Meat Science 85 (2010) 89–95

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The growth, survival and thermal inactivation of *Escherichia coli* O157:H7 in a traditional South African sausage

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ARTICLE INFO

Article history:
Received 21 October 2008
Received in revised form 15 September 2009
Accepted 9 December 2009

Keywords:
Escherichia coli O157:H7
Preservative
Boerewors
Inoculum size
Temperature

ABSTRACT

This study investigated the growth and survival of *Escherichia coli* O157:H7 inoculated into boerewors models with (B + P) and without (B – P) sulphur dioxide preservative at a low (L) and high (H) inoculum followed by storage at 0, 4 and 10 °C for 10 days. The pathogen's thermal inactivation at 50, 60, 65 and 70 °C was also evaluated in B + P. The B – P at both low and high inocula had significantly higher recoveries at all temperatures compared to B + P. The BL + P and BH + P had significant reductions in recoveries at 0 °C, declining to below detectable limits at days 8 and 10, respectively. At 4 °C, the BL + P and BH + P recoveries declined significantly at day 10. At 10 °C, significant increases were observed from days 0 to day 10 in both models and at low and high inocula. At 0 °C, the BL – P and BH – P treatments had significant declines in recoveries. The combination of sulphur dioxide preservative and low temperature demonstrated the best efficacy against *E. coli* O157:H7 survival. Thermal inactivation of *E. coli* O157:H7 was 60 min at 60 °C, 80 s at 65 °C and 60 s at 70 °C. This study demonstrated that *E. coli* O157:H7 can survive in boerewors with and without preservative and is more sensitive to heat treatment at 70 °C.

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1. Introduction

Escherichia coli O157:H7 was first associated with illness in 1982 (Doyle, Zhao, Meng, & Zhao, 1997). Since then, the pathogen has gained in notoriety due to a number of outbreaks which have occurred worldwide (Byrne, Bolton, Sheridan, Blair, & McDowell, 2002). The pathogen is an aetiological agent of haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopaenic purpura (Karmali, Steele, Petric, & Lim, 1983). It is estimated that in the United States of America, the number of *E. coli* O157:H7 foodborne illness cases are 62,500 per annum including 10,800 hospitalisations and 50 deaths (Mead et al., 1999). Although a number of foods such as lettuce (Li, Brackett, Chen, & Beuchat, 2001), unpasteurised apple cider (Besser et al., 1993), drinking water (Isaacson et al., 1993) and yoghurt (Bachrouri, Quinto, & Mora, 2002) have been associated with some *E. coli* O157:H7 outbreaks, many of them have been associated with the consumption of foods of bovine origin such as ground beef and raw milk (Griffin & Tauxe, 1991; Meng & Doyle, 1998).

Growth and survival studies of *E. coli* O157:H7 in specific ground beef products is important for the prediction of the pathogen's behaviour since broth based models may be compromised when the models are applied to the ground beef medium (Tamplin, 2002). General growth parameters for all *E. coli* include a minimum temperature of 7–8 °C, an optimum temperature of 35–40 °C, a minimum pH of 4.4, an optimum pH of 6.0–7.0; a minimum water activity of 0.935 and an optimum of 0.995 (Wilshaw, Cheasty, & Smith, 2000). Palumbo, Pickard, and Call (1997) observed the growth of *E. coli* O157:H7 in ground beef stored at 8 °C when there was little or no background flora. It has been shown that *E. coli* O157:H7 can survive in frozen ground beef patties for 9 months with little decline in numbers (Doyle & Schoeni, 1984). Therefore, contamination of beef by the pathogen poses significant risks that make it imperative for consumers to ensure adequate cooking.

Several researchers have shown that *E. coli* O157:H7 does not posses an unusual heat resistance and D-values at 50–64 °C ranging from 92.67 to 0.16 min have been reported (Ahmed, Conner, & Huffman, 1995; Doyle & Schoeni, 1984; Line et al., 1991). Murphy, Beard, Martin, Keener, and Osaili (2004) reported D-values at 55–70 °C ranging from 23.23 to 0.03 min in ground and formulated beef/turkey. The United Kingdom's Advisory Committee on the Microbiological Safety of Food (ACMSF; according to Byrne et al., 2002) recommended that minced beef burgers should be heated to an internal temperature of 70 °C for 2 min. The pathogen has a very low infective dose of about 10–100 organisms (Garbutt, 1997) and some consumers frequently subject food to inadequate refrigeration and heating leading to increased chances of infection and outbreaks.

Sulphur dioxide is used as a common preservative of some fresh processed meat, poultry and game products that are produced using comminuted meats. These products include raw unprocessed

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0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.12.010

sausage and sausage meat, mortadella, chicken and turkey loaves, frankfurters, luncheon meats, Polish salami, devon and hamburgers. It can be added to food in gaseous form, in aqueous solution as sulphurous acid, as sodium sulphite, sodium hydrogen sulphite, sodium metabisulphite, potassium metabisulphite, calcium sulphite or as calcium hydrogen sulphite (Scottish Food Co-ordinating Committee, 1992). It increases the bacterial lag phase, selects against spoilage bacteria and is effective against *Salmonella* spp. and many other *Enterobacteriaceae*, *Pseudomonas* spp., *Lactobacillus* and some yeast species. It is an effective antioxidant in sausages as well as other comminuted fat–protein–water meat emulsions and it is a reducing agent that preserves the red colour of meat (Australian Meat Industry Services, 2006). However, sulphur dioxide may cause asthma and symptoms of an allergic response in sulphite sensitive people (Australian Meat Industry Services, 2006; Rigg, 1997). Its use in foods is therefore regulated. It is not permitted in products such as fresh raw mince beef, chicken, lamb, hamburger patties, fresh cuts of raw meat, uncooked fermented meats, processed whole meat cuts, cured meat cuts and dried meats (New South Wales Food Authority, 2004). The permitted maximum level of sulphur dioxide preservative in South Africa and Scotland is 450 mg/kg for sausages, hamburgers and similar products (Scottish Food Co-ordinating Committee, 1992; South African Government, 1977), while Australia permits a maximum of 500 mg/kg for similar products (Rigg, 1997). A survey on the usage levels of sulphur dioxide in Australian sausages found that 39% of the samples failed the maximum permitted concentration of 500 mg/kg while 49% passed with a concentration of 300–400 mg/kg, and the remaining 12% passed with a concentration of less than 300 mg/kg (Rigg, 1997).

Boerewors is a very popular uncured South African traditional fresh sausage that may be manufactured with or without sulphur dioxide preservative (Steyn, 1989). There is, however, no information available on the growth, survival and thermal inactivation of contaminating *E. coli* O157:H7 in this product. *E. coli* O157:H7 was chosen because of the severity with which it causes illness and the high frequency at which it has been associated with ground beef disease outbreaks. Therefore, the aims of this study were to investigate the growth and survival of *E. coli* O157:H7 in boerewors at low and high inocula sizes, with and without sulphur dioxide preservative and at 0, 4 and 10 °C. The pathogen's thermal inactivation end point was also determined for the boerewors with preservative model at 50, 60, 65 and 70 °C.

2. Materials and methods

2.1. Preparation of boerewors models

Boerewors models were manufactured following typical industrial procedures (Hugo, Roberts, & Smith, 1993) and in compliance with the South African regulations for boerewors (Government Notice No. R.2718 of 23 November, 1990; Foodstuffs, Cosmetics and Disinfectants Act No. 54 of 1972). Table 1 shows the formulation of boerewors. Fresh meat (beef [70/30] and pork [50/50]), was purchased from a butchery in the Bloemfontein District. The models consisted of 90% total meat content formulated to contain 30% fat and 60% lean meat. The rusk (commercially dried yeastless and sugarless bread crumbs), spices, Worcester sauce and vinegar were mixed in 360 g ice water and left to stand for 5 min to allow for hydration.

The spice mixture (405 g) consisted of coriander (98.6985 g), monosodium glutamate (26.325 g), black pepper (24.3 g), nutmeg (20.25 g), cloves (16.2 g), thyme (4.05 g), sodium chloride (188.527 g), sodium metabisulphite (10.6515 g), ascorbic acid (9.315 g) and dextrose (15.066 g). The spices, rusk, vinegar and Worcester sauce were thoroughly mixed with the cubed meat and minced through a 4.5 mm plate. The boerewors with preservative (B + P) spice mixture had 450 mg/kg sulphur dioxide added as sodium metabisulphite while the boerewors without preservative (B − P) spice mixture was formulated without the addition of the sulphur dioxide. A total of 15 kg each of B − P and B + P was made for the growth and survival experiment. Each of the two models was packaged in 99 g quantities into 144 sterile stomacher bags (Whirl-Pak™; Total B − P and B + P samples analysed = 288), sealed with masking tape and stored at −18 °C until required. Additional four 99 g samples of each of the two models were also packaged to cater for batch sampling to screen for *E. coli* O157:H7. A further 7.128 kg of B + P was manufactured for the thermal inactivation experiment and packaged as 99 g in 72 sterile stomacher bags (Whirl-Pak™) and stored as above.

Table 1
Formulation used in the manufacture of boerewors models (Hugo et al., 1993).

Ingredient	Amount
Beef (70/30)	600 g/kg
Pork (50/50)	300 g/kg
Vinegar	15 g/kg
Water[a]	24 g/kg + 10 g/kg
Rusk[b]	20 g/kg
Spice mixture	27 g/kg
Worcester sauce	4 g/kg
Sodium chloride	12.6 g/kg
Sulphur dioxide[c]	450 mg/kg
Ascorbic acid	62.308 mg/kg

[a] One percent water was added with the organism at the spiking stage.
[b] Commercially dried, yeastless and sugarless bread crumbs.
[c] Sulphur dioxide was added to boerewors with preservative as sodium metabisulphite.

2.2. Microbial quality of manufactured boerewors

Screening for *E. coli* O157:H7 was done separately for the beef (70/30), pork (50/50) and the finished boerewors without preservative (B − P) and boerewors with preservative (B + P). Two 99 g samples were taken immediately after manufacture of the models and analysed within 30 min. Ten grams from each of the samples was weighed into sterile stomacher bags (Whirl-Pak™) and homogenised in 90 ml MacConkey broth (Difco) using a stomacher (Lab Blender 400, ART Medical Equipment). The mixture was aseptically transferred to sterile 100 ml Schott bottles and enriched by incubating for 18 h at 37 °C. Serial dilutions were made followed by spread plating in duplicate on Fluorocult *E. coli* O157:H7 agar (Merck). The plates were incubated at 37 °C for 24 h. The above procedure was repeated once and mean values were calculated from duplicate experiments.

Other microbial tests performed on the B + P and B − P models included aerobic plate counts (standard plate count agar; Oxoid; incubation at 32 °C for 72 h); coliform counts (Chromocult coliform agar; Merck; incubation at 37 °C for 24 h) and yeasts and moulds counts (potato dextrose agar; Biolab; incubation at 25 °C for 7 days).

2.3. Preparation of E. coli O157:H7 inoculum

E. coli O157:H7 (ATCC 43895) maintained on a nutrient agar (Oxoid) slope was streaked out on Fluorocult *E. coli* O157:H7 agar plates and incubated for 24 h at 37 °C. Colonies were confirmed by serotyping using the slide agglutination test for *E. coli* O157:H7 on heat-killed organisms (Mast Assure™; Mast Diagnostics). A loopful of culture was suspended in 10 ml brain heart infusion (BHI) broth (Biolab) and incubated at 37 °C for 24 h. The resultant culture (3 ml

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