



Review

Reduction of verotoxigenic *Escherichia coli* in production of fermented sausages

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ABSTRACT

After a number of foodborne outbreaks of verotoxigenic *Escherichia coli* involving fermented sausages, some countries have imposed regulations on sausage production. For example, the US Food Safety and Inspection Service requires a 5 log₁₀ reduction of *E. coli* in fermented products. Such regulations have led to a number of studies on the inactivation of *E. coli* in fermented sausages by changing processing and post-processing conditions. Several factors influence the survival of *E. coli* such as pre-treatment of the meat, amount of NaCl, nitrite and lactic acid, water activity, pH, choice of starter cultures and addition of antimicrobial compounds. Also process variables like fermentation temperature and storage time play important roles. Though a large variety of different production processes of sausages exist, generally the reduction of *E. coli* caused by production is in the range 1–2 log₁₀. In many cases this may not be enough to ensure microbial food safety. By optimising ingredients and process parameters it is possible to increase *E. coli* reduction to some extent, but in some cases still other post process treatments may be required. Such treatments may be storage at ambient temperatures, specific heat treatments, high pressure processing or irradiation. HACCP analyses have identified the quality of the raw materials, low temperature in the batter when preparing the sausages and a rapid pH drop during fermentation as critical control points in sausage production. This review summarises the literature on the reduction verotoxigenic *E. coli* in production of fermented sausages.

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1. Introduction

Fermented sausages originated from Mediterranean countries and have been produced since Roman times. A large variety of fermented sausages exist. Due to their combination of growth hurdles such as low pH and low water activity (a_w) (Leistner, 2000), they were traditionally considered safe. However, after a number of foodborne outbreaks involving fermented sausages, EU considers that minced and/or fermented beef, and products thereof, represent a hazard to public health (Anon, 2003). These outbreaks are costly, both in terms of human suffering, and from an economic point of view, involving extensive work identifying the source of the outbreak and organising recalls of large amounts of food. Due to these outbreaks some countries have imposed regulations on sausage production. For example, the US Food Safety and Inspection Service requires that the production process for fermented products must give a 5 log₁₀ reduction of *Escherichia coli* (Anon, 2001). Such regulations have led to a number of studies on the inactivation of *E. coli* in fermented sausages. By far most studies have been performed using *E. coli* O157:H7, whilst some studies compare with *E. coli* of other serotypes. Here we review some of the findings from these studies.

2. VTEC characterisation and foodborne outbreaks

Some *E. coli* produce Shiga toxins (also called verotoxins or verocytotoxins). These *E. coli* are designated Shiga toxin-producing or verotoxin-producing *E. coli* (STEC or VTEC). A subgroup of VTEC, capable of causing serious disease like enterohaemorrhagic colitis with bloody diarrhoea and possibly renal failure (hemolytic uremic syndrome, HUS), is designated enterohaemorrhagic *E. coli* (EHEC). Shiga toxin is an essential virulence factor in EHEC disease, but Shiga toxin is not sufficient to cause disease by itself. The pathogenesis of EHEC disease depends on the production of Shiga toxin in combination with other virulence factors, and EHEC strains typically harbour virulence genes for attachment (e.g. intimin genes) or potentiating toxin genes (e.g. subtilase cytotoxin). *E. coli* are grouped into different serotypes according to the variant of O-polysaccharide antigens on their surfaces and their types of flagellar H antigens. The most common disease causing EHEC is the serotype *E. coli* O157:H7 which can cause serious illness and death. Strains of this serotype have been responsible for large foodborne outbreaks involving meat, poultry, dairy products, apple juice, cider, vegetables, mayonnaise and water (Getty, Phebus, Marsden, Fung, & Kastner, 2000). *E. coli* O157:H7 is the most prevalent cause of HUS in children. More knowledge is therefore obtained about *E. coli* O157:H7 than other EHEC when considering

prevalence, diagnostics, virulence, survival and growth. Other serogroups of *E. coli* e.g. O26, O111, O103, O121 and O145 are also increasingly reported to be responsible for disease in humans. There are large geographical and regional differences in prevalence of different serogroups and different EHEC. Several EHEC have been implicated in foodborne outbreaks from fermented sausages (Table 1). An outbreak in Norway in 2006 caused by an *E. coli* O103:H25 was linked to dry fermented sausages where 17 cases were reported of which 10 developed HUS and one child died (Sekse et al., 2009). After this incidence we performed a series of experiments to enhance the safety of Norwegian dry fermented sausages (Heir et al., 2010; Omer et al., 2010).

Estimations indicate that as little as 50 EHEC can cause disease (Tilden et al., 1996). It is therefore necessary to consider the whole food production chain to increase low prevalence of EHEC. In addition sausage production must lead to further reduction in EHEC if present.

3. Production of fermented sausages

3.1. Sausage production

A large variety of processes for production of fermented sausages exist.

Sausages are made by mixing minced meat and fat with herbs, spices, salt, sugar, sodium nitrite and starter culture. The starter culture is usually a single species of lactic acid bacteria (LAB) or a LAB mixed with other bacteria. The mix is stuffed into casings and subjected to a fermenting step where LAB grow and produce lactic acid which leads to a lowering of the pH. The sausages are subsequently dried until the desired a_w is achieved. The fermentation and drying are performed in cabinets with controlled temperature and humidity.

Fermented sausages can be either dry or semi-dry (Getty et al., 2000). Generally, dry fermented sausages have $a_w < 0.90$, whilst semi-dry sausages are in the range between 0.90 and 0.95 (Lücke, 2000). Dry sausages such as Genoa salami, dry salami and pepperoni, contain 25–40% moisture, are heavily spiced, are not heated above 26.7 °C, have a firm texture and are usually shelf-stable. In Europe, these fermented sausages can be further divided into Northern and Mediterranean types (Demeyer et al., 2000). Northern type products often contain beef and pork and are characterised by relatively short ripening periods, up to 3 weeks and involving clearly separated fermentation and drying periods. Rapid acidulation to final pH values below 5 and smoking ensure microbiological safety and shelf-life. Mediterranean type sausages are predominately pork products and

Table 1
Reported outbreaks of EHEC caused by fermented sausage.

| Place | Year | Product | Serotype ^a | Cases/HUS/Deaths | Reference |
|-----------|-----------|--|-----------------------|----------------------------|---|
| USA | 1994 | Dry cured sausage | O157:H7 | 20/—/— | (Alexander et al., 1995) |
| Australia | 1994–1995 | Semi-dry fermented sausage (Mettwurst) | O111:NM | 53/23/1 | (Cameron, Walker, Beers, Rose, & Anear, 1995) |
| Germany | 1995–1996 | Teewurst (or Mortadella) | O157:H— | 300–600 ^b /28/— | (Ammon, Petersen, & Karch, 1999) |
| Canada | 1998 | Genoa salami | O157:H7 | 39/2/— | (Williams et al., 2000) |
| Canada | 1999 | Salami | O157:H7 | 143/5/— | (MacDonald et al., 2004) |
| Sweden | 2002 | Fermented sausage | O157:H7 | 38/12/— | (Sartz et al., 2008) |
| Norway | 2006 | Morr, Norwegian type dry fermented sausage | O103:H25 | 17/10/1 | (Schimmer et al., 2008) |
| Denmark | 2007 | Organic beef sausage | O26:H11 | 20/—/— | (Ethelberg et al., 2009) |
| Italy | 2007 | Dry-fermented pork salami | O157 | 2/—/— | (Conedera et al., 2007) |

^a NM: non motile.

^b Estimated from number of HUS cases.

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