PS6.05 Reduction of Echerichia coli O157:H7 on dry-fermented sausage slicing knife by intense light pulse treatment 206.00

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Abstract—The efficacy of intense pulsed light to inactivate Escherichia coli O157:H7 on stainless steal slicing knife was investigated in this study by evaluating the effects of treatment times after the contamination and number of successive flashes. The sterilization system generated 3 J/ cm2 per pulse for an input voltage of 3000 V. Side of inoculated round knife (Ø6,5 cm) used for slicing of dry-fermented sausage was treated after 1,5,15,30,45 and 60 minutes. The number of flashes used were 1 and 5. Maximum reduction was 6.5 log10 CFU per side of a knife achieved with only one flash, 1 minute after inoculation whereas 3.75 log10 CFU reduction after 5 minutes with the same number of flashes. Successive flashing of the side of a knife appears to be ineffective at inactivating E. coli O157:H7 to the extent significantly greater than the effect achieved with a single flash (P>0,05). Overall, this study demonstrated that a significant level of E. coli O157:H7 reduction can be achieved on a stainless steal meat slicing knife with a very short treatment time after its inoculation and with a single flash. ILP unit was laboratory-scale batch system but the technology is fully scalable to production size and semi-continuous processing.

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Index Terms — decontamination, Escherichia coli O157:H7, intense light pulse treatment, meat slicing knife.

I. INTRODUCTION

Pulsed light treatment is a novel method for the decontamination of surfaces and has been proposed as a technique for surface decontamination of food and food contact surfaces. The process involves generation of high intensity light pulses used to flash target surface with a broad spectrum white light leading to partial or complete microbial inactivation. The broad spectrum white light produced by pulsed light equipment is typically in the wavelength 200-1100 nm which comprises of 15% ultraviolet light, 50% visible light and 35% near infrared light. During the pulse, the system delivers a spectrum that is 50,000 more intense that sunlight at the earth's surface [10]. The system provides very short pulses (300μs) to the surface of the test material. This short pulse reportedly heats the surface of the material to approximately 160°C during the moment of the flash but this is a very short duration (300μs) so it is essentially a non-thermal process [10]. The mode of action of the pulsed light process is attributed to the effect of the high peak power and the UV component of the broad spectrum of the flash. Inactivation occurs by several mechanisms, including chemical modification and cleavage of DNA, protein denaturation and other cellular materials alteration [14].

Studies in which the UV component was filtered out showed marked reductions in process efficacy [12]. The UV light transforms the pyrimidine bases in the DNA and forms dimmers so the microorganism cannot replicate [13]. Under certain experimental conditions with continuous UV treatment, repair of the damaged DNA can occur, but this repair does not occur after pulsed light treatment [3] The ability of continuous ultraviolet (UV) light and visible light to inactivate cellular microorganisms is well known [1,11,6,2]. In recent years, research attention has been focused on the investigation of bactericidal effects of intense light pulses (ILP) on food surfaces and in food preparation environments [8]. It has been demonstrated that the xenon flash lamp used in ILP treatment units is an

efficient UV source for the inactivation of E. coli, which is generally accepted as a representative organism for a wide range of Gram negative bacteria. The inactivation of E. coli displays a maximum at wave length of 270 nm. No measurable inactivation was observed to occur above 300 nm [16]. Today, the literature on pulsed light is rapidly expanding, but a gap remains between fundamental and applied research with respect to decontamination and food safety [7,4,9]. The main objective of this work was to examine the preventive decontamination effect of intense light pulse treatment (ILP) on a round, stainless steal, meat slicing knife as a surce of contamination of sliced ready-to-eat meat products. E. coli O157:H7 was chosen as a typical test microorganism.

II. MATERIALS AND METHODS

A. Intense light pulse (ILP) equipment The test assembly consisted of a housing, a stroboscope and a lamp. The housing was a rectangular parallelepiped stainless steel box (length: 100 cm, width: 100 cm, depth: 60 cm). Pulse duration of 300 μs and pulse intensity of 3 J/ cm2 were used for an input voltage of 3000 V. The lamps were a 20 cm cylindrical Xenon flash lamp (Flashlamps Verre & Quartz, Bondy, France), with the spectral distribution of the light as reported by the Claranor shown in the Figure 1. Intense light pulse unit was laboratory-scale four-lamp batch system and it was used with top and bottom lamp 6 cm and left and right hand lamp 10 cm away from the treated sample (knife). Both side lamps were elevated 5° degrees above the horizontal line.

B. Bacterial strain and culture condition E. coli O157 strain 463 from Laboratory of Food Microbiology and Food Preservation (LFMFP) was used throughout the study. A reference stock of E. coli O157 LFMFP 463 was kept at -70°C in Tryptone Soy Broth (TSB; Oxoid, Basingstoke, England), supplemented with 0.6% w/w yeast extract (YE; Oxoid), with 15% v/v glycerol (Prolabo, Heverlee, Belgium). A stock culture was kept at 4°C on a Tryptone Soy Agar (TSA; Oxoid) slants supplemented with 0.6% YE. The stock culture was refreshed monthly. Working cultures were prepared by suspending a loopfull (1μl) in 10 ml of fresh TSBYE and incubated for 24 h at 37°C. During experimental setup, stationary phase cells were used and an initial inoculation level of approximately 6.5 log10 bacteria per ml was obtained by appropriate 10-fold dilutions of the working culture in TSBYE.

C. Preparation and inoculation of the knife Polished stainless steal round knife with a diameter of 6,5 cm was used. It was soaked overnight in technical alcohol, scrubbed thoroughly with a brush, rinsed three times in tap water and twice in distilled water, and autoclaved at 121°C for 15 min. Then it was used for the slicing of a dry-fermented sausage bearing a Protected Designation of Origin (PDO) mark and purchased from a local retailer. After 30 sec of a slicing the knife was inoculated with E. coli O157:H7. A single decontamination experiment involved transferring a 0.1 ml specimen of the E. coli O157:H7 suspension to the knife that had been in contact with the sausage, aseptically drying for 1, 5, 15, 30, 45, and 60 min after the inoculation at room temperature and, finally exposing the knife to 1 or 5 flashes of intense pulsed light. The inoculated knife without the ILP treatment represented the control. Throughout the experiment, the knife was handled aseptically with sterile forceps.

D. ILP treatment Treatments consisted of 1 or 5 flashes and every flash was manually started at a rate of one pulse per 2 second. The time after the inoculation till ILP treatment was accounted and ranged from 1 minute to 1 hour. The lethality of the process was evaluated establishing the degree of micro-organism inactivated by subtracting the logarithm of the count after the treatment from the logarithm of the count before the treatment. All the experiments were carried out in quadruplicate using the same culture on the same day for every pair of trials (treatment and control) to minimize sample variability.

E. Microbiological analysis Surviving cells on the complete surface of a side on the stainless steal knife were swabbed with 3M™ Sponge-Stick. Hydrated sponge with 10 mL Neutralizing Buffer on stick allowed sampling without directly handling the sponge or the knife. After the swabbing the sponge was aseptically detached from the stick and transferred to a sterile stomacher bag. One to fifty (1:50) dilution was made by adding 40 ml of peptone physiological saline (PPS; 8.5 g/l NaCl and 1 g/l neutralized bacteriological peptone (Oxoid)) and the sample was homogenized for 120 s by means of a stomacher Seward Laboratory blender 400 (UAC House, London, England).

Subsequently, enumeration on the standard selective media for detection of E. coli 0157 (CTSMAC) [5] were performed. Enumerations were preformed after 24 h incubation at 37 ±1°C. Surviving cells were enumerated as described earlier. The total number of cells on each knife side was not divided by the area of the knife but reported as log colony forming units per side of knife. The calculated limit of detection by this method was 50 CFU/side of a knife.

F. Statistical analysis Data were analyzed by using StatSoft, Inc. (2003). STATISTICA (data analysis software system), version 6. (www.statsoft.com). A t-test was used to compare pairs of means with $P \leq 0.05$; when multiple mean comparison was made, one-way ANOVA and the Fisher LSD test were used.

III. RESULTS AND DISCUSSION

The log reduction of population log (N/N0) is calculated for each 0.1 ml E. coli O157:H7 specimen. N is the population in CFU/side of a knife after UV inactivation and N0 is the population in CFU/side of knife of the control specimen, that is, the population before UV inactivation. As described earlier, each point is an average of four experimental results. For E. coli O157:H7, maximum log10 reduction was 6.54 log10 (N/N0) on a side of stainless steal knife with a ILP treatment performed less then 1 minute after the inoculation occurred (E. coli O157:H7 remained below the detection limit of 50 CFU/side of knife)), whereas 3,75 log10 (N/N0), 3,40 log10 (N/N0), 3,12 log10 (N/N0), 2,54 log10 (N/N0) and 2,12 log10 (N/N0) reduction was achieved after 5,15,30,45 and 60 minutes respectively (Figure 2).

The observed differences in log10 reduction were found siginificqnt ($p<0.05$) between each time interval. The results showed that the longer the time between inoculation and ILP treatment, the lower the lethality of the process. These findings emphasize the importance of keeping the time between possible contamination and decontamination as short as possible. During growth, bacteria are situated on top of each other in several layers forming a colony. Bacteria located on the upper layers receive the light directly and are easily inactivated. However, bacteria on the bottom layers are protected by those on the upper layers which screen the incident light and as a result, the survivor numbers are higher. When bacteria arrived to the stationary phase only the bacteria on the upper layer are inactivated and a large and constant part of the contaminating population will survive the treatment [4]. When the effect of the successive flashing was tested, the results showed them ineffective at inactivating E. coli O157:H7 to the extent greater than the effect achieved with a single flash.

The results clearly demonstrated that there was no significant difference between 3,12 log10 (N/N0) reduction achieved with 1 flash treatment and 3,52 log10 (N/N0) reduction achieved with 5 flashes, both of them performed 30 minutes after the inoculation of the knife ($p > 0.05$). These results show that when ILP is implemented in the industry for the decontamination of the meat contact surfaces, whiwh are important source of cross-contamination, one must take into account the effect of the time between contamination and decontamination. The highest efficiency of the ILP will be reached if the meat contact surfaces are flashed as soon as possible after the processing steps where contamiantion can occur. Theefficacy of decontamination lost into account of the time can not be compensated by the increased number of successive flashes.

IV. CONCLUSION

The results obtained here demonstrate that intense pulse light decontamination treatment can be successfully applied for decontamination E. coli O157 on a slicing knife, which is one of the most important sources of contamination in sliced ready-to-eat meat products. Overall, the study has shown that the decontamination efficiency of pulsed UV radiation is a function of time after the the contamination but the degree of inactivation of E. coli O157:H7 achieved by flashing was independent of the successive flashing. The fact that a significant level of microbial reduction can be achieved after a very short treatment time indicates much promise for the use of ILP as a quick method of reducing the microbial load on a stainless steal surfaces in meat-processing environments. Having in mind that in terms of the sterilization dose, pulsed light may represent the most energy-efficient mechanism to date, (4) this can only emphasize the necessity of the further research in this area.

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