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# EFFECT OF FAT CONTENT, ADDITIVES AND STORAGE TEMPERATURE ON THE HEAT RESISTANCE OF Escherichia coli 0157:H7 IN MEAT AND MEAT PRODUCTS BY

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# ABSTRACT

The effect of storage temperatures at either -18 or 5°C, holding at 25°C and salt stress on heat resistance of E. coli 0157:H7 was studied at 55°C in a nutrient medium. Cultures stored frozen at -18°C and under refrigeration at 5°C were generally more heat sensitive than those stored at 25°C which had greater heat resistance. Salt stress resulted in increase (1.5%) followed by decrease (3%) in heat resistance of E. coli 0157:H7. Effect of fat content (8 or 15%) on heat resistance of E. coli 0157:H7 was determined in ground beef 8 and 15% fat at 55°C. Results showed that ground beef containing 15% fat offered protection compared to 8% fat, indicated by higher recovery of heated E. coli 0157:H7 cells in case of 15% fat. The effects of additives and storage temperatures on heat resistance of E. coli 0157:H7 were investigated in beef burgers containing 69% meat and 31%, other ingredients (rusk, onion, salt, spices and tap water), the burgers were then stored at 5 or -18°C. This study found that additives had profound effects on the heat resistance of E. coli in fresh beef burgers, while refrigerating and freezing decreased the heat resistance of E. coli 0157:H7, as indicated by the lower D-values for these samples than the D-values for fresh beef burger samples. The results of this study will be beneficial to the food industry in designing HACCP plans to effectively eliminate E. coli 0157:H7 in the meat products.

# INTRODUCTION

Several outbreaks of Escherichia coli 0157:H7 infections have been documented since the pathogen was first identified as causing illness associated with under cooked ground beef. The infections dose of E. coli 0157:H7 is quite low, i.e., less than a few hundred cells (Doyle et al., 1997). Most outbreaks have been linked to the consumption of foods from animal origin. Although infections have also been associated with raw vegetables (Beuchat, 1998; Tharrington et al., 2004). The potential for microbial safety and spoilage problems due to new food sanitation, processing or preservation technologies must be evaluated, as their introduction may present new opportunities for emerging or acknowledged foodborne pathogens to assert themselves. For example, increased reliance on refrigeration and increased production of extended shelf life refrigerated foods may have played a role in the emergence of Listeria monocytogenes as a major

foodborne pathogen (Miller et al., 1998). Stress conditions may be encountered by bacteria in minimally processed foods or in foods that are processed using hurdle technology (Abee and Wouters, 1999). Subsequent processing or handling may allow poststress growth. Food processing methods apply a variety of stresses analogous to environmental stresses that interfere with bacterial homeostasis, prevent growth, or kill foodborne pathogens (Buncic and Avery, 1998; Leenanon and Drake, 2001; Alvarez et al., 2004). Low-temperature stress can increase synthesis of stress proteins, which can confer increased resistance to subsequent stresses, including heat (Sheridan and McDowell, 1998; Rowan, 1999). Such effects directly influence the extent to which pathogens, including E. coli 0157:H7, survive in food and hence the risks posed to consumers (Byrne et al., 2002).

In more general terms, the survival of pathogens and other bacteria in food ingredients and products is also significantly influenced intrinsic elements of the food ecosystem (Blackburn et al., 1997; Koutsoumanis et al., 1999). Thus, the composition of meat products and with particular reference to commercial burger formulation, the inclusion of non-meat ingredients, such as spices can influence the extent of survival and/or growth of bacteria present in such products.

The present work studied the effect of storage temperature, fat content and additives on the heat inactivation of E. coli 0157:H7 in a nutrient medium or in ground beef.

# MATERIALS AND METHODS

# Materials:

E. coli 0157:H7 (ATCC 69373) strain was obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Stock culture was maintained on tryptic soy agar (TSA) (Biolife), slants stored at 3°C. Every 7 to 10 days, a loopful of this culture was transferred to fresh TSA slants and incubated for 24 h at 37°C. These cultures were then stored at ambient temperature and used as stock cultures for experimental procedures (Leenanon and Drake, 2001). For inoculation, cultures were transferred to tryptic soy broth (TSB) (Biolife) and grown at 37°C for 12 h. cultures were then serially diluted in 0.1% peptone and inoculated into fresh TSB, followed by incubation at 37°C for 12 h for growth to stationary phase. Stationary phase cultures were used since such cultures have been shown to have greater heat resistance than log-phase cultures (Todd et al., 1993).

Cow meat animal (round cuts) and fat were purchased from a local market in Ismailia Governorate. The meat was cut to small pieces and divided into two portions then frozen at -18°C for 12 hrs. Frozen meat and fat (different percent ages) were minced two times (using small house mincer) and autoclaved at 121°C for 15 minutes and used for experiments. Fat content was determined according to A.O.A.C (1995).

# Thermal Resistance in a Nutrient Medium Cold and freeze stress:

0.1 ml of stationary phase culture, prepared as described above, was inoculated into 4 ml vials containing 3.9 ml of sterile TSB. Tubes were stored at  $5^{\circ}$ C  $\pm 0.5^{\circ}$ C, or  $-18^{\circ}$ C  $\pm 1^{\circ}$ C for one to seven days. After each storage period (1, 2, 3, 4, 5, 6 and 7 days) two tubes were removed for enumeration prior to heating. The remainder vials from each storage treatment were immersed in constant temperature water bath at 55°C ± 0.1. Temperature was monitored using a handheld digital thermometer (inserted into sealed vials filled with 4 ml of TSB). When sample vials reached 55°C, duplicate samples were removed and placed in an ice water bath for 30 s. these samples represented the initial (0 min.) population for the determination of heat resistance at 55°C. Duplicate vials were then removed from the water bath at 5 min for up to 60 min. cooled vials were stored at 3°C prior to analysis. Enumeration of surviving organisms in each sample was performed by serial dilutions of cultures and inoculation onto TSA using a spread plate technique. Plates were incubated at 37°C for 24 h prior to counting. Log CFU/ml survivor curves at 55°C were determined from the survival data obtained and D<sub>55</sub>-values were determined from models of the log CFU/ml linear survivor curves (Jackson et al., 1996).

# Hold stress:

To evaluate changes in thermal resistance as a result of hold stress at 25°C. inoculated vials were subjected to holding at  $25^{\circ}$ C  $\pm 1.0^{\circ}$ C for 2, 4, 6, 8, 10 and 12 h prior to heating at 55°C. survivor curve and D-value at 55°C for the samples treated were determined as described.

# Salt stress:

To evaluate changes in thermal resistance as a result of salt stress. Stationary-phase cultures were inoculated into vials supplemented with 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3% sodium chloride and the heat resistance at 55°C was determined as previously described.

# Thermal Resistance in Minced Meat

# A- Effect of fat content:

Duplicate 50 g ground beef 8 or 15% fat were weighed into sterile pouches and inoculated with  $E.\ coli\ 0157:H7$  at concentration of approximately  $10^6\ CFU/g$ . Negative controls included bags containing meat samples inoculated with 0.1 ml of 0.1% (w/v) peptone water with no bacterial cells. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample, compressed into a thin layer (Ca, 1.0 cm thick) by pressing against a flat surface excluding most of the air and then heat sealed (Byrne  $et\ al.$ , 2002).

# B- Effect of additives and storage temperatures:

Beef burger samples were prepared to contain 69% minced meat (8% fat), 2.5% spices (red pepper 11.5%; all spices 10%; black pepper 45%; coriander 12%; nutmeg 2.5%; commin 150% and clove 4%) and 28.5 other ingredients (13% rusk; 11% onion; 1.5% salt and 3% tap water) and autoclaved at 121°C for 15 minutes. Control sample prepared of minced meat (8% fat) without any

additives. Duplicate 50 g of each sample were weighed into sterile pouches and inoculated with  $E.\ coli\ 0157:H7$  at concentration of approximately  $10^6\ CFU/g$  according to (Byrne et al., 2002). To determine the influence of storage temperatures on the heat resistance of  $E.\ coli0157:H7$ , beef burger samples were frozen at  $-18^{\circ}C \pm 1^{\circ}C$  and refrigerated at  $5 \pm 0.5^{\circ}C$  for one to seven days. Bags containing samples that were not refrigerated or frozen were designated as fresh samples. Frozen and refrigerated samples were thawed to room temperature prior to heating.

# Thermal Inactivation:

Bags at room temperature were placed in a basket and then fully submerged in a temperature controlled water bath, stabilized at 55°C. the temperature was continuously monitored by a hand thermometer at the center of two uninoculated bags (negative controls). Survivor curves and D-values at 55°C for samples were determined as described by (Jackson et al., 1996).

# RESULTS AND DISCUSSION

# Thermal Resistance in a Nutrient Medium

Fig. (1) shows that cold stress decreased the heat resistance of *E. coli* 0157:H7. The decrease in heat resistance following cold stress may be due to the induction of cold shock proteins and the repression of heat shock proteins (Berry and Foegeding, 1997; Leenanon and Drake, 2001). In the present study, control cultures grown to stationary phase at 37°C were more heat resistant than similar cultures which had been stored at 5°C prior to heating. Dantur and Pizarro (2004) reported that several stressing factors including heat, pH, chemicals or physical agents, induced an elevated resistance to a subsequent stress.

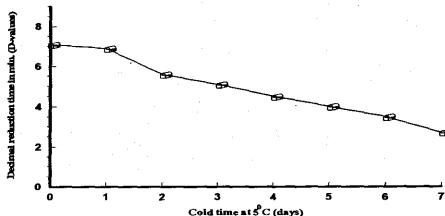


Fig. (1): D-values in minutes for E. coli 0157:H7 heated in TSB at 55°C for 60 min, after cold storage at 5°C.

Fig. (2) shows that samples stored for 1 day at -18°C had  $D_{55}$ -value of 6.8 min, while cultures stored for 2, 3, 4, 5, 6, and 7 days appeared to be more heat sensitive,  $D_{55}$ -values were 6.8, 5.3, 4.7, 4.5, 4.2 and 3.6, respectively. The

greater heat sensitivity of cultures stored at -18°C could be a result of a senstization of cells to the freezing process. These findings are in contrast to the results of Leenanon and Drake, (2001); Iwahashi et al. (1995) and Park et al. (1997) who demonstrated cell membrane flexibility is an important factor in freeze-thaw stress resistance. It is known that membrane flexibility can be modified by the binding of saccharides acting as cryoprotectants or by alteration of the phospholipid and neutral lipid compositions. Jackson et al. (1996) concluded that the variations in heat resistance can occur due to storage temperatures, growth phase as well as product formulation, sampling techniques and even strain differences within a species or serotype. On other hand, Teo et al. (2001) demonstrated that low temperature, high-pressure treatment had the potential to inactivate E. coli 0157:H7 strains in different fruit juices.

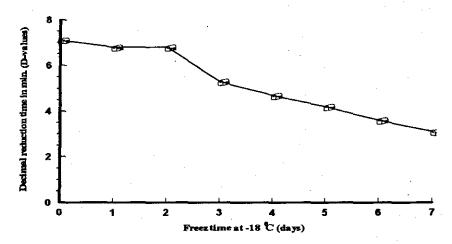


Fig. (2): D-values in minutes for *E. coli 0157:H7* heated in TSB at 55°C for 60 min. after freeze stress at -18°C.

Fig. (3) shows that  $D_{55}$  values increased from 7.1 min at zero time to 10.7 min after 12 hours of storage at 25°C. This resistance could be a result of physiological process within the cells or a combination of factors including culture age, growth phase or cell density (Jackson et al., 1996). Also Trujillo et al. (2002) reported that P. fluorescens, L. helveticus and L. innocua showed higher resistance at 25°C than at 4°C. Several studies have reported that growth and storage temperatures influence the heat resistance of microorganisms (Condon et al., 1992; Van Opstal et al., 2004). Such increase in resistance could be an important concern if conditions resulting in this increase were reproduced in a food system. However, if the increase in heat resistance is a result of high population density, it is unlikely that these conditions would occur in food. Berry and Koohmaie (2001) demonstrated the importance of temperature control during meat handling and storage to prevent the outgrowth of this pathogen and indicated that proper sanitation and processing practices that prevent and reduce contamination of carcasses with E. coli 0157:H7 are essential regardless of background microflore levels.

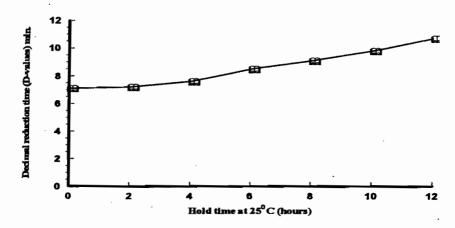


Fig. (3): D-values in minutes for E. coli 0157:H7 heated in TSB at 55°C for 60 min. after hold stress at 25°C.

Fig. (4) shows that  $D_{55}$  values increased from 7.1 in control samples to 8.5, 8.9 and 8.2 after salt stress with 0.5, 1.0 and 1.5% sodium chloride respectively. These results are in agreement with those of Kaur *et al.* (1998) who reported that a decrease in \*w (from 0.98 to 0.96) resulted in an increase in the heat resistance of *E. coli.* 0157:H7 in salt and sucrose solutions. On the other hand  $D_{55}$  values decreased to 7.0, 6.3 and 5.1 after salt stress with 2.0, 2.5 and 3.0% sodium chloride. This decrease could be a result of a physiological process within the cell.

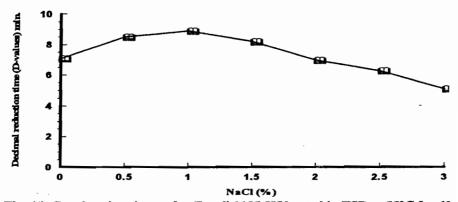


Fig. (4): D-values in minutes for E. coli 0157:H7 heated in TSB at 55°C for 60 min. after salt stress

# **Heat Resistance of Inoculated Ground Beef:**

# A- Effect of fat content

The D-values of *E. coli* 0157:H7 in ground beef (8 and 15% fat) at 55°C were determined. The D-values obtained in ground beef were 12.1 and 15.6 min after 60 min at 55°C for 8 and 15% fat, respectively (Fig., 5). Ground beef containing 15% fat offered protection compared to 8% fat, indicating by higher

recovery of heated *E. coli.* 0157:H7 cells in case of 15% fat. Similar results were observed by Juneja *et al.* (1997) who reported that the increased thermal resistance of *E. coli* 0157:H7 in beef compared to chicken may be attributed to the effect of different species and the differences in fat content between the substrates. Ahmed *et al.* (1995) reported that the D-value of *E. coli* 0157:H7 in ground beef heated at 60°C in thermal death time tubes ranged from 0.45 (beef 7% fat) to 0.47 (beef, 20% fat) min; the values ranged from 0.38 min (3% fat) to 0.55 min (11% fat) in chicken. Slight differences by previous workers may be attributed to different *E. coli* 0157:H7 strains, methodology, physiological conditions of the cells and fat content (Alvarez *et al.*, 2004; Van Opstal *et al.*, 2004).

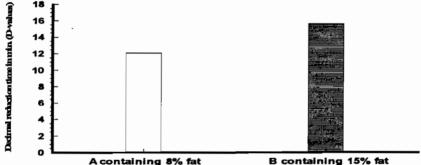


Fig. (5): Effect of fat content on heat resistance (expressed as D-values) for Escherichia coli 0157:H7 in ground beef at 55°C

# **B-Effect of additives**

The D-value for E. coli 0157:H7 in fresh minced beef containing 8% fat (control sample) was 13.0 min at 55°C (Fig., 6). These results are not in agreement with previous reports of D-values for E. coli 0157:H7 in fresh minced beef containing 8% fat. Byrne et al. (2002) found D-values of 20.8 min at 55°C, 2.7 min at 60°C and 0.6 min at 65°C. Juneja et al. (1997) reported D-values of 21.13 min at 55°C, 3.17 min at 60°C and 0.39 at 65°C. However, the D-values for E. coli 0157:H7 in fresh control sample obtained in the present study are lower than those reported in the literature by some other investigators. Such diversity in results has been recognized within a recent review of the published data on the heat resistance of E. coli 0157:H7 (Stringer et al., 2000), who reported D-values for E. coli 0157:H7 in meat at 60°C ranging from 0.3 to 10 min., such variation has been attributed to differences in test conditions and experimental procedures used in the reviewed studies. Factors shown to influence estimates of heat resistance, leading to inconsistencies between studies, include differences among strains, the physiological conditions of the E. coli 0157:H7 examined, the composition of the suspending medium and different thermal inactivation and survivor enumeration methodologies (Juneja et al., 1997; Duffy et al., 1999; Stringer et al., 2000; Byrne et al., 2002; Dantur and Pizarro, 2004). These factors impede the accurate comparison of results from different studies.

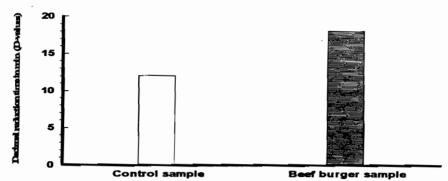


Fig. (6): Effect of additives on heat resistance (expressed as D-values) for Escherichia coli 0157:H7 in fresh beef burger at 55°C

In the present study, the D-value for *E. coli* 0157:H7 in the fresh beef burger sample was 18.1 min at 55°C (Fig., 6). The data show that D-value for *E. coli* 0157:H7 in the beef burger formulation containing 31%, non meat ingredients was higher than the D-value for the pathogen in the control sample (100% meat), this finding clearly demonstrates the impact of product formulation on heat resistance. Kotrola and Conner (1997) reported higher D-values for *E. coli* 0157:H7 in turkey products that included non meat ingredients, than in products composed of 100% turkey. Similar results reported by Byrne *et al.* (2002).

Refrigerating and freezing of beef burger decreased the heat resistance of *E. coli 0157:H7* in beef burger, as indicated by lower D-values for refrigerated and frozen samples than the D-values for fresh beef burger samples (Fig., 7). These finding are in agreement with the results of Byrne *et al.* (2002), while are in contrast to the results of Jackson *et al.* (1996) who demonstrated increased heat resistance of *E. coli 0157:H7* in frozen minced beef patties in comparison to fresh patties at 3 and 15°C. Another study reported no change in the heat resistance of *E. coli*0157:H7 in minced beef after freezing (Juneja *et al.*, 1997). Such disparities between results may be due to some factors, including the differences in experimental procedures used (Tharrington *et al.*, 2004).

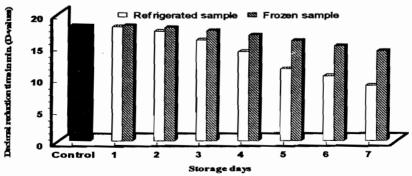


Fig. (7): Effect of storage temperatures on the heat resistance of *E. coli* 0157:H7 in beef burger at 55°C

In this study, the D-values of E. coli 0157:H7 in broth were different to those in the beef burger formulations at the same temperature. This result is supported by the findings of Williams and Ingham (1997), who demonstrated that the heat resistance of the pathogen was different in beef than in tryptone soya broth. It is also in line with the more general recognition that treatment can significantly influence the thermal resistance of E. coli 0157:H7 (Blackburn et al., 1997; Kaur et al., 1998; Koutsoumains et al., 1999). Liquid media studies may not be representative of the conditions in a food, particularly in heterogenous foods such as comminuted meat products, which are difficult to accurately reproduce in a model system (Byrne et al., 2002).

This study demonstrated that variations in heat resistance can occur due to product formulation and storage temperatures. Product formulation should be a critical consideration as an integral part in the determination of risk associated with E. coli 0157:H7 in beef burgers and also in the development and application of cooking processes which can adequately reduce the risks posed by such pathogens in foods. The study also demonstrated that food service handling conditions should then be optimized to place the organism in a more heat sensitive state, thereby maximizing the effectiveness of cooking treatments.

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# تأثير محتوى الدهن والإضافات ودرجة حرارة التخزين على المقاومة الحرارية لميكروب E. coli 0157:H7 في اللحوم ومنتجاتها

آمال عبد الفتاح جاب الله ، عادل ابو بكر شطا قسم الصناعات الغذائية - كلية الزراعة بالإسماعيلية- جامعة قناة السويس

فى هذا البحث تم دراسة تأثير درجة حرارة التخزين على  $^{\circ}$ م أو  $^{\circ}$ ام أو الحفظ على  $^{\circ}$ م كذلك تأثير ملح كلوريد الصوديوم على المقاومة الحرارية لميكروب  $E.\ coli\ 0157:H7$  على  $^{\circ}$ 00 فى البيئة المغذية.

كما تم دراسة تأثير نسبة الدهن في اللحم المفروم وتأثير كلا من الإضافات والتخزين على ٥٥م أو -١٨٥م في البيف برجر على المقاومة الحرارية لنفس المبكروب.

وقد أوضحت النتائج أن المزارع التي تم تخزينها على ٥°م أو -١٨°م اقــل مقاومة للحرارة من التي تم حفظها على ٢٥°م، تأثير الملــح أدى الِــي زيــادة فــي المقاومة الحرارية حتى تركيز ١,٥% تبعها نقص بزيادة التركيز إلى ٣٣.

أوضحت النتائج أيضا أن زيادة نسبة الدهن إلى ١٥% فى اللحم المفروم أدت إلى زيادة المقاومة الحرارية للميكروب مقارنة بنسبة دهن ٨٨.

اظهر ميكروب E. coli 0157:H7 مقاومة حرارية عالية في عينات البرجر وذلك نتيجة للإضافات مقارنة باللحم الخام (الكنترول) ، كما ادى التخرين مسواء بالتبريد أو التجميد الى نقص المقاومة الحرارية في عينات البرجر.

وتعتبر نتائج هذه الدراسة مفيدة عند تصميم نظام الله HACCP في صاعة اللحوم للتخلص بنجاح من ميكروب E. coli 0157:H7 في اللحوم ومنتجاتها.