Meat Science 95 (2013) 185–189

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Efficacy of knife disinfection techniques in meat processing

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ARTICLE INFO

Article history:
Received 3 July 2011
Received in revised form 6 April 2013
Accepted 8 April 2013

Keywords:
Cleaning and disinfection
Knives
Lactic acid
Ultrasound
Heat

ABSTRACT

EU Regulation 853/2004 requires that knives used in meat processing be disinfected by submerging them in hot water (+ 82 °C). Alternative procedures are permitted if the efficacy is proved to be equivalent. In the present study, various time–temperature combinations together with pure water, water with lactic acid (2 %), and the use of ultrasound with and without lactic acid (2%) were investigated. Steel plates were covered with fat and protein and then inoculated with a standardized bacterial contamination assembled according to the composition of bacterial contamination found in a previous field trial conducted with regard to the slaughter of pigs. Several combinations with diverse temperatures and time intervals were tested until no microbial load was detectable by using a wet–dry-swab technique that had previously been tested to ensure maximum bacterial recovery. The following were effective in bringing the tested bacterial flora below the detection limit:

- 70 °C water bath for 10 s
- 60 °C water bath + ultrasound for 5 s
- 40 °C water bath + lactic acid (2%) for 10 s
- 40 °C water bath + ultrasound + lactic acid (2%) for 5 s

In particular, the use of lactic acid permitted a relevant reduction of the temperature, while providing effective sterilization. The use of such non-hazardous food-safe additives is particularly suitable for successful disinfection at lower temperatures.

1. Introduction

During meat processing, knives must be kept clean to prevent cross-contamination. Ideally, knives should be mechanically clean prior to treatment in water at 82 °C (Eustace, 2005; Eustace et al., 2007; Eustace, Midgley, Small, Jenson, & Sumner, 2008; Goulter, Dykes, & Small, 2008; SCVPH, 2001; Weise & Levetzow, 1976). According to Thiaudiere (1992), the type of mechanical cleaning has a significant impact on the microorganisms present on knife blades after treatment. However, sanitization procedures are often brief, incomplete and are carried out infrequently. Indeed, the short exposure to 82 °C does not destroy bacterial populations (Goulter et al., 2008; Taormina & Dorsa, 2007). Peel and Simmons (1978) have reported that water at 82 °C eliminates *S. typhimurium* on steel after 2 s, but the time necessary for this increases to 8 s when organic matter is introduced. These authors recommend dipping knives for 10 s into water at 82 °C. Snijders, van Logtestijn, Mossesl, and Smulders (1985) concluded that 1 s at 82 °C is sufficient to eliminate bacterial contaminants on a clean surface. In the presence of fat or protein, however, these authors regarded even 10 s as insufficient.

Disinfection of knives at 82 °C in a water bath or a procedure of equivalent efficacy is mandatory in establishments such as an abattoir (EU Regulation 853/2004 Annex III, Section I, Chapter II, No. 3, resp. Chapter III, No. 5 and Section IV, Chapter I No. 5). Usually hot water at 82 °C is used for disinfection purposes; however, the original source of this method is not known (Midgeley & Eustache, 2003). Moreover, the efficacy of disinfection is dependent on temperature and time of exposure; an acknowledged time–temperature combination has not been established in the field of legislation. The legislation only demands the specific temperature of 82 °C, with no demands for a specific time of exposure.

The denaturation of proteins commences at about 50 °C to 60 °C (Goodson & Rowburry, 1989). During slaughter and further processing, fatty tissue and proteins may adhere to knife surfaces, thus providing a protective layer. During the denaturation of proteins on the knife surface, bacteria might be entrapped and thus remain alive (Schütt-Abraham, Trommer, & Levetzow, 1988, 1992). Moreover, the melting temperature of fats depends on the structure and quantity of their fatty acids, and temperatures should be higher than their melting point of between 40 °C and 60 °C (Fries, 2002).

The aim of this study has been to examine combinations of physical (heat, ultrasound) and chemical (lactic acid) factors for disinfection purposes.

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http://dx.doi.org/10.1016/j.meatsci.2013.04.035

2. Materials and methods

2.1. Test strains

In a preliminary field trial, the qualitative and quantitative bacterial exposure of knives at various working positions in a pig-slaughter line was examined. By using Standard 1 agar (Merck 1.07881 — main components: peptone and yeast-extract) and blood agar (Standard 1 agar with 5% 'defibrinized' sheep blood) plates, 60% Micrococcaceae, 20% gram-negative, and 20% gram-positive rods were differentiated down to their genera. Based on these results, a defined test flora was prepared by using DSM (German Collection of Microorganisms) and ATCC (American Type Culture Collection) strains, which were available: 60% *S. aureus* (DSM 6538), 20% *Enterobacter aerogenes* (ATCC 13046), and 20% *Listeria monocytogenes* (DSM 20600) representing gram-positive cocci, gram-negative, and gram-positive rods, respectively. The growth rates and the population composition of the test strains were determined after culture in trypton-soy–nutrient solution (Oxoid CM0129). Following 18 h at 30 °C, the population of the test bacteria consisted of 10^9/ml *S. aureus* and *E. aerogenes* and 10^8/ml *L. monocytogenes*. The required amounts were then combined into a mixed culture with the above-mentioned percentages prior to the trials.

Based on field data (Einschütz, 2004) namely the surface of an average blade is 54 cm^2 with a mean burden of log 4.84 cfu (median aerobic plate count [APC])/knife, a burden of log 4 cfu per plate (10 cm^2 high-grade steel plate replacing the knives) was used as the microbial test load. In total, 0.01 ml test flora were necessary for each test plate.

In order to ensure that the initial status could be replicated each time that a new mixture was set up, the population of the mixed cultures was examined using APC.

2.2. Preparation of the test surface

The surface was prepared with sterile fatty tissue. Normal bacon rind was singed with a Bunsen burner on the inner side, following which the burned top layer was removed in a sterile manner, and the test surface was slightly pressed onto the sterile bacon. Then, 0.01 ml sterile commercial bovine serum (reflecting protein contamination) was dispersed with a spatula over the surface. Subsequently, the test plates were placed on a sterile tray and briefly heated over a Bunsen burner until coagulation became visible. Coalescence of the fat and serum was thus achieved. The test surface was then inoculated with the test suspension of the bacterial culture (0.01 ml) and then the bacterial suspension was mixed with the fat-protein load using the tip of the sterile pipette. The contamination load was subsequently allowed to attach to the surface for 20 min.

2.3. Test combinations

In total, 30 time–temperature combinations together with physical (ultrasound 35 kHz) and/or chemical treatment (lactic acid, 2%) were used (Table 1). For each combination, 10 individual test plates were prepared and examined after treatment. In addition to these 10 replications, one control plate for the initial bacterial load on the surface was prepared. In total, 300 individual test plates underwent the individual sterilization procedure, with 30 control plates not receiving such treatment. In the case of a negative result of bacterial recovery at a given temperature, the next strongest impact, i.e., the next higher temperature of this technical combination was not tested. Each combination was tested within one day, including the control, i.e., 10 independent and individual tests per combination plus the control plate.

Table 1
Mean log APC per test surface (10 cm^2) under several test conditions (log x_{mean}, (sd)).

Combination	Time (s)			Temperature		
		40 °C	50 °C	60 °C	70 °C	80 °C
Water	1				3.3 (0.5)	
	5				0.8 (1.3)	
	10	3.9 (0.3)	3.9 (0.4)	4.1 (0.3)	n.d	n.d.
	30	4.9 (0.4)	4.0 (0.4)	3.2 (0.4)		
	60	4.3 (0.5)	4.0 (0.7)	n.d.	n.d.	n.d.
Ultrasound (US)	1			2.7 (1.1)		
	5			n.d.		
	10	3.5 (0.5)	3.7 (0.4)	n.d.		
	30	3.8 (0.4)	3.3 (0.3)			
	60	3.6 (0.4)	1.5 (1.6)			
Lactic acid (LA)	1	3.6 (0.2)				
	5	1.7 (1.8)				
	10	n.d.				
US plus LA	1	3.0 (1.5)				
	5	n.d.				
	10	n.d.				

Number of samples tested for each of the listed 30 time–temperature combinations: $n = 10$.
n.d.: none detected; spare areas: not done in case of negative result to be expected; sd: standard deviation.

2.4. Recovery of bacteria

In preliminary experimental approaches, the performance of the wet-dry swab technique was considered to be favorable. In accord with the sampling in the field trial and following the technical impact, this technique was used in collecting the bacteria and entailed streaking the surface 10 times under slight pressure, first with the wet swab and then with the dry swab (DIN, 10113–1, 1997). Both swabs were then suspended in 20 ml of a saline–peptone dilution in an Erlenmeyer flask (No. 1) and rotated (1500 rpm) for 1 min. These swabs were then transferred into another Erlenmeyer flask (No. 2) with 20 ml saline–peptone. This was performed four times in order to recover as many bacteria as possible from the swabs. From each of the four Erlenmeyer flasks, 1 ml was taken, and a decimal dilution sequence was prepared from each flask (10^0, 10^{-1} and 10^{-2}), of which 0.1 ml was transferred to duplicate Standard 1 agar plates (spread plating procedure) and incubated at 30 °C for 48 h. The APC was calculated according to the German Standard (DIN)/European Standard (EN) (DIN 10 162, EN 180 6887-1 and DIN 10 161-1) for each flask. For each single analysis, the detection limit was 2.3 log/cm^2. Finally, results from the four Erlenmeyer flasks were added together, representing the whole bacterial load that could be recovered from the surface. This procedure was carried out in all replications (10 steel plates) for each combination. From the results of these ten replications per combination the mean value for the respective combination was calculated, that may be below the analytical detection limit of 2.3 log/cm^2. Resuscitation of injured or stressed bacteria was not conducted. Internal quality assurance was performed by testing the results of the APC on the basis of ISO 14461-2. The burden of the control plate (log 4 cfu per plate) was used to establish the success of inoculation and the recovery method. The positive control was acceptable in all samples.

2.5. Statistics

Mean value and standard deviation of the APC were calculated for each test condition at the tested time–temperature combinations (Table 1). Thus, some calculated values are below the detection limit. The independent *t*-test using IBM-SPSS-Statistics-20 was performed for three different scenarios. The null-hypothesis was set as follows: no difference exists between the influences of the compared parameters. For *p*-values of ≤ 0.05, the null-hypothesis was rejected. In this case the alternative hypothesis is accepted, assuming that a correlation