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Control of *Escherichia coli* O157:H7 in Beefburgers

The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 29

Contents

CONTROL OF *ESCHERICHIA COLI* 0157:H7 IN BEEFBURGERS

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SUMMARY

The inactivation of *E. coli* O157:H7 by heating, freezing, pulsed electric field, sodium lactate, lactic acid and citric acid, alone or in combination was investigated. The industrial process for beefburger manufacture did not significantly reduce *E.coli* O157:H7 numbers regardless of the burger recipe and method of tempering used. Fast freezing of the burgers (to –18°C in 30 minutes as opposed to 36 hours), pulsed electric field, sodium lactate, lactic acid and citric acid, individually and in combination, did not significantly reduce *E. coli* O157:H7 numbers when applied at different stages throughout the beef burger manufacturing process. Beefburger safety is therefore reliant on proper storage, handling and thermal processing in the domestic or catering kitchen. The lethal effect of thermal processing may be enhanced by the addition of sodium lactate to the burger during mixing. These results are presented and discussed.

INTRODUCTION

It is well established that beefburgers are an important source of *E. coli* O157:H7. Indeed *E. coli* O157:H7 is nick-named the 'burger bug' because of its association with beefburgers in the USA in 50% of reported outbreaks between 1982 and 1994 (Meng and Doyle, 1998). Up to 3.2% of Irish beef carcasses are contaminated with this pathogen (McEvoy *et al.*, 2001). Given that the trimmings from beef carcasses are pooled from multiple sources, one lot of *E. coli* O157:H7 contaminated beef has the potential to contaminate a large volume of product. Armstrong *et al.* (1996), for example, estimated that *E. coli* O157:H7 from one contaminated carcass could result in the contamination of several tonnes of beefburgers.

The inactivation of *E. coli* O157:H7 in beefburgers is currently achieved through the application of heat during cooking. The recommended minimal heating treatment in Ireland and the UK for cooking ground beef and beefburgers is to an internal temperature of 70°C for 2 minutes (FSAI, 1999; ACMSF, 1995). However, given the potential for cross contamination and inadequate cooking in domestic and catering establishments (Griffith *et al.*,

1994; Scott, 1996; Tarsitani *et al.*, 1998; Jin *et al.*, 1998), it is desirable that the product be free of the pathogen when it leaves the manufacturing plant (Jordan *et al.*, 1999).

Controlling *E. coli* O157:H7 in beefburgers is reliant on the development of strategies to reduce or eliminate this pathogen during burger manufacture. Apart from thermal treatments, there are several other potential strategies worthy of investigation. These include freezing, formulation using bacteriocidal ingredients such as sodium lactate, lactic acid and citric acid, applying pulses of high voltage electricity and the use of high pressure treatments. The objective of this project was to investigate the effectiveness of each of these technologies in the destruction of *E. coli* O157:H7 during commercial beefburger manufacture with the exception of high pressure application, which will be the subject of another research project.

THE SURVIVAL OF *E. COLI* O157:H7 DURING BEEFBURGER MANUFACTURE

The first task was to establish whether the current process of beefburger manufacture effected the destruction of any *E. coli* O157:H7 that entered the process on contaminated beef trimmings. In co-operation with manufacturers, the process and temperatures used in commercial beefburger manufacture were established (Figure 1).

A laboratory system comprising a Lauda waterbath with ramping facility, a PC with Wintherm software and a temperature microprocessor monitoring system was then developed to mimic the temperature profile of beefburger manufacture in industry.

The effect of each stage of manufacturing on the survival of added *E. coli* O157:H7 was investigated individually and in combination using 2 commercial recipes (a 100% beefburger (recipe 1) and a burger containing rusk, seasoning, frozen onion, salt and soya concentrate in addition to the meat component (recipe 2)). Modifications such as tempering using microwaves (as opposed to convection heating) were also examined.

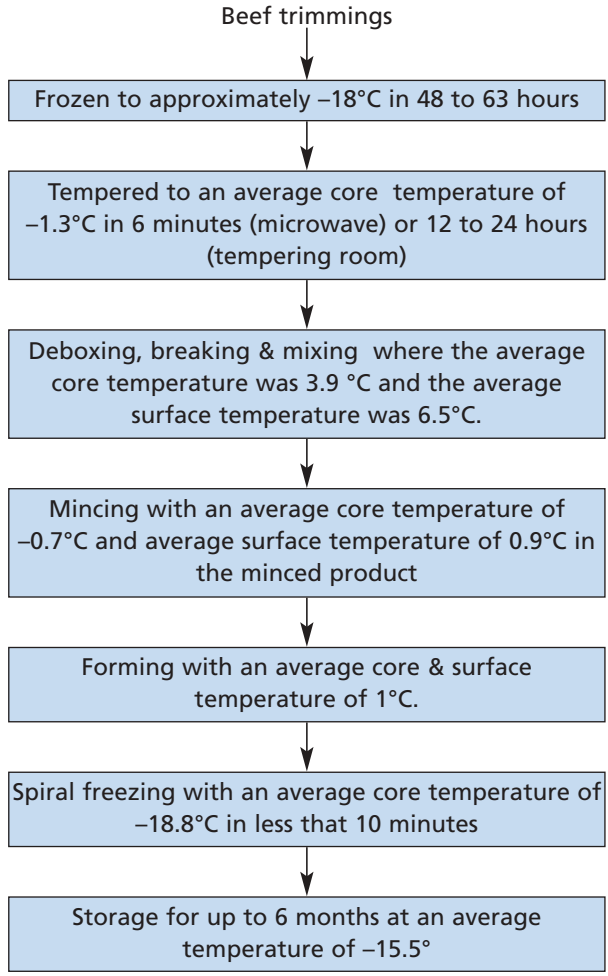

Figure 1: A summarised flow diagram of the beefburger manufacturing process.

Decreases in *E. coli* O157:H7 numbers as a result of the manufacturing process ranged from 0.2 to 0.7 $\log_{10}$ cfu/g depending on the recipe and method of tempering used. Decreases were statistically insignificant ($P > 0.05$) which is consistent with other similar studies (Sage and Ingham, 1998; Ansay, 1999).

However, Sage and Ingham (1998) suggested that the freezing and thawing steps of the beefburger production process would provide an additional safety margin against *E. coli* O157:H7 infection by killing a proportion of the cells present. The effects of the rate of beef trimming freezing and frozen storage of the beef burgers on *E. coli* O157:H7 survival were therefore investigated.

THE EFFECT OF FREEZING AND FROZEN STORAGE ON THE SURVIVAL OF *E. COLI* O157:H7

Bacterial cells may be injured during freezing (Mossel and Netten, 1984; Ray, 1986; Musarrat and Ahmad, 1988). Two different freezing regimes were tested in order to investigate *E. coli* O157:H7 destruction during the freezing of beef trimmings. The latter were inoculated with *E. coli* O157:H7 and frozen slowly to –18°C over a time period of 36 hours as is current practice. Similar samples were also frozen to the same temperature within 30 minutes. The slower freezing regime showed a similar result to that achieved above (Table 1) while faster freezing effected a greater, but statistically insignificant ($P > 0.05$) reduction in the numbers of organisms (Table 2).

The effect of frozen storage on the survival of *E. coli* O157:H7 in beefburgers was also investigated. Beefburgers were prepared in a manner similar to the commercial process using beef trimmings and then inoculated with the pathogen. Burgers containing approximately 2.7 $\log_{10}$ cfu/g (recipe 1) and 2.5 $\log_{10}$ cfu/g (recipe 2) *E. coli* O157:H7 per gram were frozen to –18°C and stored for 2 months. The decrease in pathogen numbers was statistically insignificant ($P > 0.05$) (Table 3). Doyle and Schoeni (1984) reported a similar change in *E. coli* O157:H7 levels after 2 months storage of beefburgers at –20°C. Freezing and frozen storage are undertaken to prevent proliferation of pathogenic and spoilage bacteria but are not effective in destroying *E. coli* O157:H7 already present in the raw materials and in the product. These findings agree with those of Heuvelink *et al.* (1999) who concluded that raw meat contaminated with *E. coli* O157:H7 will remain a hazard even if the meat is stored at freezing temperatures.

Table 1. The numbers of added *E. coli* O157:H7 surviving after each stage during beefburger manufacture.

Stage	Numbers of *E. coli* O157:H7 (log_{10}) per gram of beefburger				
Recipe/tempering method	R1M[1]	R1T[2]	R2M[3]	R2T[4]	Average
Inoculation	3.1	3.4	3.1	3.4	3.3
Frozen (36 hours to –18°C)	2.9	3.2	2.9	3.2	3.1
Tempered to –3°C	3.0	3.2	3.0	3.2	3.1
Mincing, mixing & forming	2.6	2.9	2.8	3.1	2.9
Rapid freezing to –18°C	2.5	2.7	2.8	3.1	2.8

R1M[1] = recipe 1 with tempering using a microwave
R1T[2] = recipe 1 with tempering using a tempering room
R2M[3] = recipe 2 with tempering using a microwave
R2T[4] = recipe 2 with tempering using a tempering room

Table 2. The effect of rate of freezing on the survival of added *E. coli* O157:H7 on beef trimmings

Rate of freezing	Numbers of *E. coli* O157:H7 (log_{10}) per gram of beef trimming
Control (untreated)	3.1
Slow (-18°C in 36 hours)	2.9
Fast (-18°C in 30 minutes)	2.6

Table 3. The effect of frozen storage on the survival of added *E. coli* O157:H7 in uncooked beefburgers

	E. coli O157:H7 (log_{10}) per gram of beef trimming	
	Recipe 1	Recipe 2
Control	2.7	2.5
After 2 months storage	2.6	2.2

THE EFFECT OF SODIUM LACTATE IN BEEFBURGERS ON THE SURVIVAL OF *E. COLI* O157:H7 DURING BEEFBURGER MANUFACTURE AND THERMAL PROCESSING

Sodium lactate, the sodium salt of lactic acid, is naturally present in beef and has GRAS (generally regarded as safe) status from the US Food and Drug Administration. In the EU, lactic acid and lactic acid derivatives may be added to foodstuffs in general 'quantum satis'. This means that no maximum level is specified (Lamers, 1996).

This salt, which has specific anti-microbial properties against *E. coli* O157:H7 (Miller and Acuff, 1994), was added (4%, w/w) to each burger recipe prepared using beef trimmings inoculated with *E. coli* O157:H7. There was no significant decrease in pathogen count with recipe 1, but a statistically significant 1.8 log reduction was obtained in burgers prepared using recipe 2 ($P < 0.05$) (Table 4).

When the thermal resistance of the pathogen was examined in beefburgers with and without sodium lactate it was discovered that this salt also enhanced the killing effect of heat. The D-values (time required to effect a 90% reduction in *E. coli* O157:H7 numbers) at 50, 55 and 60°C decreased significantly ($P < 0.05$) (Table 5).

Therefore, the inclusion of 4% sodium lactate in beefburgers could provide some protection against *E. coli* O157:H7, directly through formulation and indirectly by reducing the thermostability of the organisms thereby increasing the safety margin during cooking. However, at concentrations above 2.4%,

Table 4: The effect of sodium lactate on the survival of added *E. coli* O157:H7 in raw beefburgers

Treatment	*E. coli* O157:H7 (log_{10} cfu/g) Recipe 1[1]	Recipe 2[2]
Control	4.9	5.5
Control plus NaL (4%)	4.4	3.7

1 Recipe 1 = 100% beefburger

2 Recipe 2 = beef, rusk, seasoning, frozen onion, salt & soya concentrate

Table 5. The effect of sodium lactate on the thermal resistance of *E. coli* O157:H7 in beefburgers

	D-value (minutes)			
	Recipe 1		Recipe 2	
Temperature of cooking	0% sodium lactate	4% sodium lactate	0% sodium lactate	4% sodium lactate
50°C	151	69	185	57
55°C	13	10	11	7.6
60°C	2.6	1.1	3.5	2.3

sodium lactate may give a salty flavour to food products. This aspect requires research before these findings can be applied commercially.

THE EFFECT OF SODIUM LACTATE, LACTIC ACID AND CITRIC ACID IN BEEFBURGERS ON THE SURVIVAL OF *E. COLI* O157:H7

Although a reduction was obtained with sodium lactate in recipe 2 burgers, it is desirable that ingredients specifically added for bacteriocidal purposes are not dependent on synergistic interactions with other ingredients as there is a large variation in the latter and in the recipes commercially used. Indeed, the

most commonly used recipe contains only beef trimmings. The research therefore focused on reducing *E. coli* O157:H7 in beefburgers made from recipe 1 (beef trimmings only).

In addition to sodium lactate (4% w/w), lactic acid (2%, v/v) and citric acid (0.5%, v/v) are also GRAS substances and are known to effect the destruction of bacterial pathogens in food. Inoculated beefburgers were prepared as before, but sodium lactate (4% w/w), lactic acid (2% v/w) and citric acid (0.5% v/w) were added during mixing. These were frozen to a core temperature of –18°C within 10 minutes as is currently the case in commercial manufacture. The effect on *E. coli* O157:H7 was minimal and not statistically significant (Table 6). The addition of these acids to burgers therefore conferred no food safety advantage.

Table 6: The effect of sodium lactate, lactic acid and citric acid on the survival of *E. coli* O157:H7 in uncooked beefburgers

Treatment	*E. coli* O157 counts (log_{10} cfu/ml)
Control	7.3
Sodium lactate	6.8
Lactic acid	7.3
Citric acid	6.7

THE EFFECT OF PULSED ELECTRIC FIELDS (PEF) ON THE SURVIVAL OF *E. COLI* O157:H7 IN BEEFBURGERS

Pulsed electric field pasteurisation is a promising technique for non-thermal food preservation. A trans-membrane voltage is induced across the bacterial cell membrane which induces increased permeability. When the voltage applied exceeds approximately 1 volt the bacterial cell membrane is damaged

(Sale and Hamilton, 1967). In liquid media, bacteria like *E. coli* O157 are readily destroyed by PEF treatment (Zhang *et al.*, 1995; Dutreux *et al.*, 2000). This technology was successfully applied to apple juice (Evrendilek *et al.*, 1999) and liquid eggs (Martin-Belloso *et al.*, 1998) but has not been tested in solid foods such as beefburgers.

Using a custom build PEF unit, inoculated beefburgers prepared using recipe 1 were treated with varying numbers of 40kV pulses of electricity. Regardless of the number of pulses, this treatment had no effect on the survival of added *E. coli* O157:H7 levels (Table 7).

Table 7: The effect of PEF on the survival of added *E. coli* O157:H7 in uncooked beefburgers

Number of pulses	Beefburgers *E. coli* O157:H7 ($\log_{10}$ cfu/g)
0 (Control)	8.0
10	7.7
100	7.9
500	7.9
1000	7.8
5000	8.2

The effectiveness of PEF in the destruction of bacteria is dependent on a number of factors including the chemical composition and electrical resistivity of the food or medium. The ineffectiveness of PEF in the destruction of *E. coli* O157:H7 in beefburgers was attributed to the high protein and lipid concentration in the beef, both of which increase microbial resistance to electrical pulses.

THE EFFECT OF FREEZING, LACTIC ACID AND PULSED ELECTRIC FIELDS (PEF) ON THE SURVIVAL OF *E. COLI* O157:H7 ON FILTER PAPER AND BEEF TRIMMINGS

While the PEF treatment did not destroy *E. coli* O157:H7 it was possible that the permeability of the cell membranes was increased which would facilitate entry of anti-microbial agents into the cells. *E. coli* O157:H7 cells were inoculated onto beef trimmings which were subsequently used to prepare burgers using recipe 1 to which sodium lactate (4% w/w), lactic acid (2% v/v) or citric acid (0.5% v/v/) were added. The additional hurdle of freezing was also added. These treatments, however, did not significantly reduce *E. coli* O157:H7 levels (Table 8).

Given the importance of the medium in which the bacterial cells are suspended on the effectiveness or otherwise of PEF, it was decided to repeat the above experiment using the combinations of lactic acid, PEF and freezing against *E. coli* O157:H7 cells spray inoculated onto filter paper. Once again, the individual treatments were ineffective and there was no significant difference between *E. coli* O157:H7 counts before and after treatment with lactic acid, PEF or freezing (Table 9). Lactic acid and PEF were similarly ineffective but the combinations of lactic acid and freezing and lactic acid, PEF and freezing both gave an approximate 6 $\log_{10}$ cfu/ml reduction which was statistically significant ($P < 0.05$).

Table 8: The effect of lactic acid, sodium lactate and citric acid as beefburger ingredients with PEF and freezing treatments on the survival of added *E. coli* O157:H7 in uncooked burgers

Treatment	*E. coli* O157: H7 counts ($\log_{10}$ cfu/g)
Control	7.1
Sodium lactate & PEF & freeze	6.7
Lactic acid & PEF & freeze	6.9
Citric acid & PEF & freeze	6.5

Table 9: The effect lactic acid in combination with PEF and freezing treatments on the survival of *E. coli* O157:H7 spray inoculated onto filter paper

Treatment	*E. coli* O157: H7 counts (log_{10} cfu/ml)
Control	7.3
Lactic acid	6.2
PEF	6.5
Freeze	6.7
Lactic acid & PEF	6.5
Lactic acid & freeze	1.4
Lactic acid & PEF & freeze	1.6

These findings could best be applied at the start of the commercial beefburger manufacturing process. *E. coli* O157:H7 contamination on beef trimmings is restricted to the surface, unlike burgers, where bacterial cells are mixed into the product and may lie in the core and thus be protected by surrounding protein and lipids. Beef trimmings were therefore spray inoculated with *E. coli* O157:H7 and treated with lactic acid, citric acid, PEF and freezing individually and in combination. On beef trimmings there was no significant reduction in *E. coli* O157:H7 levels (Table 10). This may be due to absorption of the anti-microbials into the beef (Cutter, 2000) which are therefore unavailable for anti-microbial activity.

Table 10: The effect lactic acid and citric acid in combination with PEF and freezing treatments on the survival of *E. coli* O157:H7 spray inoculated onto beef trimmings

Treatment	*E. coli* O157: H7 counts (log_{10} cfu/ml)
Control	7.3
Lactic acid	7.4
Citric acid	6.8
PEF	6.9
Freeze	6.7
Lactic acid & PEF & freeze	6.9
Citric acid & PEF & freeze	6.5

CONCLUSIONS

- The currently used beefburger manufacturing process of freezing, tempering, deboxing, breaking, mixing, mincing, forming, freezing and frozen storage does not provide protection against the threat of *E. coli* O157:H7
- Freezing and frozen storage does not effect a reduction in *E. coli* O157:H7 levels
- Sodium lactate will effect an approximate 2 log_{10} cfu/g reduction in *E. coli* O157:H7 levels in recipe 2 burgers and significantly reduce the thermal resistance of the organism. This warrants further investigation.
- The incorporation of sodium lactate, lactic acid or citric acid during formulation will not reduce *E. coli* O157:H7 in the beefburgers
- Pulsed Electric Field (PEF) is similarly unsuitable as a treatment to reduce the risks associated with of *E. coli* O157:H7 in beefburgers
- While the combination of lactic acid treatment and freezing was effective against the pathogen, spray inoculated onto filter paper, the

action against the *E. coli* O157:H7 is lost when the paper is replaced by beef

RECOMMENDATIONS TO INDUSTRY

- The microbial quality of beefburgers is wholly determined by the microbial quality of the beef raw materials used (Gill *et al.*, 1996). Hazard Analysis and Critical Control Point (HACCP) implementation during beef slaughter, as detailed in 'HACCP for Irish Beef Slaughter' (Bolton *et al.*, 2000) is currently the most effective means of ensuring that the raw materials used to manufacture beefburgers are free of *E. coli* O157:H7. Beefburger manufacturers should only accept beef trimmings from beef plants which have effective, verifiable HACCP systems in operation.
- The application of heat during cooking is still the only means of destroying *E. coli* O157:H7 in beefburgers. Beefburger packaging should contain advice on handling and cooking (stating that the core must be heated to a minimum temperature of 70°C for at least 2 minutes).

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