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Effectiveness of inactivation of foodborne pathogens during simulated home pan frying of steak, hamburger or meat strips

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ABSTRACT

In order to evaluate the effect of simulated home pan frying of raw meat and meat preparations of different animal species on the thermal inactivation of pathogens, the heat resistance (D-value) of three strains of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and two strains of generic *E. coli* was validated in BHI and adjusted BHI (i.e. pH 5.6 and 1.5% NaCl) at 60 °C. The D-values were obtained of the linear phase of the survivor curves created in GInaFiT, a freeware tool to fit models to experimental data. The obtained D-values corresponded to those previously published in literature and confirmed *L. monocytogenes* to be the most heat resistant pathogen among them. Heat treatment in adjusted BHI significantly increased heat-resistance of *E. coli* O157:H7 and generic *E. coli*. Subsequently, the thermal inactivation of *L. monocytogenes*, *Salmonella* spp., *C. jejuni* and *E. coli* O157:H7 was evaluated using a standardized procedure simulating commonly used home pan frying of various types of meat including steaks or filets, hamburgers and meat strips from various animal species such as pork, beef, chicken, lamb and some turkey, horse, kangaroo and crocodile meat. Corresponding F_{70}-values were calculated based upon measured core time/temperature profiles. It was noted that a core temperature of 70 °C was not always achieved and, moreover, a heat treatment equivalent to 2 min at 70 °C was also not always obtained. This was in particular noted in hamburgers although the meat was visually judged well done. On several occasions, residual survivors of the initial inoculated (4 log CFU/g) food borne pathogens could be recovered either by enumeration (limit of detection 1 log CFU/g) or by the presence/absence testing per 25 g. Pan frying of hamburgers yielded the highest number of surviving pathogenic bacteria (46%), followed by well-done filets and steaks (13%) and meat strips (12%). Taking only steaks (beef, horse, kangaroo, crocodile and turkey) into account, residual detection of pathogens occurred for all levels of doneness: 18% for well-done, 71% for medium and even 90% for rare steaks. Numbers of *L. monocytogenes* recovered after heat treatment ranged from <1 log CFU/g to 2.6 log CFU/g. Although, the prevalence of pathogens in meat might be low, and the numbers present in case of natural contamination are probably lower than the current used inoculum of 4 log CFU/g, consumers could still be exposed to surviving food borne pathogens in case of these commonly used pan frying of raw meat and meat preparations at consumer's home.

1. Introduction

Despite preventive measures during slaughter and good hygiene and good manufacturing practices during further processing, raw meat and meat preparations are still occasionally contaminated with pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp. and pathogenic verotoxin-producing *E. coli* (VTEC) (Frank et al., 2011; Hendriksen et al., 2011; Kirkpatrick and Tribble, 2011; Milillo et al., 2012; Scallan et al., 2011; Söderström et al., 2008; Taylor et al., 2012). In 2012, the Belgian government analyzed 2401 samples of meat and 3028 samples of meat preparations. From these analyses it was concluded that *Campylobacter* was present in 6.3% and *Salmonella* in 4.1% of the meat samples. *Salmonella* was also present in 0.5% of the meat preparations, while *L. monocytogenes* and *E. coli* O157 were present in 0.2% of meat preparations samples (FASFC, 2013). The presence of pathogens in (undercooked) meat can present a serious food safety threat and result in a food-borne outbreak (Takhar et al., 2009). European strong-evidence food-borne outbreaks are

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summarized by EFSA and ECDC in their annual EU summary report on zoonoses, zoonotic agents and food-borne outbreaks. In 2012, *Salmonella* spp. were the most frequently reported cause of food-borne outbreaks (28.6%) in the EU, with pig meat, broiler meat, bovine meat and their derived products responsible for respectively 5.8%, 3.7% and 2.0% of these *Salmonella* strong-evidence food-borne outbreaks. *Campylobacter* spp. were responsible for 9.3% of the food-borne outbreaks in 2012, with broiler meat and derived products as the most commonly reported cause of strong-evidence outbreaks (44.0%). Although verotoxigenic *E. coli* was responsible for only 0.8% of the total number of reported food-borne outbreaks, the main food vehicle in strong-evidence outbreaks was bovine meat and products thereof (50.0%), followed by pig meat (16.7%) (EFSA and ECDC, 2014).

The main settings where strong-evidence food-borne outbreaks have occurred are households/domestic kitchens of consumers (39.7%) and restaurants, cafés, pubs, bar and hotels (23.9%) (EFSA and ECDC, 2014). A large part of these food-borne outbreaks and most of the separate cases of food borne infections or poisonings can be attributed to careless actions by consumers during the preparation of the food (FASFC, 2012; Sampers et al., 2012). In a study of Fischer et al. (2007) participants claimed to prefer convenience and taste over food safety and effort. Among the regular occurrence of cross-contamination events, another inadvertence by consumers during the preparation of food is undercooking (Sampers et al., 2012). However, the heat treatment of raw meat in consumer domestic kitchens or food service operations is of great importance, in addition to a clean environment and the preservation of the cold chain, to provide sufficient inactivation of possible pathogenic bacteria present (Murphy et al., 2004). It is generally accepted that when meat (including hamburgers or any other comminuted meat) is subjected to a core temperature of 70 °C for 2 min or was subjected to a heat treatment equivalent to 2 min at 70 °C, it will accomplish a substantial inactivation (6 log reduction) of pathogens and therefore renders the meat safe to eat (ACMSF, 2007). In the case of steak or filet it is assumed that the meat is internally sterile and that high temperatures on the surface during pan frying are sufficient to inactivate any pathogens present. However, it is not always clear which temperatures are actually reached during home pan frying of raw meat. Although measuring the internal temperature of meat is a useful method to assess readiness for consumption, the use of a thermometer to assess the doneness of food is currently uncommon in European households (Bearth et al., 2014). Besides, thermal inactivation of pathogens and presence of residual survivors in meat may also vary depending upon the exact nutritional composition (e.g. fat content), texture (e.g. fiber structure) and the initial number of micro-organisms present (Jay, 2000; Tuntivanich et al., 2008).

The effectiveness of thermal inactivation processes during home cooking procedures should gain more attention as 36.6% of the total fresh meat bought on the Belgian market are mixed meat preparations (sausages, mixed minced meat, hamburgers) (VLAM, 2014). These meat preparations have more opportunities for introducing pathogens in the meat, but also have an increased risk to contain pathogens in the core of the food product because they are more extensively handled and undergo extensive manipulations. This increases the risk of survival and cross-contamination of pathogenic micro-organisms in undercooked meat (Sampers et al., 2012). Besides, Bergsma et al. (2007) and de Jong et al. (2012) showed in their studies unsuspected survival of pathogens during consumer style cooking techniques. Therefore, the objective of the present study is to evaluate the effect of simulated home pan frying of raw meat and meat preparations of different animal species on the thermal inactivation of pathogens.

2. Materials and methods

2.1. Selection of bacterial strains and culture conditions

In this study, 3 strains of *L. monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*, (nalidixic acid resistant) *E. coli* O157:H7 and 2 strains of generic *E. coli* were used (Table 1). The strains were obtained from the culture collection of the Laboratory of Food Microbiology and Food Preservation (LFMFP) of Ghent University (Ghent, Belgium) and from the culture collection of the Belgian Veterinary and Agrochemical Research Centre (CODA, Brussels, Belgium). Stock cultures of *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7 and generic *E. coli* strains were kept at −75 °C in Tryptone Soy Broth (TSB, Oxoid, Bastingstoke, UK), supplemented with 0.6% yeast extract (YE, Oxoid) and 15% glycerol (Prolabo, Heverlee, Belgium). Working stocks were stored refrigerated at 4 °C on Tryptone Soy Agar (TSA, Oxoid) slants (supplemented with 50 μg/ml nalidixic acid for *E. coli* O157:H7) and were renewed monthly. Working cultures were activated by transferring a loop culture from slants into 10 ml of Brain Heart Infusion broth (BHI, Oxoid) (supplemented with 50 μg/ml nalidixic acid for *E. coli* O157:H7) and incubation at 37 °C for 24 h. A reference stock culture of *C. jejuni* strains was kept at −75 °C in full-horse blood (E&O Laboratories, Bonnybridge, England). A swab of each strain was transferred into 10 ml of selective Bolton broth (Oxoid) and incubated at 41.5 °C for 48 h under microaerobic conditions provided by Campygen packs (Oxoid) in closed jars. Working stocks were stored at 4 °C under microaerobic conditions, and were renewed monthly. The working cultures were prepared by transferring 0.1 ml of each stock culture into 10 ml of fresh Bolton broth and incubation under microaerobic conditions at 41.5 °C for 48 h to stationary phase. Purity and verification of all the cultures concentration (8–9 log CFU/ml) were confirmed by 10-fold serial dilutions from working cultures into Peptone Physiological Salt solution (PPS, containing 1 g/l neutralized bacteriological peptone and 8.5 g/l NaCl) and spread plating 0.1 ml from selected dilutions onto duplicates of TSA plates.

2.2. Determination of D-values of bacterial strains

2.2.1. Heat challenge

A stationary phase culture of each tested bacterial strain was diluted in BHI or Bolton broth, in case of *C. jejuni* strains, to around 6 log CFU/ml. At set time points (i.e. 0, 2, 4, 6, 7, 8 and 9 min), 1 ml of the diluted culture was used to inoculate 9 ml of pre-heated heat challenge medium to establish heat inactivation curves. The heat challenge medium BHI (or Bolton broth in case of *C. jejuni* strains) was dispensed in test tubes, submerged in a water bath (Memmert, WNB 10, Schwabach, Germany) and preheated to the target inactivation temperature of 60 °C before being inoculated. The temperature of the medium was monitored using a Testo 177-T4 temperature data logger (Testo AG, Lenzkirch, Germany) in a test tube with non-inoculated BHI or Bolton broth throughout the duration of the heat treatment (i.e. 10 min). Ten minutes after the first inoculation all inoculated test tubes were taken together from the hot water bath and placed in an iced water bath to cool down before enumeration. The heat treatment was performed in triplicate for each strain.

In addition, the heat resistance of all strains was also determined in a heat challenge medium (BHI or Bolton broth) adjusted towards pH 5.6 and 1.5% NaCl (w/w); both values mimicking those measured in ground pork meat. Lactic acid (10 mol/l) (Roland Chemicalien, Brussels, Belgium) was used to adjust the medium to pH 5.6 (after autoclaving). The added volume of lactic acid did not significantly affect the volume of the challenge medium. The pH and a_w values of the adjusted broth were confirmed with a digital pH-meter (pH flash seven easy, Mettler-Toledo, Zaventem, Belgium) and an a_w-cryometer (NAGY AWK-30, NAGY Messysteme, Gaufelden, Germany).

2.2.2. Enumeration of surviving organisms

The number of surviving organisms was determined by tenfold dilutions of the inoculated heat medium in PPS, followed by plating on appropriate selective isolation media. Enumeration of *L. monocytogenes* was performed by spread plating 0.1 ml on Agar Listeria Ottaviani & Agosti (ALOA) (Biolife, Milano, Italy), while *Salmonella* was enumerated