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Effect of frozen storage, different thawing methods and cooking processes on the survival of *Salmonella* spp. and *Escherichia coli* O157:H7 in commercially shaped beef patties

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ABSTRACT

The effect of common handling practices (i.e., freezing, thawing and cooking) of beef patties on the survival of *Salmonella* spp. and *Escherichia coli* O157:H7, was evaluated. Inoculated ground beef was stored at −22 °C for 5 and 75 days. After thawing at 4 °C/16 h, 20 °C/12 h, in microwave/22–24 min, or without prior thawing, beef patties (90 g) were shaped and cooked in oven-broiler or in pan-grill to internal temperatures of 60 °C or 71 °C. Cooking in oven-broiler was more effective compared to pan-grill, especially when cooked to 71 °C. Defrosting methods did not affect significantly ($P \geq 0.05$) the survival of the pathogens during subsequent cooking. Frozen storage for 75 days enhanced the survival of *E. coli* O157:H7, as the pathogen survived 3.1 logCFU/g when cooked in oven-broiler at 71 °C. Results may supplement the existing guidelines for the appropriate practices, associated with freezing, thawing and cooking of patties in households or catering services.

1. Introduction

Beef patties constitute conventional ready to cook meat products, ranked among the top most frequently consumed meat products worldwide. These products may be prepared in-house from ground beef with or without the addition of other ingredients and seasonings, or they may also be found available in the form of pre-shaped frozen patties, which is highly convenient for Quick Service Restaurants. Over the past decade, ground beef has been linked with several outbreaks of foodborne diseases caused by various serotypes of *Salmonella* spp. or Shiga-toxin producing *Escherichia coli* (Centers for Disease Control & Prevention (CDC), 2002, 2010, 2011, 2012a, 2012b; Schneider et al., 2011; McLaughlin, Castrodale, Gardner, Rafiq, & Bradford, 2006). The common cause of all these outbreaks is traced to poor hygienic conditions during production and improper handlings by the food handlers, either in the domestic environment or in restaurants. Therefore, the Food Safety Inspection Service of the U.S. Department of Agriculture (USDA-FSIS) has issued a series of specific guidelines for safe practices during storage and preparation of ground beef. Through these guidelines it is recommended that ground beef should be stored in refrigerators for 1–2 days or in freezer up to 4 months. Before cooking, the necessary portion should be thawed in refrigerator, in microwave or under regularly changed cold water, while thawing in hot water or at room temperature on countertop is not recommended. Finally, a minimum internal temperature of 160 °F (71.1 °C) should be targeted when cooking beef burgers or patties by using a precise thermometer, in order to eliminate the risk of surviving pathogenic bacteria (United States Department of Agriculture—Food Safety & Inspection Service, 2013a). Although the type of cooking method will have different impacts on the survival of pathogens (D'Sa, Harrison, Williams, & Broccoli, 2000; Rhee, Lee, Hillers, McCurdy, & Kang, 2003; Shen, Georgnaras, Belk, Smith, & Sofos, 2011a; Shen et al., 2010), no specific references have been made in the issued guidelines.

Despite these recommendations, the storage and cooking practices being applied by the food handlers, either in households or in catering services and restaurants, are generally based on personal preferences and convenience for handling and consuming foods (Badrie, Gobin, Dookeran, & Duncan, 2006; Gilbert et al., 2007; Jevšnik, Hlebec, & Raspor, 2008; Karabudak, Bas, & Kiziltan, 2008). Phang and Bruhn (2011) reported that 16% of the consumers are thawing ground beef patties on counter top, while 87% did not know the proper cooking temperature of ground beef. In the same study, 51% of the consumers reported that they use the color of patties as a criterion of doneness, with 23% preferring pink interior. However, the color in the center of cooked ground meat products may be affected by many factors other than temperature, giving false indications for the safety of the final product (King (née Turner) & Whyte, 2006).

Many researchers have studied the single effect of frozen storage (Ansay, Darling, & Kaspar, 1999; Byrne, Bolton, Sheridan, Blair, &

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McDowell, 2002), different thawing (Lianou & Koutsoumanis, 2009; Sage & Ingham, 1998), or cooking (D'Sa et al., 2000; Rhee et al., 2003) methods and degree of doneness (Gill, Moza, & Barbut, 2009; Passos & Kuaye, 2002) on the survival or heat tolerance response of pathogenic bacteria, in fresh ground meat. Heat resistance of *L. monocytogenes* and *Salmonella* Enteritidis following frozen storage and thawing has been tested at 57 °C, in cells suspended in laboratory medium (Lianou & Koutsoumanis, 2009) but not immobilized in the frozen/thawed ground meat sample and cooked at realistic internal temperatures. During frozen storage and thawing of beef patties, pathogens encounter various stresses, mainly associated with the temperature accepted by the cells. The exposure of microorganisms to such stresses has been found to affect their heat tolerance (Abee & Wouters, 1999). Therefore, it is essential to simulate the combined effect of all the common handling practices that are being used during preparation of ground beef, on the potential of pathogens to survive the heat process during cooking. According to the above, we aimed to evaluate the single and synergistic effect of frozen storage, different thawing and cooking methods and degree of doneness of commercial-style beef patties on the survival of *Salmonella* spp. and *E. coli* O157:H7, simulating common practices used by the food handlers in households or restaurants.

2. Materials and methods

2.1. Bacterial cultures and preparation of inocula

A five-strain composite of *Salmonella* spp. consisted of two strains of *S.* Typhimurium (calf bowel and epidemic isolates), *S.* Agona, *S.* Infantis, *S.* Reading (animal feeds isolates) and a three-strain composite of *Escherichia coli* O157:H7 (NCTC 12079, NCTC 13125, NCTC 13127) were used in the present study. All microorganisms were maintained on tryptone soy agar (TSA; LAB M, LAB011, Lancashire, UK) slants at 4 °C, which were replaced every 30 days. Each strain was activated separately by transferring a single colony from the slants into 10 ml tryptic soy broth (TSB; LAB M, LAB004) for 24 h at 37 °C, followed by a second activation step in TSB (37 °C, 18 h). Prior to inoculation, the strains were centrifuged at 3600 rpm at 4 °C for 10 min. The supernatant was rejected and the pellet was resuspended in 10 ml maximum recovery diluent (MRD; LAB M, LAB103). This washing procedure was followed twice. The washed strains were mixed equally in a sterile 100 ml—container and the strain composite of each microorganism was used for the inoculation of the samples. The level of the strain-composite inoculum was 1.5×10^9 CFU/ml.

2.2. Inoculation of ground beef

Freshly produced ground beef, which contained approximately 20% fat was obtained from a local meat processing industry on the day of each experimental trial and was kept at 4 °C before inoculation. The ground beef had not been subjected to any intervention for reduction of its initial microbial load. This approach was chosen so that we avoid interference with the natural ecosystem of the samples and minimize any potential impact of decontamination on the interactions between the spoilage flora and the pathogens, especially in cases where significant growth of the target organisms may occur (i.e., during defrosting at 20 °C). Portions (400 g) of ground beef was placed into a sterile stomacher bag (BagLight® PolySilk 400, BagLight®, France) and were inoculated with 5 ml of the strain composite of *Salmonella* spp. or *E. coli* O157:H7 in order to yield an initial inoculation level of approximately 6.5–7.0 log CFU/g. The inoculated samples were mixed thoroughly by hand-massage of the bag for 5 min and were stored at 4 °C for 30 min for the attachment of the inoculum. To avoid any differences in temperature during freezing or thawing, a constant shape (14 × 9 × 3.5 cm) was given to each inoculated 400-g sample. In parallel, beef patties of commercial dimensions (90 g; diameter 9 cm × height 1.5 ± 0.2 cm) were manually prepared and they were designated for cooking without prior thawing. All 400 g samples were stored at −22 °C for 5 or 75 days, representing a short- or a long-term storage under freezing that may take place in households or catering services.

2.3. Thawing and cooking of beef patties

The frozen 400 g samples of ground beef were thawed using three different methods, simulating realistic scenarios that are regularly practiced by food handlers. In particular, samples were left (i) at 4 °C for 16 h, simulating thawing in a refrigerator, (ii) at 20 °C for 12 h, simulating thawing on the kitchen counter overnight or (iii) they were thawed in a commercial microwave (defrosting mode; Easy Grill, Whirlpool) for 22–24 min. After thawing, ground beef was shaped in patties (90 g; diameter 9 cm × height 1.5 ± 0.2 cm) using sterile petri dishes as matrix and they were held at the temperature reached after thawing (i.e., 4 °C for refrigerator, 20 °C for kitchen counter and microwave) for less than 30 min before cooking.

Beef patties were cooked in a commercial oven-broiler (Davoline 4503 Futura, Thessaloniki, Greece) preheated at 200 °C, or in a pan-grill (Tefal Specifics Grill Pan 26 cm, Tefal, France) at maximum power (1500 W), representing two conventional methods of cooking. Thermal application was halted when the temperature at the geometrical center of the samples reached 60 °C, simulating undercooking of patties, or 71 °C, as suggested by the USDA-FSIS. During cooking, samples were flipped over once when the internal temperature reached 35 °C, in order to avoid extensive browning of the patties surface. This temperature was chosen based on preliminary experiments, where thermocouples (SE030, Pico Technology, Cambridgeshire, UK) were fitted on the top, the middle and the bottom side of the patties. Results from these trials showed that the rate of temperature increase during cooking in oven-broiler was much higher at the lower side compared to the upper side of the patties (and vice versa when cooking in pan-grill) and therefore, the final temperature at the two sides differed approximately 20 °C when the internal temperature was 60 or 71 °C. By turning the patties once, this difference was limited at approximately 3–5 °C (data not shown). For the same reason, beef patties cooked directly from the frozen form were turned twice (at 0 °C and 35 °C).

Changes in temperature during freezing, thawing or cooking of samples were monitored using a time–temperature data logger (Pico-PP22, TC-08 Thermocouple Data Logger, Pico Technology, Cambridgeshire, UK) equipped with Type K thermocouples (SE030, Pico Technology, Cambridgeshire, UK). Each thermocouple was fitted from one side to the geometrical center of the ground beef block or patties before freezing, to monitor the temperature changes during freezing and thawing. The same procedure was followed for the beef patties which were formed after thawing and were intended for cooking. Due to the small thickness of the patties, small deviations of the thermocouple from the geometrical center could significantly affect the logging of the internal temperature. Therefore, a sterile metallic wire was used to fully penetrate the patties while they remained in the petri dishes, from one precisely measured spot on the one side of the petri dish to another spot at the other side. The petri dishes were broken to remove the patties without changing the form of the patties or the position of the thermocouple. The obtained data in all cases were recorded and analyzed using the PicoLog software (ver. 5.21.9).

The *F*-values of each treatment were calculated according to Murphy, Beard, Martin, Keener, and Osaili (2004), using the equation:

$$F = \int_0^t 10^{(T(t)-T(ref)/z)} dt \quad (1)$$

with T the temperature in the center of the beef patties at t = 0 to t = t_i and $T(ref)$ the reference temperature, which is a theoritical temperature at which D value should be known. In the present study, the z values by Juneja (2003) in ground beef (25% fat) were used for *Salmonella* (z = 8.06 °C) and *E. coli* O157:H7 (z = 6.33 °C), while