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pH dependent *C. jejuni* thermal inactivation models and application to poultry scalding

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ABSTRACT

Campylobacter jejuni related outbreaks and prevalence on retail poultry products pose threats to public health and cause financial burden worldwide. To resolve these problems, it is imperative to take a closer look at poultry processing practices and standards. Using available data (*D*-values) on the thermal inactivation of *C. jejuni* we develop a comprehensive inactivation model, taking into account the variation of strain-specific heat resistance, experimental method, and suspension pH. Utilizing our *C. jejuni* thermal inactivation model, we study the poultry scalding process. We present a mechanistic model of bacteria transfer and inactivation during a typical immersion scald in a high-speed industrial plant. Integration of our *C. jejuni* inactivation model into the scalding model culminates in validation against industrial processing data. In particular, we successfully predict bacteria concentrations in the scald water and link key factors such as scald water pH and temperature to cross-contamination and overall microbiological quality of carcasses. Furthermore, we demonstrate the applicability of our inactivation model for scalding operations at seven Canadian poultry plants. In addition to providing recommendations for best-practice and a review of scalding research, our work is intended to act as a modular foundation for further research in the interest of public health and financial well-being.

1. Introduction

Campylobacter jejuni, a major cause of foodborne illness, continues to cause millions of cases of illness in humans (e.g. gastroenteritis) annually. An estimated 20–150 cases are reported per 100,000 people in industrialized nations each year (Olson et al., 2008). A primary driver is consumption of undercooked poultry products (FAO/WHO, 2009). Modern studies report *Campylobacter* prevalence of retail poultry carcasses to be 57.7% in Canada, 58.8% in Japan, and 90% in the United Kingdom (Suzuki and Yamamoto, 2009; Moran et al., 2009). Furthermore, an estimated 24% and 46% of processors be will unable to pass stricter 2015 FSIS-USDA *Campylobacter* performance standards for raw chicken carcasses and not-ready-to-eat communicated chicken parts, respectively (US Department of Agriculture and Service, 2015).

While *C. jejuni* originates in the gastrointestinal tract of poultry, each bacterium finds its way onto the skin and feathers through external means (FAO/WHO, 2009). Birds are contaminated externally due to excreta buildup in densely populated living conditions. Contamination may not be eliminated during processing before retail sale and consumption. Of steps bringing a live chicken to retail, the slaughterhouse is a site of concern. The scalding process has been identified as a site providing opportunity for cross-contamination by the World Health Organization (FAO/WHO, 2009). In addition, cross-contamination between birds has been shown to be highly prevalent in scalding (Mulder et al., 1978).

Scalding background: Scalding is one of many stages in the poultry process. Typically, it is conducted immediately after chickens have been killed and bled out. As the first cleaning stage of poultry processing, bacteria and organic material levels are among the highest prior to scalding (FAO/WHO, 2009). Hence the opportunities for cross-contamination are abundant in this early processing stage. The two methods of scalding currently used in large-scale processing plants are *immersion* and *steam*, where the former is most widely adopted and the main focus of this work. During a

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typical two minute immersion scald, birds are immersed in 50–60°C aerated (scald) water to prepare birds for defeathering, wash off dirt and organic material, and reduce bacteria levels. Many foodborne pathogens associated with poultry, including *C. jejuni*, are heat sensitive and are inactivated thermally in the scalding temperature range (FAO/WHO, 2009).

Chemical additives in scalding: It is popular among poultry processors to utilize chemical additives and antimicrobials in scald water to enhance the killing of bacteria. By altering water chemistry and pH, the rate of bacterial inactivation also changes. However, conflicting experimental results on the efficacy of chemical additives indicate a more detailed understanding of the bacterial inactivation process may prove useful in pathogen control. We invite the reader to see our review of experiments using additives including bacteria counts and prevalence in AppendixA (Lillard et al., 1987; Okrend et al.; Humphrey and Lanning, 1987; Berrang et al., 2011).

In order to gain predictive insight on how scald water temperature and pH affect the inactivation of *C. jejuni*, we construct and utilize mathematical models. The power here is that, along with industrial scale data, such models can test mechanistic hypotheses as well as provide quantifiable connections between processing parameters and resulting bacteria levels in both the scald water and on chicken carcasses. In light of this perspective, the paper is organized as follows: first, in Section 2, we develop an inactivation model linking pH and temperature to death rates of *C. jejuni* in scald water using recent experimental data. Second, in Section 3, we present a mathematical model describing inactivation and transfer of *C. jejuni* in the immersion scalding process. In Section 4, we validate our findings by successfully predicting experimental bacteria counts in scald water using the model developed herein. Also, we provide general guidelines for combating cross-contamination and improving overall microbiological quality at the scalding stage. In Section 5 we discuss the affects of pH and temperature on cross-contamination using the steady-state concentration of bacteria in scald water (see Section 5.1). Furthermore, we illustrate the applicability and relevance of our work by using Canadian processor survey data to give alternative scalding strategies and operating conditions (see Section 5.3). Finally, we provide directions for further study and call for specific future experiments needed to fill gaps in present knowledge and data (see Section 5.4).

2. Thermal inactivation of *C. jejuni*

2.1. Experimental results

The time to kill 90% of an initial population is called a decimal reduction time, or *D-value*. All available experimentally determined *C. jejuni* D-values across six experiments have been compiled for this study, totaling 17 *C. jejuni* strains (Al Sakkaf and Jones, 2012; Doyle and Roman, 1982; Sörqvist, 1989; Blankenship and Craven, 1982; Waterman, 1982; Nguyen et al., 2006). These D-values give the killing rate of *C. jejuni* in neutral pH media such as brain-heart infusion broth (BHI) and skim milk. The high variation in killing rate with temperature is captured in Fig. 1A. While suspension temperature is crucial, pH also plays a pivotal role in bacteria inactivation (Bazin and Prosser, 1988; Humphrey and Lanning, 1987). As pH drifts away from neutral to acidic or alkaline, the rate of killing is increased (Bazin and Prosser, 1988). In other words, neutral pH 7 is the highest point of thermal resistance (Bazin and Prosser, 1988). Experimental results of *C. jejuni* D-values across the pH spectrum are shown in Fig. 1B. To further complicate the situation, scald water contains high levels of organic material (e.g. excreta, blood, fat, proteins, etc.), buffering the bacterial inactivation process (Humphrey and Lanning, 1987; Yang et al., 2001). If the processor accounts for the least sensitive strain (highest thermal resistance), then the remaining strains will also be inactivated. In Section 2.2, we provide an effective range which covers a wide variety of strains regardless of their individual thermal resistances. We invite the reader to see a more complete discussion regarding these items in AppendixB.1 and AppendixB.2.

2.2. Determining inactivation rate during scalding process

To gain insights into the scalding process, we develop a model accounting for the items mentioned above. Specifically, we wish to address:

1) The variation across experiments in killing rate versus temperature (Fig. 1A)
2) The effects of pH on thermal inactivation (Fig. 1B)
3) The buffering effects of organic material present in scald water (Yang et al., 2001).

To capture the combined effects of pH and temperature on D-values in scalding water, relative to each of the 17 *C. jejuni* strains from the available data, we propose the following inactivation model:

$$D_{w_i}(pH, T) = D(pH)[D_i(T)] \tag{1}$$

where i refers to a particular *C. jejuni* strain, T is the temperature of the scald water, and the forms of $D(pH)$ and $D_i(T)$ are dictated by experimental data (see Fig. 1A and B). In particular, we use $D(pH) =$

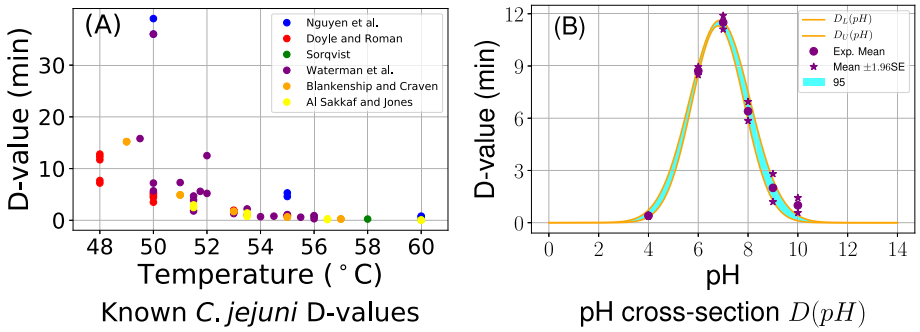

Fig. 1. (A) All known *C. jejuni* D-values with respect to temperature (Al Sakkaf and Jones, 2012; Doyle and Roman, 1982; Sörqvist, 1989; Blankenship and Craven, 1982; Waterman, 1982; Nguyen et al., 2006). D-values from a given paper are assigned the same color. High D-value variation is seen especially at low temperatures. As temperature increases, D-value variation appears to decline. (B) Experimental *C. jejuni* D-values taken in 52°C scald water with 13 mg/ml total solids and 6.2 mg/ml proteins (Humphrey and Lanning, 1987). The filled region represents a 95% confidence interval for the *C. jejuni* D-values as suspension pH varies. For data fitting details see AppendixB. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)