## Heat resistance of *Escherichia coli* O157:H7 in foods

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### ABSTRACT

Studies in laboratory media have demonstrated that when heated cells of *E. coli* O157:H7 are recovered in anaerobic conditions, their measured heat resistance can be up to ten-fold higher than when they are recovered in aerobic conditions. These findings have important implications for the safety of minimally heat-treated foods that are packed under vacuum or an anaerobic atmosphere. We have now assessed these implications. A number of different foods were prepared both aerobically (under an atmosphere of 20% oxygen, balance nitrogen) and anaerobically (under an atmosphere of 10% hydrogen, balance nitrogen). The foods tested included meat, poultry and vegetables. Cells of *E. coli* O157:H7 were inoculated into the foods and defined heat treatments given. Recovery and subsequent growth took place in the food. Decimal reduction times (D-values) were determined using the end point method. In most cases, measured D-values were higher in anaerobically prepared food than in the same food prepared aerobically. A protective effect of some foods was also noted.

### INTRODUCTION

Vero cytotoxin-producing *Escherichia coli* first came to prominence in 1982 as the causative agent in outbreaks of bloody diarrhoea linked to a fast food restaurant chain in the USA (Riley *et al.*, 1983). Since then strains of serotype O157:H7 have been implicated in several outbreaks. Suspected foods include undercooked hamburgers, pasteurised milk, cooked meat and gravy, apple juice and school dinners (Smith, 1997). Many of the reported outbreaks implicate foods that had undergone a heat treatment. The heat resistance of *E. coli* O157:H7 in foods is therefore of paramount importance. Studies have been made of the heat resistance in laboratory media, ground beef and chicken, and apple juice. One particularly interesting feature is the observation that the measured heat resistance of *E. coli* O157:H7 is considerably increased when cells are recovered in anaerobic (reduced) conditions rather than in aerobic (oxidised) conditions (Murano and Pierson, 1992, 1993; Czechowicz *et al.*, 1996; Bromberg *et al.*, 1998; George *et al.*, 1998). The aim of this work was to examine the heat inactivation of *E. coli* O157:H7 in foods prepared under anaerobic or aerobic gas mixtures. Beef, chicken, and mushrooms were tested and heat treatments were carried out at 59° and 61°C. The number of cells surviving the heat treatment and capable of growth in the food were measured and approximate D-values calculated using the end point method.

### METHODS

**Food preparation**: The food was cooked with water, then blended to a smooth puree with more water to a final concentration of up to one part water to one part food. Glucose (0.2%) was added to the beef and chicken to encourage gas production. The purees were divided into two and heated in a boiling water bath for 15 min to drive off air. Anaerobic (10%$H_2$: balance $N_2$) or aerobic (20%$O_2$: balance $N_2$) gas was then bubbled through the puree whilst cooling on ice. The foods were dispensed by pipetting 20 ml lots under the appropriate gas mixture into tubes flushed out with gas and closed with rubber septa and aluminium seals before sterilising at 121°C for 15 min. The foods tested were frozen minced beef, fresh chicken breast and fresh mushrooms. Laboratory medium was also tested (George *et al.*, 1998).

**Inoculum**: A 24 h culture of *E. coli* O157:H7 strain 204-P, was serially diluted in peptone salt dilution fluid to give $10^3$ to $10^9$ cells $ml^{-1}$ estimated by viable counts on aerobic TSA plates. Sets of five replicate tubes of both aerobic and anaerobic media were inoculated with 0.1 ml of each of three or more dilutions.

**Heat Treatment**: Tubes were immersed in a waterbath pre-heated to the test temperature and heated for up to 27 min at 59°C and up to 2 min at 61°C. At the end of the heating time the tubes were plunged into a second waterbath filled with iced water, and agitated to cool quickly to 30°C. Temperatures were logged as described by Fernández and Peck (1997) and the equivalent heating time at temperature was calculated. Tubes were then incubated at 30°C for two weeks.

**Detection of growth**: The number of survivors capable of growth in the foods was measured by tubes showing growth as judged by gas production, and calculated from 5-tube Most Probable Number tables. Approximate D-values were calculated using the equation: $D = t/(\log N_0 - \log N)$, where t is the equivalent time at temperature, $N_0$ is the inoculum level calculated from the viable count on aerobic plates, and N is the most probable number (MPN) of survivors calculated from the number of tubes showing growth after two weeks at 30°C. D-values are the mean of two experiments.

**Measurement of redox potential, pH and headspace composition**: Redox potentials of the foods were measured by positioning the electrode tip approximately 10 mm below the surface of the food for top measurements and as deep as possible (60-80 mm below the surface) for bottom measurements. The headspace was flushed with either aerobic or anaerobic gas during measurement. The pH was also measured under a flow of gas. Measurements were taken from three tubes of each food under each gas atmosphere. The oxygen concentration of the gas headspace in the food tubes was measured using a portable oxygen meter (Portamap 1, Systech Instruments, UK.). Samples were measured from up to five tubes of each food prepared under both gas mixtures.

## RESULTS AND DISCUSSION

The oxygen concentration in the headspace of the food tubes after autoclaving was 0.9 - 1.7% in the tubes containing foods prepared anaerobically and 10.4 - 12.0% in the tubes containing aerobically produced foods. With the exception of the aerobic beef medium, the pH of the foods was 6.1 or above. Redox potential measurements show that beef, chicken and mushroom were all very reduced at the bottom of the tubes even when prepared under 20% $O_2$. Thus, even when the three foods were prepared "aerobically" they remained at least partially reduced, as judged by the low redox potential at the bottom of the tubes (Table 1).

There is some variation in the D-values obtained in different foods. Beef and chicken provided considerable protective effect, whilst a smaller protective effect of mushroom was noted (Table 1). These confirm observations made previously.

In general terms, cells of *E. coli* O157:H7 appeared more heat resistant when they were heated and recovered in foods prepared "anaerobically" compared with foods prepared "aerobically". This was particularly clear in mushroom, TSYGB and in beef heated at 61°C. A failure to observe the effect in all circumstances may be related to difficulties in producing foods that are consistently "aerobic", that is of a high redox potential.

Table 1. Approximate D-values of E.coli O157:H7 in foods prepared aerobically and anaerobically

| Food | | Headspace $O_2$ (%v/v) | pH | Redox potential (mV) top | Redox potential (mV) bottom | D-value (min) 59°C | D-value (min) 61°C |
|---|---|---|---|---|---|---|---|
| beef | aerobic | 5.4 | 5.6 | -138 | -164 | 5.4 | 0.8 |
| | anaerobic | 0.9 | 6.1 | -221 | -208 | 5.3 | >1.3 |
| chicken | aerobic | 10.4 | 6.2 | +57 | -184 | 4.0 | 0.4 |
| | anaerobic | 1.7 | 6.1 | -337 | -326 | 3.7 | 0.5 |
| mushroom | aerobic | 12.0 | 6.5 | +95 | -134 | <2.6 | 0.4 |
| | anaerobic | 1.1 | 6.6 | -223 | -201 | 4.0 | 1.0 |
| TSYGB | aerobic | 12.0 | 6.6 | | +273 | 0.9 | 0.2 |
| | anaerobic | 0.8 | 6.4 | | -300 | 4.1 | 0.4 |

## CONCLUSION

Packaging of foods under a low oxygen atmosphere (either anaerobic gas or vacuum) is likely to increase the probability of *E. coli* O157:H7 surviving a mild heat treatment. Although most foods have a low redox potential which will encourage recovery compared with a high redox potential, removing oxygen from the package may further enhance this effect. Determining the heat resistance of *E coli* O157:H7 using aerobic plate counts may not give a realistic indication of the heat resistance in food. It is important that this is taken into consideration when the effectiveness of a heat-treatment is assessed.


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