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An investigation of high intensity ultrasonication and chemical immersion treatments on *Campylobacter jejuni* and spoilage bacteria in chicken

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ABSTRACT

High intensity ultrasonication (US) alone or in combination with chemical immersion treatments of lactic acid (3% LA), sodium decanoate (3% SD) and trisodium phosphate (10% TSP) were investigated to reduce populations of *Campylobacter jejuni* and spoilage organisms in raw chicken. Different experimental conditions were used including a range of temperatures (4 °C, 25 °C and 54 °C) and exposure times (1, 2 and 3 min). All combination treatments significantly reduced *C. jejuni* compared to their individual treatments while only the combination US + SD significantly reduced Total Viable Count (TVC). Multiple linear regression predicted bacterial reductions resulting from changing treatment, temperature and time or each group of microorganisms. Increasing temperature from 4 °C to 54 °C would enhance *C. jejuni*, TVC and Total Enterobacteriaceae Count (TEC) reductions by 0.73, 1.02 and 1.37 $\log_{10}$ cfu/g respectively. Increasing time from 1 to 3 min enhanced bacterial dependent of *C. jejuni* and TEC by 0.49 and 0.31 $\log_{10}$ cfu/g respectively.

Industrial relevance.

This study demonstrates the potential application of high intensity ultrasomication alone or in combination with chemical treatments to reduce bacterial contamination of chicken carcasses. Different tempretures and times were investigated to optimize the most effective treatments conditions in chicken abattoirs.

1. Introduction

Campylobacter jejuni is the most frequently reported bacterial gastrointestinal foodborne pathogen in the EU since 2005. The number of confirmed case of human campylobacteriosis in Europe has been estimated as 229,213 with an infection rate of 65.5 per 100,000 for 2015 (European Food Safety Authority (EFSA), 2015). The Health Protection Surveillance Center (HPSC) in Ireland reports that the numbers of notified campylobacteriosis cases has increased over the last 5 years in Ireland, with a total of 2451 cases were recorded (equivalent to a crude incidence rate of 53.4 per 100,000) in 2015 (HPSC, 2016). In addition, it has been indicated that the economic costs associated with campylobacteriosis to the public health systems and to lost productivity is €2.4 billion annually (European Food Safety Authority, 2014). Poultry meat is considered one of the main sources of *C. jejuni* worldwide and the prevalence of this pathogen is frequently high in raw poultry meat within the EU (EFSA, 2013; Food Safety Authority of Ireland, 2011; Whyte et al., 2004). Quantitative microbiological risk assessments have indicated that even partial reduction in *C. jejuni* numbers on chicken carcasses (> 1 $\log_{10}$ per carcass) can significantly reduce the infection rate in humans (Lindqvist & Lindblad, 2008). Several quantitative risk assessments of *Campylobacter* in chicken indicated that the most effective intervention measures were those aimed at reducing the *Campylobacter* concentrations, rather than reducing the prevalence of contaminated carcasses (Nauta et al., 2009). Control of *Campylobacter* requires enhanced practices at all stages of the broiler production chain to limit exposure risks to consumers. Improved biosecurity may be the most efficient strategy to minimize the risk of colonization of this microorganism in the intestinal tract of birds (Smith et al., 2016). However, such preventative measures at farm level may increase costs, need to be consistently applied at all stages of production and have not always resulted in reduced *Campylobacter* levels in flocks (Food Safety Authority of Ireland, 2002; Patriarchi et al., 2009; Rosenquist et al., 2009). Effective interventions during slaughtering and processing stage are desirable, economically feasible and can be applied to high risk batches of birds in order to decrease contamination and to reduce the

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risk of human exposure to contaminated chicken meat (Bolder, 1997; Del Río, Panizo-Morán, Prieto, Alonso-Calleja, & Capita, 2007; Loretz, Stephan, & Zweifel, 2010; Mani-López, García, & López-Malo, 2012).

Organic acids and trisodium phosphate (8–12%) are both categorized as 'generally recognized as safe' (GRAS) for use in food production (Demirci & Ngadi, 2012; USDA-FSIS, 1996). These chemicals have been used in the USA and Canada for many years as sprays or in immersion systems to reduce bacterial contamination and improve shelf life (Capita, Alonso-Calleja, Garcia-Fernandez, & Moreno, 2002; USDA-FSIS, 1996). In contrast, the European regulations have supported the application of strict hygiene precautions along the production process rather than using chemical interventions.

However, recently the European Commission has approved the use of lactic acid for bacterial decontamination of beef carcasses (The European Commission, 2013). The mechanism of action of organic acids is thought to result in the permeation of the cell membrane, lowering of intracellular pH, and disruption of important cellular processes; while medium chain fatty acids such as capric acid may damage the lipid bilayer causing cell contents to leak (Alexandre, Mathieu, & Charpentier, 1996; Dibner & Buttin, 2002). Physical methods such as ultrasonication are considered as emerging technologies with potential applications in the food industry. Ultrasonication has been assessed as a technology to aid in the tenderization of meat, speed up maturation and mass transfer, decrease cooking energy, and increase the shelf life of meat by reducing microbial populations without effecting the quality and sensory characteristics of meat (Alarcon-Rojo, Janacua, Rodriguez, Paniwnyk, & Mason, 2015; Awad, Moharram, Shaltout, Asker, & Youssef, 2012). Ultrasound waves produce alternating compression and decompression within liquids which leads to the formation of cavitation bubbles, which generate very high local temperatures and pressures when they grow and suddenly collapse (Cárcel, García-Pérez, Benedito, & Mulet, 2012). The irregular collapse of a cavitation bubble leads to a liquid jet accelerating through the center of the collapsing bubble producing high energy shock waves - which can cause damage to the cell wall of bacteria (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). Additionally, the effect of localized high temperatures can produce free radicals which may cause DNA injury, and microstreaming which results in thinning of cell membranes leading to loss of cell viability (Bermúdez-Aguirre, Mobbs, & Barbosa-Cánovas, 2011). The cumulative effect of such localized high temperatures is an increase in the general temperature of the liquid medium (Chen et al., 2012). Susceptibility of microorganisms to ultrasound is dependent on a range of factors. In general, endospores and viruses show increased resistance, while Gram-negative bacteria are more susceptible than Gram-positive bacteria. Cell morphology can also affect susceptibility with larger cells typically being more sensitive than small cells and rod shaped bacteria more susceptible than cocci (Torley & Bhandari, 2004). It has been previously suggested that ultrasonication technology could be used in broiler processing as the relatively small carcasses could be immersed in dedicated ultrasonication tanks (Bolder, 1997). Furthermore, the effectiveness of ultrasonication could be enhanced by it combining with heat (Chandrapala et al., 2012; Haughton et al., 2012), or with chemical treatments (Koolman, Whyte, Meade, Lyng, & Bolton, 2014). The objective of the current study was to investigate the effectiveness of ultrasonication treatments applied alone, or in combination with chemical immersion, to reduce *Campylobacter* and spoilage bacteria at different times and temperatures.

2. Materials and methods

2.1. Preparation of bacterial suspensions and inoculation of samples

Suspensions of *C. jejuni (1146 chicken isolate)* were prepared by inoculating 20 ml aliquots of Mueller-Hinton Broth (MHB) (Oxoid,UK, CM0405) containing *Campylobacter* growth supplement with a single colony of the isolate and incubated for 24 h at 42 °C under microaerobic conditions. A total of ten of the 20 ml aliquots were then combined to make up 200 ml volumes, and diluted with 300 ml of maximum recovery diluent (MRD), (OxoidCM0733) to give a 500 ml volume containing a cell concentration of approximately 7 $\log_{10}$ cfu/ml. Chicken thigh pieces were purchased from retail outlets and dipped in the 500 ml volumes of the *C. jejuni* suspension for 60 s then left for 30 min to allow attachment to occur. Background levels of TVC and TEC were determined in control samples and compared to those following treatment in order to calculate bacterial reductions achieved for each treatment. Levels of *C. jejuni* on chicken skin samples following inoculation were confirmed as 5.7 $\log_{10}$ cfu/g and reductions caused by treatments were calculated based on the difference in counts between control and treated samples.

2.2. Chemical and ultrasonication treatments

Each experiment was repeated in triplicate on three separate occasions at three different temperatures (4, 25 and 54 °C). For each of the temperatures, chicken thighs were immersed for 3 different exposure times (1, 2 or 3 min) in 3% lactic acid (LA) (Sigma Aldrich, USA, W261114), sodium decanoate (SD) (Sigma-Aldrich, USA, C4151) and 10% trisodium phosphate (TSP) (Sigma-Aldrich, USA, 222,003) alone, or in combination with ultrasonication. The ultrasonication bath used in the study was a Quirumed 534 C200 (Quirumed S.L.,Valencia, Spain) with a frequency of 40 kHz, ultrasound power of 120 W, temperature range: 20–80 and a 5 l capacity. A thermocouple (Traceable VWR, USA) was used to monitor temperatures in each experiment. A temperature of 4 °C was maintained by immersing cooler packs in the US bath. Treatment temperatures (25 and 54 °C) were controlled by an in-built heating element and thermostat within the US bath. A volume of 1.5 L of each solution was used in the bath for all treatments. In addition, immersion treatments in water with and without sonication were also carried out. Following treatment, samples were immersed in 1.5 L distilled water for 15 s to rinse off any residual chemical. After washing three 5 g pieces of skin were aseptically removed from each thigh for analysis. Untreated control samples were microbiologically analyzed directly without any chemical or washing treatment step. Washed control (WC) samples were immersed in distilled water only then rinsed in another 1.5 L distilled water prior to microbiological analysis.

2.3. Microbiological analysis

Samples were stomached (Colworth Stomacher 400, UK) for 30 s in 45 ml MRD, and serially diluted (1:10) in MRD before being plated in duplicate onto tazobactam modified Charcoal Cefoperazone Deoxycholate Agar (TmCCDA) (Smith et al., 2015) (Oxoid, UK, CM0739) containing a selective supplement (Oxoid, UK, SR0155E) and incubated microaerobically at 42 °C for 48 h - in order to enumerate *C. jejuni*. Samples were also plated in duplicate for total viable counts on Plate Count Agar (PCA) (Oxoid, UK, CM0325) at 30 °C for 48 h and for total Enterobacteriaceae counts (TEC) on Violet Red Bile Glucose Agar (VRBGA) (Oxoid, UK, CM1082) at 37 °C for 24 h.

2.4. Statistical analysis

Microbial counts were converted to $\log_{10}$ cfu/g. A multiple liner regression model was then run to predict the significant effect of treatment, temperature and time on bacterial reductions between various treatment groups. To compare significant differences between treatments a 1-way ANOVA was used followed by Tukey multiple comparison tests. Significance was determined at the $P < 0.05$ level. Data was analyzed using IBM SPSS software (IBM SPSS statistics 24 Software, Armonk, New York, United States, www.IBM.com).