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FINAL REPORT

Project Armis No. 4368

Development of a Critical Control Step for *E.coli* 0157:H7 in Pepperoni

The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 18

Contents

DEVELOPMENT OF A CRITICAL CONTROL STEP FOR *E.COLI* O157:H7 IN PEPPERONI

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SUMMARY

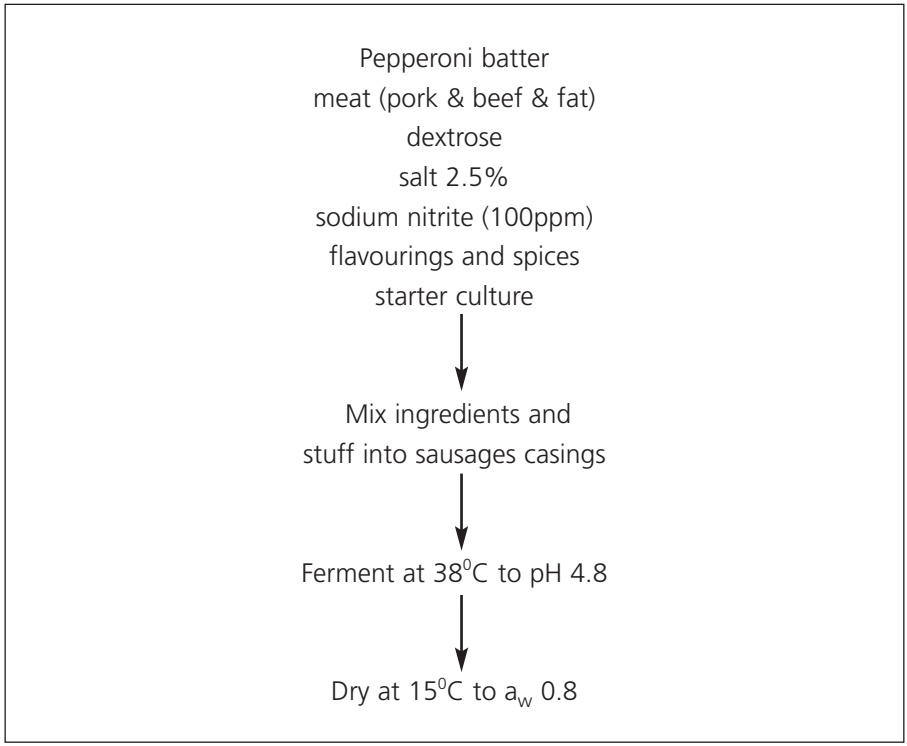
Verocytotoxin producing *Escherichia coli* (VTEC) and particularly strains of serogroup O157, have emerged as food poisoning pathogens which can cause a severe and potentially fatal illness. The symptoms of VTEC infection include haemorrhagic colitis with bloody diarrhoea and severe abdominal pain. The infection may lead to renal failure as a result of haemolytic uraemic syndrome. Because of the severity of the illness and the low infectious dose, this pathogen is classed as a serious food safety issue. It is recommended by the United States Department of Agriculture that the production process for ready to eat foods such as fermented meats (pepperoni, salami etc.) should be capable of addressing a worst case scenario ie. the production process should be able to yield a $\log_{10}$5.0cfu /g (10^5 cfu/g) reduction in numbers of *E. coli* O157:H7 on the raw meat. The aim of this study was to develop an industrially viable critical control step(s) which could be implemented into the pepperoni production process.

- In pepperoni produced from a typical commercial formulation (2.5% salt, 100 ppm sodium nitrite, pH 4.8) and inoculated with *E. coli* O157:H7 ($\log_{10}$7.0 cfu/g) the pathogen numbers declined by $\log_{10}$0.82 cfu/g during the fermentation and drying processes. Pepperoni was then made with varying concentration of salt, sodium nitrite and final pH to determine if modification of any of these intrinsic factors would yield a greater reduction in the numbers of *E. coli* O157. Significantly greater reductions ($P<0.001$) ($\log_{10}$4.81 cfu/g) were noted in samples with low pH (4.4) and increased salt (3.3%) and sodium nitrite (300 ppm) levels. While this reduction is close to the $\log_{10}$5.0 cfu/g required the formulation would not yield a commercially acceptable product.
- Subsequent studies focused on the inclusion of a heating step into the production process. The relationship between heat resistance, pH and acid adaptation of *E. coli* O157:H7 was also investigated. Pepperoni batter was inoculated with acid adapted or non acid adapted *E. coli* O157:H7, fermented to pH 4.8 or 4.4 and than heated at 55, 58, 60 or 62^{0}C and the time and temperature required to inactivate *E. coli* O157:H7 calculated. *E. coli* O157 cells which were non acid adapted

and were inoculated into pepperoni fermented to pH 4.8 were found to have the greatest heat resistance and therefore represented a 'worst-case-scenario' for *E. coli* O157:H7 survival in pepperoni. The heating step developed was based on these 'worst case' conditions.

- A critical control point heating step was developed (18 min at 62^{0}C) for pepperoni which yielded a $\log_{10}$5.0 cfu/g reduction in the number of *E. coli* O157:H7. This step was included in the process between fermentation and drying of the product.
- Sensory trials carried out by the Industrial partner showed that heating the pepperoni for 18 min at 62^{0}C did not adversely affect the sensory qualities of the product and will be an industrially viable process step to ensure product safety.

Figure 1. Standard pepperoni production process



Pepperoni production process

Pepperoni was produced on a pilot scale in the laboratory according to an industrial formulation as outlined in Fig.1.

Survival of *E. coli* O157 in standard and ingredient modified pepperoni

The survival of *E. coli* 0157:H7 (strain 380-94, from US salami outbreak in 1994) was investigated by inoculating pepperoni meat batter with *E. coli* 0157:H7 ($\log_{10}$7.00 cfu/g) and then monitoring the survival of the organism during the fermentation and drying process. In addition to the standard pepperoni product (pH 4.8, salt 2.5%, 100 ppm NaN02), a range of product formulations with different concentrations of salt (2.5, 3.3 and 4.8%), sodium nitrite (100, 200, 300 and 400 ppm) and final pH (4.4, 4.8 and 5.5) were prepared and then at various stages during fermentation and drying, the product was examined for the presence of the pathogen by plating onto Sorbitol McConkey Agar (SMAC).

In pepperoni prepared to the standard commercial formulation [salt (2.5%), sodium nitrite (100 ppm) and pH (4.8)] *E. coli* 0157:H7 numbers declined by approximately $\log_{10}$0.39 cfu/g during fermentation and a further $\log_{10}$0.43cfu/g during subsequent drying (8 days) (Fig 2). Significantly greater ($P<0.001$) reductions in pathogen numbers were noted in samples with increased salt (3.3%) and sodium nitrite (300 ppm) (4.02cfu/g) (Fig 3) but the levels of salt and sodium nitrite would render the pepperoni product organoleptically unacceptable. In other formulations the decline in numbers of *E. coli* O157:H7 ranged from $\log_{10}$0.37 to 2.51 cfu/g. It was concluded that it would not be possible to achieve a $\log_{10}$5.0 cfu/g reduction in *E. coli* O157:H7 numbers by modifying the product formulation.

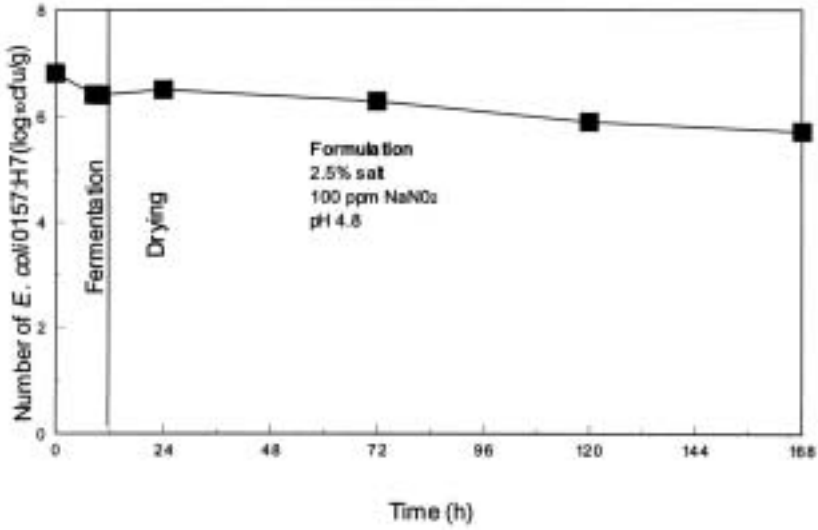


Figure 2. Decline in *E. coli* 0157:H7 numbers (log_{10}cfu/g) in standard pepperoni production process

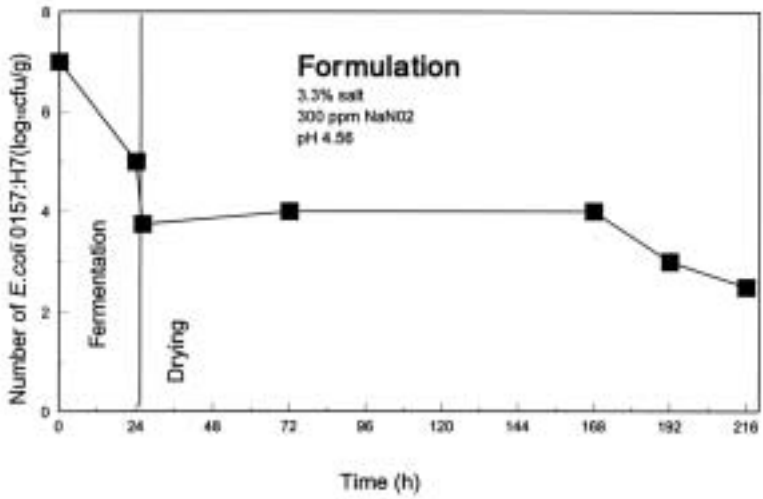


Figure 3. Decline in *E. coli* 0157:H7 numbers (log_{10}cfu/g) in modified pepperoni production process

Effect of heat and acid adaptation on the survival of *E. coli* O157:H7 in pepperoni

This study established the temperature and exposure time required to heat inactivate *Escherichia coli* O157:H7 in pepperoni. The study also aimed to establish if pH or acid adaptation of *E. coli* O157:H7 cells affected the thermal resistance profile of the pathogen (Fig 4). It was hypothesised that protective effects arising from acid adaptation might also confer some additional heat protection on the *E. coli* 0157: H7 cells and that higher D values [time in minutes at a particular temperature to reduce the number of bacteria by 10 fold (1 log)] might be recorded for acid adapted cells.

E. coli O157:H7 (strain 380-94) were grown for 18 h at pH 5.6 (acid adaptation) or pH 7.4. (non acid adapted). These cells were inoculated into pepperoni batter which was then made into sausages. The sausages were fermented to either pH 4.8 or 4.5 and a sample of the pepperoni was then removed and heated for various lengths of time at 55, 58, 60 or 62^{0}C. At each temperature, the length of heating time was plotted against the number of surviving *E. coli* O157:H7 and the D value was calculated (Fig 5).

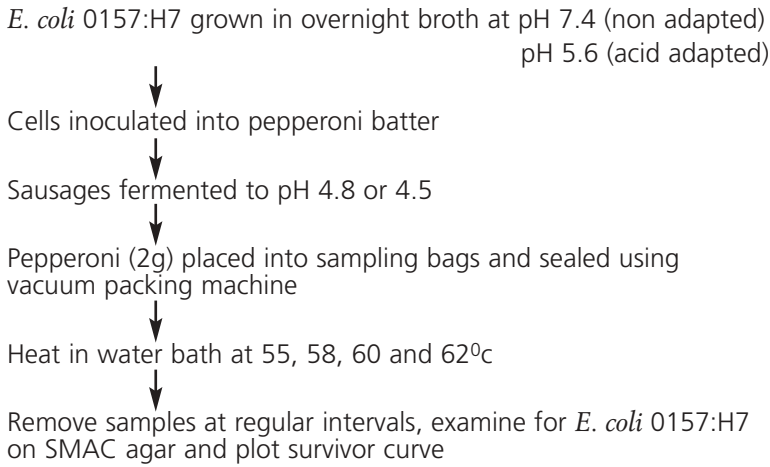


Figure 4. Flow diagram of experiment to determine the effect of heat and acid adaptation on the survival of *E. coli* 0157:H7

The D value at four different temperatures (55, 58, 60, 62°C) for *E. coli* O157:H7 in different physiological states (acid adapted/ non adapted) and in pepperoni fermented to different pHís (4.4, 4.8) are shown in Table 1. The results showed that the pH of the pepperoni and the physiological state (acid adapted/non adapted) of the cells had a significant effect on the thermal resistance profile (D value) of the pathogen. *E. coli* O157:H7 inoculated into pepperoni which had been fermented to pH 4.8 were significantly more heat resistant ($P<0.01$) than cells in pepperoni fermented to a lower pH (4.5) (Fig 6). *E. coli* O157:H7 which had been acid adapted prior to inoculation in the pepperoni batter were less resistant to heat than the non adapted cells (Fig 7)

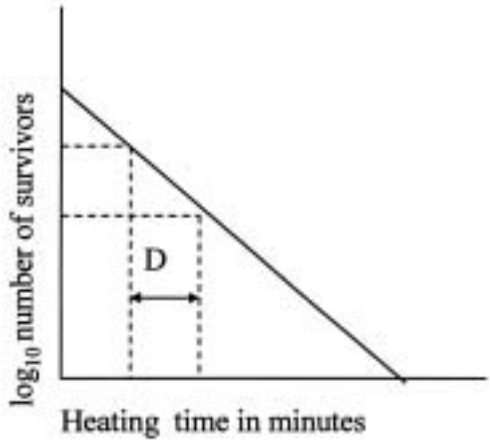


Figure 5. Bacteria survivor curve and calculation of heat resistance

Table 1: D values at 55, 58, 60 and 62°c for *E. coli* 0157:H7 (acid adapted/non adapted) in pepperoni fermented to pH 4.8 or 4.4

Final pH	D value			
	55°c	58°c	60°c	62°c
a) non adapted cells (pH 7.2)				
4.8	62.02	14.35	6.12	2.53
4.4	16.01	4.10	2.35	1.16
b) acid adapted cells (pH 5.6)				
4.8	22.10	5.22	4.37	1.64
4.4	17.62	4.51	2.90	1.25

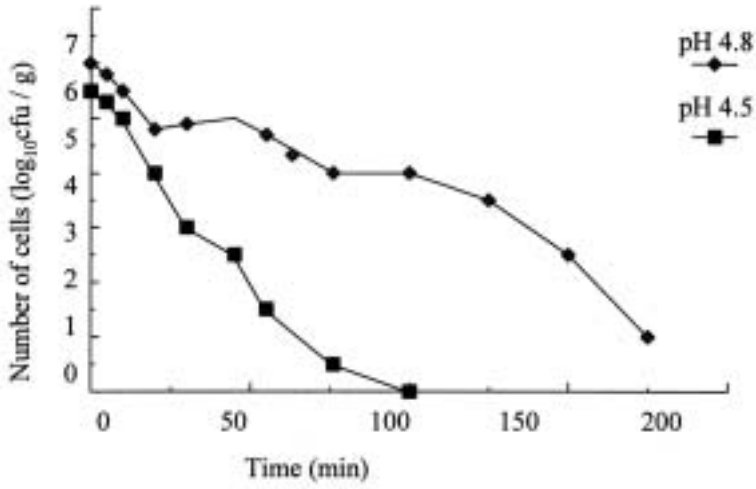


Figure 6. Survivor curve for *E. coli* O157:H7 at 55°C in pepperoni fermented to pH 4.8 or pH 4.5

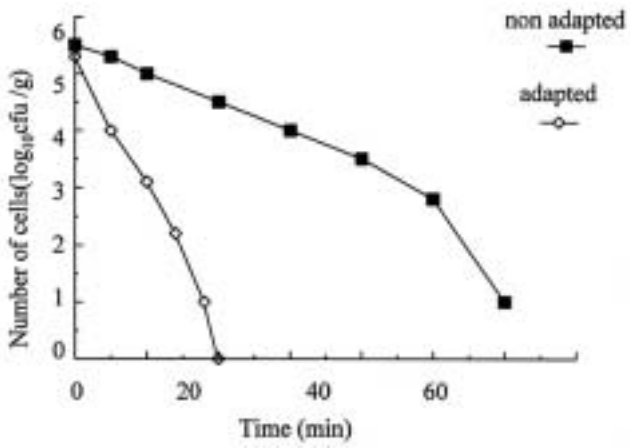


Figure 7. Survivor curve for *E. coli* 0157:H7 (non adapted and acid adapted cells) at 58^{0}C in pepperoni fermented to pH 4.8

A heating step for pepperoni

It was concluded that the most heat resistant *E. coli* O157:H7 were those cells which were not acid adapted and were in product fermented to pH 4.8. These conditions represented the 'worst case scenario' for survival of *E. coli* O157:H7 and so were used in developing a heating step to achieve a $\log_{10}$5.0 cfu/g reduction in numbers of the pathogen in pepperoni. The data generated on the D values were used to calculate the length of heating time at each temperature to achieve a 5 log reduction in *E. coli* O157:H7 (Table 2). These heating times were validated by inoculating pepperoni batter with *E. coli* O157:H7 ($\log_{10}$5.0 cfu/g), fermenting the sausage to pH 4.8 and then heating the sausage at the time and temperature outlined in Table 2. The sausages were then dried as normal to an a_w of 0.8. The final product was examined and no *E. coli* O157:H7 was detected in the pepperoni product.

Table 2. Heating time at four temperatures to achieve a 5 log reduction in numbers of *E. coli* O157:H7

279 min @ 55^{0}C
61 min @ 58^{0}C
29 min @ 60^{0}C
18 min @ 62^{0}C

Sensory trials

Once the efficacy of the critical control point heating step had been established, sensory analysis studies were carried out on the pepperoni product on a pilot scale by the industrial partner in the project. Two temperatures (58 and 62^{0}C) were chosen for this industrial trial. Triangle tests were carried out to detect any differences in the sensory qualities (appearance, colour, flavour and texture etc.) between the standard pepperoni product and the heated product. The studies showed that product heated at 58^{0}C for 61 minutes was of lower sensory quality that the standard product. Trials showed that pepperoni which was heated for a shorter period of time at a slightly higher temperature (62^{0}C for 18 minutes) was indistinguishable from the standard pepperoni product.

CONCLUSIONS

The main achievement of this project has been the development of a commercially viable heating step which can be used in the production of pepperoni. This critical control step ensures a $\log_{10}5.0$ cfu/g reduction as recommended by the United Department of Agriculture in the number of *E. coli* O157:H7 on raw meat during pepperoni production.

In addition, a significant amount of data has been generated on the physiology and biochemistry of the survival of *E. coli*, in particular the effect of acid and heat stress on the survival and development of resistance by the pathogen, which will have a wide application in terms of food safety.

Final trials are ongoing in industry with a view to implementing this critical control point heating step in to a commercial pepperoni production process.

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