different orders to investigate their potential combined inactivation efficiency, with a view to optimising the treatment process. The effects of the individual treatments were determined and the sum of their effects was compared to the combined treatments.

3.1. Treatment of bacterial suspensions with ultra-violet radiation

A suspension (1.0 ml) of bacteria was pipetted into the wells of a multiwell plastic dish (each well was of 1.7 cm internal diameter) and exposed to the UV light for the appropriate time period. Before exposure and immediately after UV exposure, 100 µl samples of the bacterial suspension were removed for viable counting.

3.2. Treatment of bacterial suspension with microwave energy

For the controlled application of microwave treatment, 50 ml of bacterial suspension was placed in a sterile 200 ml conical flask. The flask was placed in the centre of the cavity (without rotating) of the microwave oven and exposed for the appropriate period. The temperature of the bacterial suspension was obtained by inserting a digital thermometer before and immediately after treatment into the suspension. Also, before and after treatment, 100 µl aliquots of the bacterial suspension were removed for viable counting. Experiments (data not shown) indicated that a microwave treatment of 15 s yielded a temperature rise of between 49.6 and 58.8 °C.

3.3. Treatment of bacterial suspension with Nd:YAG laser radiation

1 ml of the bacterial suspension was pipetted into the wells of a multiwell dish and exposed to the laser beam for the appropriate time