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Effect of different storage conditions on *E. coli* O157:H7 and the indigenous bacterial microflora on lamb meat

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Abstract

Lamb chops inoculated with 2.23–2.83 log cfu/g of *E. coli* O157:H7 strain NCTC 12900 were packed in air (AP), vacuum (VP), and two modified atmospheres (MAP) consisting of 100% CO_2 and a commercial mixture of 35% CO_2/35% O_2/30% N_2. All samples (initial total counts <3.5 log cfu/g) were stored in a commercial cold storage facility set at 4 °C and one AP trial also at 12±1 °C in a temperature controlled incubator. Pathogen and indigenous flora evolution, physicochemical and sensory changes, surface packages temperature and MAP gas composition were monitored throughout the lamb meat shelf life. Temperature monitoring revealed that during chilled storage packed chops exceeded 7 °C about 3% of the time for periods of 10–20 min at 6 h intervals corresponding to defrosting cycles. In AP samples under these conditions, the *E. coli* O157:H7 strain had an overall increase of 0.48 log cfu/g by day 12. This increase, which may be regarded as an artefact of the sampling procedure, might also be a response to fluctuating temperatures. Regardless of rapid proliferation of the background microflora on AP lamb meat kept at 12±1 °C, the pathogen significantly increased by 2.35 log cfu/g after nine days. There was a slight decrease (0.20 log cfu/g) of the pathogen numbers after four weeks cold storage in VP despite a significant increase in lactic acid bacteria (LAB). With a relatively small outgrowth of LAB, chilled storage in 100% and 35% CO_2 resulted in significant differences compared to similar conditions in air (decrease from initial numbers of 0.80 and 0.45 log cfu/g, respectively). Our data confirm the importance of effective temperature control to prevent pathogen growth on raw meat and also that contaminated meat remains hazardous regardless of refrigeration and protective packaging. Further studies are needed to determine the behaviour of *E. coli* O157:H7 at temperatures that fluctuate around the minimum for growth.

Keywords: *E. coli* O157:H7; Lamb meat; Modified atmosphere packaging

1. Introduction

E. coli O157:H7 was first recognised as a human pathogen in 1982 (Wells et al., 1983). Since then, this serotype has been identified in many countries as the predominant cause of haemorrhagic colitis and subsequent severe and sometimes fatal conditions, haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Because of the severity of these illnesses and the apparent low infective dose (<100 cells, Strachan et al., 2005), *E. coli* O157:H7 is considered one of the most serious of known food borne pathogens (Meng et al., 2001; Blanco et al., 2003).

Cattle are thought to be the main source of *E. coli* O157:H7 and bovine products have often been implicated in food borne infections. Small ruminants have been subjected to fewer epidemiological surveys than cattle but available data suggest that sheep also may represent a source of transmission of this bacterium to humans (Kudva et al., 1996; Chapman et al., 2001; Meng et al., 2001; Blanco et al., 2003; Rey et al., 2003).

Whilst the behaviour of *E. coli* O157:H7 on beef stored in air and under vacuum (VP) or modified atmospheres (MAP) has received considerable attention, similar information on lamb meat is scant. Beef and lamb are both red meats but some differences are apparent between them. Thus, many of the muscles of lamb carcasses tend to have an ultimate pH value higher than 5.8. Moreover, there is a layer of fat over most superficial muscle surfaces. As a result lamb stored in air or under oxygen-reduced conditions is considered more conducive

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Table 1
Sampling days for each packaging regime and storage conditions

Packaging regime	Storage condition	Sampling days
Air	Cold room set at 4 °C	0–2–5–7–9–12
Air	12±1 °C	0–2–5–7–9
Vacuum	Cold room set at 4 °C	0–7–14–21–28
100% CO_2	Cold room set at 4 °C	0–2–5–7–9–12–14
Commercial mixture 35% CO_2/35% O_2/30% N_2	Cold room set at 4 °C	0–2–5–7–9–12–14

to growth of *Brochothrix thermosphacta* and psychrotrophic Enterobacteriaceae than beef (Dainty and Mackey, 1992; Prieto et al., 1993; Gill, 1995; ICMSF, 1998).

Pathogen behaviour on raw meats, as with many other foods, is controlled by several interrelated factors such as temperature of storage, atmosphere composition and competitive microflora (ICMSF, 1998). The current assumption is that *E. coli* O157:H7 does not multiply below 7 °C, which is the endpoint generally accepted by the EU as an appropriate temperature to prevent mesophilic pathogen growth (Anon, 2004). However, conditions at both retail and wholesale level can provide an opportunity for numbers to increase as a result of temperature abuse or temperature fluctuations (ICMSF, 2002). In reduced oxygen packaging, an additional concern is the effect of competitive spoilage flora and the lengthened storage life on survival and growth of *E. coli* O157:H7 (Tamplin, 2002).

Spain is one of the largest EU lamb producers (234,912 tonnes in 2005) with a *per capita* consumption of *ca.* 6 kg per year (FAOSTAT, 2004). In current practice, whole fresh lamb carcasses or vacuum packed primal cuts are delivered to retailers and caterers. At the retail level, lamb meat is sold in traditional butcher shops or in supermarkets in overwrapped trays or as MAP meat.

This study was aimed at determining the effect of different storage conditions on growth of *E. coli* O157:H7 on lamb meat.

2. Materials and methods

2.1. Organism used for inoculation

The verocytotoxin-negative strain *E. coli* O157:H7 NCTC (National Collection of Type Cultures, Colindale, London, UK) 12900 was employed throughout this study. The stock culture was maintained at −40 °C in Brain Heart Infusion broth (BHI, Oxoid, Basingstoke, UK) containing 20% (v/v) glycerol (Panreac, Barcelona, Spain).

2.2. Growth characteristics of the inoculum organism

To assess whether the growth characteristics of the inoculum bacterium were similar to those reported for verotoxigenic *E. coli* O157:H7 strains, BHI (Oxoid) was inoculated with *ca.* 10^5 cfu/ml. Aliquots (400 µl) were dispensed into microtiter plate wells and absorbance at 580 nm was measured for 48 h using a Bioscreen C instrument fitted with Biolink software (Labsystems Co., Helsinki, Finland). Growth rates were determined at 30 and 37 °C with NaCl concentrations between 0.5 and 6% and initial pH values between 3.5 and 6.5.

The growth characteristics of the strain *E. coli* O157:H7 NCTC 12900 were closely similar to those reported for mixes of three/four verotoxigenic *E. coli* O157:H7 strains of human and meat origin (Buchanan et al., 1993; Sutherland et al., 1995), the optimal conditions being: 0.5% NaCl, pH 6.5 and 37 °C.

2.3. Meat preparation and spiking

Twenty-four hours *post-mortem* lamb carcasses (8–10 kg) were collected at a local abattoir and transported to the laboratory under refrigeration in sterile plastic bags. Carcasses were fabricated into primal cuts and rib, loin and sirloin chops (50–60 g) were obtained under aseptic conditions.

Before inoculation or spiking, strain *E. coli* O157:H7 NCTC 12900 was grown three times under optimal conditions at 24 h intervals in BHI (Oxoid). The resulting stationary-phase culture was subsequently diluted in 0.1% peptone saline (Oxoid) solution (pH 6.5) to levels appropriate for inoculation onto the lamb meat (2–3 log cfu/g). For each packaging regime, temperature and sampling day (Table 1), 0.5 ml volumes were evenly distributed over each one of the two surfaces of five chops (four for microbiological analysis and one for the remaining analyses) and the packaging was completed within 1 h. For each trial packaging, five un-inoculated control chops were randomly selected and tested for the presence of *E. coli* O157:H7 as described below.

Table 2
Changes in numbers of inoculated *E. coli* O157:H7, naturally occurring flora, pH values, extract release volume (ERV) and L-lactic content during aerobic storage of lamb chops held in a cold room set at 4 °C

Day	*E. coli* O157:H7	APC [a]		*Pseudomonas*	*B. thermosphacta*	LAB	Coliforms	Enterobacteriaceae	pH	ERV (ml)	L-lactic acid (%)
		30 °C	7 °C								
0	2.83±1.13	3.25±0.51	3.14±0.65	2.54±1.62	2.79±0.26	2.67±1.69	2.93±0.66	3.02±0.98	5.93±0.06	19.83±2.84	0.483±0.072
2	3.14±0.71	4.94±0.34	4.60±0.22	4.31±0.44	3.93±0.41	3.76±0.56	3.27±0.58	3.67±0.75	5.95±0.05	13.67±4.04	0.413±0.078
5	3.23±0.76	5.94±0.33	5.86±0.67	5.79±0.81	4.41±0.67	4.31±0.74	3.96±0.79	4.23±0.46	5.99±0.10	10.67±1.15	0.363±0.089
7	3.23±0.44	7.02±0.49	7.19±0.75	6.84±0.78	5.10±1.06	4.21±0.46	4.43±0.74	4.61±0.44	6.08±0.15	8.17±1.44	0.283±0.045
9	3.25±0.51	7.57±0.44	7.76±0.63	7.33±0.35	5.83±1.34	4.81±0.44	4.72±0.51	5.02±0.67	6.19±0.01	6.83±1.89	0.203±0.057
12	3.31±0.42	7.82±0.31	7.89±0.65	7.63±0.42	6.46±0.52	5.22±0.33	4.28±0.89	5.47±0.78	6.26±0.14	6.12±0.02	0.183±0.066

Mean±standard deviation.
[a] Aerobic plate counts (log cfu/g).

Table 3
Changes in numbers of inoculated *E. coli* O157:H7, naturally occurring flora, pH values, extract release volume (ERV) and L-lactic content during aerobic storage of lamb chops held at 12±1 °C

Day	*E. coli* O157:H7	APC[a]		*Pseudomonas*	*B. thermosphacta*	LAB	Coliforms	Enterobacteriaceae	pH	ERV (ml)	L-lactic acid (%)
		30 °C	7 °C								
0	2.80±1.10	3.46±0.53	3.23±0.68	2.64±1.30	2.72±0.26	2.63±0.69	2.90±0.65	3.12±0.88	5.91±0.06	20.73±2.64	0.483±0.082
2	3.44±0.71	5.24±0.34	5.16±0.22	5.11±0.44	5.23±0.41	4.16±0.56	3.56±0.44	4.21±0.63	5.96±0.05	10.67±4.04	0.403±0.073
5	4.23±0.76	7.54±0.33	7.26±0.67	6.19±0.81	6.11±0.67	4.81±0.74	4.56±0.59	5.66±0.39	6.11±0.10	8.77±1.15	0.283±0.072
7	4.33±0.44	8.02±0.49	7.89±0.75	7.84±0.78	6.92±1.06	5.21±0.46	5.43±0.74	6.93±0.34	6.28±0.15	6.17±1.44	0.153±0.037
9	5.15±0.51	8.97±0.44	8.76±0.63	8.53±0.35	7.83±1.34	5.81±0.44	6.43±0.39	7.23±0.39	6.59±0.01	6.13±1.89	0.088±0.076

Mean±standard deviation.
[a] Aerobic plate counts (log cfu/g).

2.4. Packaging and storage conditions

For aerobic storage (AP), inoculated chops were individually placed in expanded polystyrene trays and overwrapped with oxygen permeable (6000–8000 cm^3 m^{-2} 24 h^{-1}) polyvinylchloride film (Wrap Film Systems Ltd., Shropshire, UK). The overwrapped trays were then placed side-by-side into polypropylene trays (26 by 16 by 6.4 cm) and held for 9 d at above chill temperatures (12±1 °C) in a Heraeus low temperature incubator (Heraeus S.A./Controltecnica instrumentación científica S.L., Madrid, Spain) or for 12 d in a commercial cold storage room (5 m^3) (Chiloverg, León, Spain) set at 4 °C.

For VP experiments, inoculated chops were individually placed on Cryovac U-BRT trays in Cryovac BB4L bags (Cryovac, Barcelona, Spain), which were immediately evacuated and sealed using a tabletop Multivac A300 packaging machine (Multivac Verpackungsmaschinen, Wolfertschwenden, Germany). For MAP assays, the air in the bag was replaced by 100% CO_2 or a commercial mixture containing 35% CO_2/35% O_2/30% N_2 (Carburos Metálicos S.A., Barcelona, Spain). The BB4L bags had an oxygen transmission rate of 30 cm^3 m^{-2} 24 h^{-1} at 23 °C and 0% relative humidity (RH) and a CO_2 transmission rate of 150 cm^3 m^{-2} 24 h^{-1} at 23 °C and 0% RH. Following VP and MAP, packages were kept in the cold storage room, as described above, for 28 and 14 d, respectively.

Throughout cold storage, both air and displayed tray temperatures were simultaneously monitored using a Testo175-T2 (Instrumentos Testo S.A., Cabrils, Barcelona, Spain) data logger with internal sensor and external probe programmed to read every 5 min with an accuracy of 0.2 °C. Data were downloaded to a computer and exported to an Excel spreadsheet for further analysis and display.

2.5. Microbiological analysis

For each sampling day (Table 1), microbiological testing was conducted for each trial using four inoculated samples of meat and duplicate plates for each dilution. Before opening, the outside surface of each pack was wiped with an alcohol swab and the concentrations of CO_2 and O_2 were determined in the MAP trays using an Oxy-Check 8003 gas analyser (Temac Instruments, Copenhagen, Denmark).

After pack opening, each lamb chop was aseptically transferred to sterile Stomacher bags (Seward Medical, London, UK) containing sufficient 0.1% (w/v) peptone (Oxoid) solution to make a 1:5 dilution. Samples were then repeatedly squeezed by hand for 2 min and the rinse fluid was serially ten-fold diluted in peptone solution. *E. coli* O157:H7 viable cells were enumerated on plates of sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC, Oxoid), incubated at 37 °C for 24 h. Aerobic plate counts (APC) were determined on Plate Count agar (Oxoid) incubated at 30 (mesophilic aerobic plate counts) and 7 °C (psychrotrophic counts) for 2 and 10 d, respectively. *Pseudomonas* numbers were determined, after 2 d incubation at 25 °C, on *Pseudomonas* agar base (Oxoid) to which CFC (cetrimide, fucidin, cephaloridine; Oxoid) supplement was added. From each set of countable plates, five colonies were randomly selected, streaked to purity on tryptone soy agar (TSA, Oxoid) and examined for Gram-reaction, cell morphology, motility, oxidase and catalase

Table 4
Changes in numbers of inoculated *E. coli* O157:H7, naturally occurring flora, pH values, extract release volume (ERV) and L-lactic content during storage under vacuum of lamb chops held in a cold room set at 4 °C

Day	*E. coli* O157:H7	APC[a]		*Pseudomonas*	*B. thermosphacta*	LAB	Coliforms	Enterobacteriaceae	pH	ERV (ml)	L-lactic acid (%)
		30 °C	7 °C								
0	2.72±0.91	3.41±0.63	3.08±0.72	2.58±0.46	2.80±0.47	3.07±0.43	2.83±0.88	2.92±0.96	5.86±0.04	23.67±2.52	0.496±0.061
7	2.56±0.75	4.28±1.21	4.29±1.01	3.81±1.03	4.29±1.49	3.52±0.32	4.31±0.65	4.46±0.85	5.93±0.04	20.17±1.61	0.432±0.043
14	2.46±0.39	6.23±0.85	6.06±0.11	4.31±1.01	5.42±0.57	4.84±1.05	5.09±0.96	5.36±0.39	5.95±0.03	16.59±0.87	0.448±0.056
21	2.53±0.34	6.85±1.17	7.11±0.33	4.42±0.95	6.55±0.68	5.76±1.04	5.23±0.74	6.73±0.64	5.98±0.11	18.17±1.04	0.457±0.067
28	2.52±0.39	7.17±039	7.39±0.69	4.64±0.75	7.18±0.63	6.68±0.64	5.15±0.65	6.23±0.79	6.01±0.16	14.40±2.42	0.436±0.034

Mean±standard deviation.
[a] Aerobic plate counts (log cfu/g).

Table 5
Changes in numbers of inoculated *E. coli* O157:H7, naturally occurring flora, pH values, extract release volume (ERV), and O_2 and CO_2 content during storage under 100% CO_2 of lamb chops held in a cold room set at 4 °C

Day	*E. coli* O157:H7	APC[a]		*Pseudomonas*	*B. thermosphacta*	LAB	Coliforms	Enterobacteriaceae	pH	ERV (ml)	O_2 (%)	CO_2 (%)
		30 °C	7 °C									
0	2.23±0.67	3.31±0.54	3.14±0.65	2.54±1.62	2.79±0.26	2.56±0.87	2.44±1.02	2.52±0.85	5.93±0.06	19.83±2.84		
2	2.05±0.55	3.44±0.64	3.74±0.22	3.21±1.44	2.93±0.41	2.76±0.06	2.23±0.71	2.66±0.75	5.85±0.05	15.67±4.04	3.13±0.61	88.47±6.41
5	1.52±0.59	3.84±0.30	3.86±1.17	3.37±0.91	3.41±1.17	3.01±0.64	2.49±0.86	2.26±0.49	5.87±0.10	11.47±2.15	3.70±0.56	83.23±3.41
7	1.93±0.66	4.20±0.69	4.49±0.65	3.58±0.98	3.10±1.46	3.31±0.46	2.63±0.34	2.69±0.24	5.89±0.11	11.17±1.44	3.37±0.47	84.27±3.55
9	1.74±0.49	4.37±0.64	4.76±0.73	4.03±0.30	3.63±1.44	3.51±0.44	2.55±0.25	2.83±0.19	5.83±0.05	9.83±1.89	3.70±0.80	79.90±4.16
12	1.67±0.95	4.82±0.11	4.86±0.65	3.70±0.32	4.06±0.72	4.01±0.23	2.41±0.10	3.13±0.49	5.84±0.04	11.00±0.02	2.85±0.64	86.55±1.34
14	1.43±0.71	4.93±0.32	4.96±0.75	3.97±0.53	4.66±0.87	4.71±0.38	2.32±0.26	3.43±0.49	5.82±0.13	11.55±0.02	2.95±0.25	84.55±1.15

Mean±standard deviation.
[a] Aerobic plate counts (log cfu/g).

reactions and anaerobic growth. *B. thermosphacta* was enumerated on streptomycin sulphate cycloheximide thallous acetate agar (STAA, Oxoid). *B. thermosphacta* presumptive colonies were differentiated from *Pseudomonas* by performing an oxidase test using Oxidase Touch Sticks (Oxoid). Lactic acid bacteria (LAB) were counted using overlaid plates of MRS agar (Oxoid) incubated at 35 °C for 5 d. Confirmatory presumptive tests for LAB were performed as described by González et al. (2000). Enterobacteriaceae counts were determined on overlaid plates of violet-red bile glucose agar (VRBGA; Oxoid) after incubation at 37 °C for 24 h. Coliforms were obtained on 3 M Petrifilm Coliform count plates (3 M Microbiology Products, St. Paul, MN, USA). Colonies were counted according to manufacturers' instructions following 24 h incubation at 37 °C.

Rinses of un-inoculated control chops were screened for the presence of naturally occurring *E. coli* O157:H7 by a culture method including an immunomagnetic separation (IMS) step with Dynabeds anti-*E. coli* (Dynal A. S., Oslo, Norway) according to the manufacturers' instructions. Briefly, the protocol consisted of a 10 ml rinse pre-enrichment (37 °C for 18 h) in 90 ml buffered peptone water (BPW, Oxoid) followed by IMS and subsequent plating onto CT-SMAC (Oxoid). Suspect colonies were directly streaked onto plates of a selective chromogenic agar (O157:H7 ID medium, bioMérieux España, Madrid, Spain). Sorbitol-negative strains that did not express β-glucuronidase were tested for phenotypic traits (Meng et al., 2001) and also inoculated into API 20E strips (bioMérieux).

2.6. Sensory evaluation

For each sampling day, one inoculated lamb chop was evaluated for both appearance and odour acceptability by a trained panel of five people. Appearance was assessed using a structured hedonic scale with numerical scores from 7 (excellent) to 1 (extremely undesirable). A mean value of 3.5 was considered the borderline of consumer acceptability. Odour acceptability was evaluated using a 3-point scale (1, off-odour; 2, neutral; 3, normal). A mean value of 1.5 was considered the borderline of acceptability. Shelf life was arbitrarily defined as the time in days to reach mean values of 3.5 or 1.5 on the appearance and odour scales, respectively (Greer and Murray, 1988; Land and Shepherd, 1988).

2.7. Physicochemical analysis

The mean pH value was determined from three measurements. Extract release volume (ERV) was measured as described by Kirk and Sawyer (1991). In brief, minced meat (15 g) was mixed with 60 ml of extraction reagent (50 ml 0.2 M KH_2PO_4 and 3.72 ml 0.2 M NaOH diluted up to 200 ml with distilled water; pH, 5.8) and homogenized for 2 min in a Sorvall Omni Mixer (Pacisa, Madrid, Spain). The homogenate was filtered through filter paper (Whatman No. 1, 18.5 cm diameter), the ERV being considered as the volume collected in 15 min.

The configuration and amount of L-lactic acid were determined enzymatically by using a commercial kit (ATOM, SA., Barcelona, Spain).

Table 6
Changes in numbers of inoculated *E. coli* O157:H7, naturally occurring flora, pH values, extract release volume (ERV), and O_2 and CO_2 content during storage under a commercial atmosphere mixture (35% CO_2/35% O_2/30% N_2) of lamb chops held in a cold room set at 4 °C

Day	*E. coli* O157:H7	APC[a]		*Pseudomonas*	*B. thermosphacta*	LAB	Coliforms	Enterobacteriaceae	pH	ERV (ml)	O_2 (%)	CO_2 (%)
		30 °C	7 °C									
0	2.49±0.29	3.44±0.32	3.33±0.68	3.25±1.03	2.45±0.66	2.71±0.13	2.63±0.62	2.74±0.76	5.91±0.07	18.17±2.47		
2	2.54±0.24	3.90±0.16	3.94±0.64	3.57±0.78	2.93±0.74	2.81±0.12	2.86±0.56	2.90±0.58	5.87±0.09	13.33±2.08	30.50±2.15	30.40±2.76
5	2.13±0.19	4.39±0.49	4.78±0.54	3.69±0.68	4.25±0.81	3.00±0.20	1.83±0.67	2.28±0.56	5.90±0.2	12.18±0.73	30.10±1.47	29.33±1.80
7	2.10±0.25	4.55±0.67	5.02±0.58	4.63±1.11	4.57±1.00	3.37±0.17	2.42±0.57	2.12±0.10	6.06±0.12	10.43±0.76	28.60±1.13	28.57±4.70
9	2.25±0.22	4.88±0.70	5.40±0.45	5.04±1.00	3.87±1.78	3.79±0.24	2.69±0.70	2.47±0.28	6.03±0.07	9.67±0.88	28.60±0.82	29.37±2.63
12	2.14±0.63	5.55±0.44	5.91±0.43	5.15±1.05	4.29±1.04	3.81±0.33	2.62±0.50	2.67±0.54	6.02±0.09	10.27±1.32	29.03±1.80	29.27±1.59
14	2.04±0.75	5.95±0.62	6.11±0.53	5.39±0.98	4.52±0.84	4.12±0.53	2.42±0.68	2.97±0.54	6.07±0.06	10.45±1.67	29.34±1.45	29.87±2.15

Mean±standard deviation.
[a] Aerobic plate counts (log cfu/g).

2.8. Statistical analysis

Microbiological and physicochemical data were statistically analysed (mean, standard deviation, variance and correlation coefficient) using the Statistica for Windows Release 5.5 software (Statsoft, Chicago IL, USA).

3. Results

3.1. Occurrence of E. coli O157:H7 in un-inoculated lamb meat

No *E. coli* O157:H7 was detected in lamb chops rinses before inoculation.

3.2. Temperature during cold storage

During AP (12 d), the air temperature of the cold room (evaluated from the internal sensor) ranged between 3.9 °C and 13 °C, the mean value being 4.14 °C±0.79 °C. Displayed trays surface temperatures (evaluated from the external probe) fluctuated between −1.1 °C and 17.2 °C, the mean value being 3.52±1.45 °C. Trays exceeded 7 °C for 2.69% of the time for periods of 10–15 min mainly at 6 h intervals during defrosting cycles. The average temperature above 7 °C was 9.50±1.96 °C.

During VP (28 d), the air temperatures of the cold room ranged between 3.9 °C and 13.3 °C, the mean value being 4.08 °C±0.52 °C. Packed chops surface temperatures ranged between −1.1 °C and 14.3 °C, the mean value being 3.53±1.65 °C. Packed chops exceeded 7 °C for 3% of the time for periods of 10–20 min at 6 h intervals during defrosting cycles. The average temperature above 7 °C was 10.34±1.93 °C.

During MAP (14 d), the air temperatures of the cold room ranged between 3.9 °C and 16.1 °C, the mean value being 4.12 C±0.73 °C. The surface of packed lamb chops temperatures fluctuated between −1.1 °C and 13.9 °C, the mean value being 3.51±1.49 °C. Packages exceeded 7 °C about 2.71% of the time for periods of 10–15 min mainly at 6 h intervals during defrosting cycles. The average temperature above 7 °C was 9.68±1.90 °C.

3.3. Behaviour of E. coli O157:H7 and the indigenous bacterial microflora on overwrapped lamb chops

Tables 2 and 3 show the initial numbers and evolution of *E. coli* O157:H7 strain NCTC 12900 and the autochthonous flora on inoculated lamb chops stored in air under refrigerated conditions and at 12±1 °C, respectively. Changes in ERV, pH values and L-lactic acid content are also given. Under refrigeration, an increase ($p>0.05$) of the *E. coli* O157:H7 strain was observed as the initial level of the organism rose by 0.48 log cfu/g during the 12 d holding period. At 12 °C, *E. coli* O157:H7 significantly increased ($p<0.05$) by 2.35 log cfu/g, after 9 d storage.

In both experiments, significant increases ($p<0.05$) were noted for the storage flora examined, with *Pseudomonas* spp.

followed by *B. thermosphacta* being the dominant microorganisms. As storage progressed, pH significantly ($p<0.05$) increased while ERV and L-lactic content significantly ($p<0.05$) decreased. For both appearance and odour, lamb chops spoiled when mean APC and *Pseudomonas* numbers were *ca*. 7.5 log cfu/g. At the last sampling day, all samples exhibited a visible slime layer.

3.4. Behaviour of *E. coli* O157:H7 and the indigenous bacterial microflora on vacuum packed lamb chops stored at refrigeration

Table 4 shows the behaviour of the *E. coli* O157:H7 strain and changes in the naturally occurring flora on inoculated vacuum packed lamb chops kept at refrigeration. Data on physicochemical changes are also given. After four weeks, samples were unacceptable because greening of drip and a sour odour was noticeable at pack opening. By that time, there was a small decrease (0.20 log cfu/g, $p>0.05$) in *E. coli* O157:H7 numbers. At the onset of spoilage, all bacterial groups had significantly increased although APC counts were significantly lower than those attained in air at refrigeration. *B. thermosphacta* followed by LAB were the dominant organisms.

3.5. Behaviour of *E. coli* O157:H7 and the indigenous bacterial microflora on MAP packed lamb chops stored at refrigeration

The change in numbers of *E. coli* O157:H7 strain and the background microflora on refrigerated lamb chops packed under 100% CO_2 and the 35% CO_2/35% O_2/30% N_2 mixture are shown in Tables 5 and 6, respectively. The profiles of the changes in pH values, ERV and CO_2 and O_2 concentrations are also given.

With APC levels of *ca*. 5 log cfu/g, lamb chops packed under 100% CO_2 (Table 5) became unacceptable because of discolouration. By this time, numbers of *E. coli* O157:H7 had significantly ($p<0.05$) decreased by 0.8 log cfu/g. When mean APC levels were close to 6 log cfu/g, samples packed under the commercial gas mix (Table 6) were considered unacceptable due to abnormal odours and deterioration of texture. This storage condition resulted in an *E. coli* O157:H7 reduction of 0.45 log cfu/g. In 100% CO_2, *B. thermosphacta* and LAB, which reached similar numbers, were the predominant microflora while in the commercial atmosphere, there was a mixed population of *Pseudomonas*, *B. thermosphacta* and LAB.

4. Discussion

E. coli O157:H7 inoculation numbers (2.23 to 2.83 log cfu/g) likely represent a much higher level of contamination than that naturally occurring on raw meat, but preliminary work revealed difficulties enumerating, by direct plating, lower pathogen inoculum numbers in the presence of increasing numbers of the background microorganisms (data not shown). The initial mesophilic counts, which resulted in numbers lower than 3.5 log cfu/g, did not exceed the *m*-value set by the EU for sheep carcasses after dressing (European Commission, 2005).

Although temperature is probably the most important extrinsic factor affecting the growth and viability of microorganisms, many studies describing the behaviour of *E. coli* O157:H7 and other mesophilic pathogens on/in inoculated foods do not provide information on monitoring temperature history nor on the real temperature of samples.

The minimum temperature for multiplication of *E. coli* O157:H7 is considered to be 7 °C (ICMSF, 1996, 2002) although Tamplin et al. (2005) reported that using sterile irradiated raw ground beef, growth occurred at 6 °C. Rapid carcass chilling and temperature control during refrigerated holding are critical control points that should prevent *E. coli* O157:H7 growth on raw meat (ICMSF, 2002). However, during commercial storage, temperatures may fluctuate and opportunities exist for temperature abuse. In this study, temperature monitoring revealed that throughout chilled storage our samples were regularly exposed (four times a day), during 10–20 min defrost cycles, to temperatures above 7 °C.

When inoculated lamb chops were stored at 4 °C under aerobic conditions, the *E. coli* O157:H7 strain had an overall increase of 0.48 log cfu/g by day 12. In non-sterile ground beef held at 4 °C for 14 d, Barkocy-Gallagher et al. (2002) also observed minor increases for six *E. coli* O157:H7 strains, which significantly increased (0.9–1.5 log cfu/g) at 7 °C over this period. Slight increases at 4 °C may be regarded as an artefact of the sampling procedures or, as did the above authors, so small that they may not reflect meaningful growth. However, studies on the behaviour of *E. coli* at temperatures below the minimum for growth with periodic fluctuations to temperatures above the limit for growth have shown that cells behave differently under constant compared to fluctuating temperatures. Thus, Jones et al. (2004) reported that some fractions of *E. coli* cells elongated during incubation at 2 and 4 °C, and some elongated cells subsequently divided at 4 °C when temperatures fluctuated at 6 h intervals, but not when temperatures were constant. For *E. coli* O157:H7, Rajkowski and Marmer (1995), reported that transitory abuse leads to more rapid growth than expected. *E. coli* O157:H7 growth at temperatures that fluctuate around the minimum for growth also appears to be influenced by test matrices. Tamplin et al. (2005) observed that ten strains of this serovar grew in sterile ground beef at 6 °C but not in BHI broth.

On aerobically stored lamb meat kept at the abusive temperature of 12±1 °C, *E. coli* O157:H7 strain NCTC 12900 significantly increased by 2.35 log cfu/g after nine days storage. This is in agreement with many studies on the fate of *E. coli* O157:H7 on inoculated raw meat held in the range 10–15 °C under aerobic conditions (Palumbo et al., 1997; Nissen et al., 2000; Vold et al., 2000; Berry and Koohmaraie, 2001; Li and Logue, 2005; Tamplin et al., 2005).

Along with temperature, atmosphere gas composition is a major environmental factor that can influence microbial growth. The simplest form of modified atmosphere packaging is VP. Here, levels of CO_2 rapidly rise to 10–20% as the food product and contaminating microorganisms consume residual O_2 and produce CO_2. VP is, therefore, a form of MAP with CO_2 (Farber and Dodds, 1995). In this study, four weeks cold storage in VP resulted in a slight decrease (0.20 log cfu/g) of pathogen numbers although final populations were lower than those attained after 12 d cold storage in air. Our results support those

reported by others, who found that numbers of *E. coli* O157:H7 on beef were basically unaffected during chilled storage in VP (Berry and Koohmaraie, 2001; Dykes et al., 2001; Uyttendaele et al., 2001; Logue et al., 2005).

Two gases, N_2 and CO_2 are commonly used in MAP, the latter being mainly responsible for the bacteriostatic effect on microorganisms. For fresh meat in which red colour maintenance is important, O_2 is generally used so that the formation of metmyoglobin, which gives a brown colour to the meat, will be retarded for a period of time (Farber and Dodds, 1995). During chilled storage of inoculated lamb chops in 100% CO_2 and the commercial atmosphere of 35% CO_2, the pathogen showed significant differences compared to similar conditions in air. On inoculated beef slices packed in 40% CO_2, Uyttendaele et al. (2001) obtained a 0.93 log cfu/g reduction throughout one week storage at 4 °C. At the abusive temperature of 10 °C, work done by Nissen et al. (2000) with ground beef stored in 30 and 60% CO_2 showed that growth of *E. coli* O157:H7 was almost totally inhibited regardless of CO_2 concentrations.

Our data suggest that VP or MAP will not likely increase the risk of the pathogen growth on chilled lamb meat despite temperature fluctuations. At temperatures from 10 to 30 °C, Sutherland et al. (1997) found that up to 80% CO_2 permitted growth of pure cultures of this bacterium when inoculated on sterile pads soaked with tryptone soya broth (TSB). Experiments developed for pure cultures in broth cannot always predict growth profiles on foods because of the effects of different matrices and native microflora.

Much attention has been given to the antagonistic activity, via competition or antibiosis, of the normal meat microflora on *E. coli* O157:H7, with most studies reporting that an increase in the natural bacterial flora has an adverse effect on growth or survival (Palumbo et al., 1997; Ajjarapu and Shelef, 1999; Vold et al., 2000; Tamplin, 2002; Vimont et al., 2006).

In aerobic conditions, the microbiological and physicochemical changes observed on inoculated lamb chops held under chilled or abusive temperatures were in agreement with other works on un-inoculated lamb meat, the storage flora being dominated by *Pseudomonas* and *B. thermosphacta* (Prieto et al., 1993; Drosinos and Board, 1995). *Pseudomonas* spp. are important potential competitors due to their fast growth at low temperatures (0–15 °C) (Dainty and Mackey, 1992) and the ability of some species to produce siderophores (Cheng et al., 1995). However, for *E. coli* O157:H7, Duffy et al. (1999) found that the inhibitory effect of *P. fragi*, a major contributor to meat spoilage, was minimal and completely absent when *B. thermosphacta* was also present. Later, Samelis and Sofos (2002) demonstrated that glucose plays a role in the inhibition of this pathogen by *Pseudomonas* spp., concluding that glucose concentration in meat may be insufficient for these bacteria to inactivate or fully suppress the growth of *E. coli* O157:H7. Consistent with our data in air, Berry and Koohmaraie (2001) observed no reduction in numbers at 4 °C and growth at 12 °C on beef tissue regardless of different microflora populations. The fate of strain NCTC 12900 in the presence of numerous competitors suggests that some other factors such as sample temperature and, perhaps, low initial ratios of competitors to the pathogen were likely to be mainly responsible for its behaviour on lamb chops.

Under anaerobic conditions, suppression of *E. coli* O157:H7 growth or reduction in numbers has been attributed to the combination of anaerobic metabolism and the antagonistic activity of LAB (Vold et al., 2000; Nissen et al., 2001). In this study, reduction in numbers was not related to LAB numbers. Thus, during VP prolonged storage, strain NCTC 12900 showed good survival although LAB developed to 6.68 log cfu/g whereas packaging under 100% CO_2 showed a significant reduction despite the relative small LAB outgrowth. As it appears that large initial LAB populations (up to *ca*. 8 log cfu/g) are required to produce adverse effects on *E. coli* O157:H7 (Brashears et al., 1998; Vold et al., 2000; Smith et al., 2005), significant inhibition was probably due to meat temperature during storage and CO_2.

In conclusion, the behaviour of strain NCTC 12900 in AP demonstrates that effective temperature control is very important to ensure no growth of *E. coli* O157:H7 on lamb meat regardless of rapid proliferation of the spoilage microflora. Irrespective of LAB numbers and fluctuating temperatures, cold storage in VP or MAP resulted in a reduction compared to AP. However, the survival of the organism indicates that contaminated lamb meat is hazardous if insufficiently cooked. Because of the apparent low infective dose, handling and cross-contamination may also be a health hazard. Additional studies are needed to determine the behaviour of *E. coli* O157:H7 at temperatures that fluctuate around the minimum for growth.

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References

Ajjarapu, S., Shelef, L.A., 1999. Fate of pGFP-bearing *Escherichia coli* O157:H7 in ground beef at 2 and 10 °C and effects of lactate, diacetate, and citrate. Applied and Environmental Microbiology 65, 5394–5397.

Anon, 2004. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union L226, 22–82 (25/06/2004).

Barkocy-Gallagher, G.A., Kang, D.-H., Koohmaraie, M., 2002. Fate of field-isolated *Escherichia coli* O157 in ground beef at different storage temperatures. Journal of Food Protection 65, 1106–1109.

Berry, E.D., Koohmaraie, M., 2001. Effect of different levels of beef bacterial microflora on the growth and survival of *Escherichia coli* O157:H7 on beef carcass tissue. Journal of Food Protection 64, 1138–1144.

Blanco, M., Blanco, J.E., Mora, A., Rey, J., Alonso, J.M., Hermoso, M., Hermoso, J., Alonso, M.P., Dahbi, G., González, E.A., Bernárdez, M.I., Blanco, J., 2003. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. Journal of Clinical Microbiology 41, 1351–1356.

Brashears, M.M., Reilly, S.S., Gilliland, S.E., 1998. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. Journal of Food Protection 61, 166–170.

Buchanan, R.L., Bagi, L.K., Goins, R.V., Phillips, J.G., 1993. Response surface models for the growth kinetics of *Escherichia coli* O157:H7. Food Microbiology 10, 303–315.

Chapman, P.A., Ellin, M., Ashton, R., 2001. A comparison of immunomagnetic separation and culture, Reveal(TM) and VIP(TM) for the detection of *E. coli* O157 in enrichment cultures of naturally-contaminated raw beef, lamb and mixed meat products. Letters in Applied Microbiology 32, 171–175.

Cheng, C.M., Doyle, M.P., Luchansky, J.B., 1995. Identification of *Pseudomonas fluorescens* strains isolated from raw pork and chicken that produce siderophores antagonistic towards foodborne pathogens. Journal of Food Protection 58, 1340–1344.

Dainty, R.H., Mackey, B.M., 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. Journal of Applied Bacteriology 21, 103S–114S.

Drosinos, E.H., Board, R.G., 1995. Microbial and physicochemical attributes of minced lamb: sources of contamination with pseudomonads. Food Microbiology 12, 189–197.

Duffy, G., Whiting, R.C., Sheridan, J.J., 1999. The effect of a competitive microflora, pH and temperature on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiology 16, 299–307.

Dykes, G.A., Moorhead, S.M., Roberts, S.L., 2001. Survival of *Escherichia coli* O157:H7 and *Salmonella* on chill-stored vacuum or carbon dioxide packaged primal beef cuts. International Journal of Food Microbiology 64, 401–405.

European Commission, 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L338, 1–26 (22/12/2005).

FAOSTAT. 2004. URL: http://faostat.fao.org/. Last accesed July 2006.

Farber, J.M., Dodds, K.M. (Eds.), 1995. Principles of Modified-Atmosphere and Sous Vide Product Packaging. Technomic Publishing, Lancaster, PA.

Gill, C.O., 1995. MAP and CAP of fresh, red meats, poultry and offals. In: Farber, J.M., Dodds, K.M. (Eds.), Principles of Modified-Atmosphere and Sous Vide Product Packaging. Technomic Publishing, Lancaster, PA, pp. 105–136.

González, C.J., Encinas, J.P., García-López, M.L., Otero, A., 2000. Characterization and identification of lactic acid bacteria from freshwater fishes. Food Microbiology 17, 383–391.

Greer, G.G., Murray, A.C., 1988. Effects of pork muscle quality on bacterial growth and retail case life. Meat Science 24, 61–71.

ICMSF, 1996. Microorganisms in Foods 5: Characteristics of Microbial Pathogens. Blackie Academic & Professional, London.

ICMSF, 1998. Microorganisms in Foods. 6. Microbial Ecology of Food Commodities. Blackie Academic and Professional, London.

ICMSF, 2002. Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. Kluwer Academic/Plenum Publishers, New York.

Jones, T., Gill, C.O., McMullen, L.M., 2004. The behaviour of log phase *Escherichia coli* at temperatures that fluctuate about the minimum for growth. Letters in Applied Microbiology 39, 296–300.

Kirk, R.S., Sawyer, R., 1991. Pearson's Composition and Analysis of Foods, ninth edition. Longman Scientific & Technical, Essex.

Kudva, I.T., Hatfield, P.G., Hovde, C.J., 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. Journal of Clinical Microbiology 34, 431–433.

Land, D.G., Shepherd, R., 1988. Scaling and ranking methods. In: Piggott, J.R. (Ed.), Sensory Analysis of Foods. Elsevier Applied Science, London, pp. 155–185.

Li, Q.Z., Logue, C.M., 2005. The growth and survival of *Escherichia coli* O157:H7 on minced bison and pieces of bison meat stored at 5 and 10 °C. Food Microbiology 22, 415–421.

Logue, C.M., Sheridan, J.J., Harrington, D., 2005. Studies of steam decontamination of beef inoculated with *Escherichia coli* O157:H7 and its effect on subsequent storage. Journal of Applied Microbiology 98, 741–751.

Meng, J., Doyle, M.P., Zhao, T., Zhao, S., 2001. Enterohemorragic *Escherichia coli*, In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology: Fundamentals and Frontiers, second ed. ASM Press, Washington, DC, pp. 193–213.

Nissen, H., Alvseike, O., Bredholt, S., Holck, A., Nesbakken, T., 2000. Comparison between the growth of *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* spp. in ground beef packed by three commercially used packaging techniques. International Journal of Food Microbiology 59, 211–220.

Nissen, H., Maugesten, T., Lea, P., 2001. Survival and growth of *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Salmonella enteritidis* on decontaminated and untreated meat. Meat Science 57, 291–298.

Palumbo, S.A., Pickard, A., Call, J.E., 1997. Population changes and verotoxin production of enterohemorrhagic *Escherichia coli* strains inoculated in milk and ground beef held at low temperatures. Journal of Food Protection 60, 746–750.

Prieto, M., García-López, M.L., García-Armesto, M.R., Otero, A., López, T.M., Moreno, B., 1993. Factors affecting spoilage microflora succession on lamb carcasses at refrigeration temperatures. Journal of Applied Bacteriology 74, 521–525.

Rajkowski, K.T., Marmer, B.S., 1995. Growth of *Escherichia coli* O157:H7 at fluctuating incubation temperatures. Journal of Food Protection 58, 1307–1313.

Rey, J., Blanco, J.E., Blanco, M., Mora, A., Dahbi, G., Alonso, J.M., Hermoso, M., Hermoso, J., Alonso, M.P., Usera, M.A., González, E., Bernárdez, M.I., Blanco, J., 2003. Serotypes, phage types and virulence genes of Shiga-producing *Escherichia coli* isolated from sheep in Spain. Veterinary Microbiology 94, 47–56.

Samelis, J., Sofos, J.N., 2002. Role of glucose in enhancing the temperature-dependent growth inhibition of *Escherichia coli* O157:H7 ATCC 43895 by a *Pseudomonas* sp. Applied and Environmental Microbiology 68, 2600–2604.

Smith, L., Mann, J.E., Harris, K., Miller, M.F., Brashears, M.M., 2005. Reduction of *Escherichia coli* O157:H7 and *Salmonella* in ground beef using lactic acid bacteria and the impact on sensory properties. Journal of Food Protection 68, 1587–1592.

Strachan, N.J.C., Doyle, M.P., Kasuga, F., Rotariu, O., Ogden, I.D., 2005. Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. International Journal of Food Microbiology 103, 35–47.

Sutherland, J.P., Bayliss, A.J., Braxton, D.S., 1995. Predictive modelling of growth of *Escherichia coli* O157:H7: the effects of temperature, pH and sodium chloride. International Journal of Food Microbiology 25, 29–49.

Sutherland, J.P., Bayliss, A.J., Braxton, D.S., Beaumont, A.L., 1997. Predictive modelling of *Escherichia coli* O157:H7: inclusion of carbon dioxide as a fourth factor in a pre-existing model. International Journal of Food Microbiology 37, 113–120.

Tamplin, M.L., 2002. Growth of *Escherichia coli* O157:H7 in raw ground beef stored at 10 °C and the influence of competitive bacterial flora, strain variation, and fat level. Journal of Food Protection 65, 1535–1540.

Tamplin, M.L., Paoli, G., Marmer, B.S., Phillips, J., 2005. Models of the behavior of *Escherichia coli* O157:H7 in raw sterile ground beef stored at 5 to 46 °C. International Journal of Food Microbiology 100, 335–344.

Uyttendaele, M., Jozwik, E., Tutenel, A., De Zutter, L., Uradzinski, J., Pierard, D., Debevere, J., 2001. Effect of acid resistance of *Escherichia coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of *Escherichia coli* O157:H7 on red meat. Journal of Food Protection 64, 1661–1666.

Vimont, A., Vernozy-Rozand, C., Montet, M.P., Lazizzera, C., Bavai, C., Delignette-Muller, M.L., 2006. Modeling and predicting the simultaneous growth of *Escherichia coli* O157:H7 and ground beef background microflora for various enrichment protocols. Applied and Environmental Microbiology 72, 261–268.

Vold, L., Holck, A., Wasteson, Y., Nissen, H., 2000. High levels of background flora inhibits growth of *Escherichia coli* O157:H7 in ground beef. International Journal of Food Microbiology 56, 219–225.

Wells, J.G., Davis, B.R., Wachsmuth, I.K., Riley, L.W., Remis, R.S., Sokolow, R., Morris, G.K., 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. Journal of Clinical Microbiology 18, 512–520.