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Full Length Research Paper

Effect of short-time microwave heating on survival of *Escherichia coli* O 157 in beef hamburgers

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Survival of three *Escherichia coli* strains O 157: B 032, LMG 8223, IVK 805 from the collection of the Department of Veterinary Protection of Public Health, Faculty of Veterinary Medicine, University of Gent, Belgium, in breaded and unbreaded beef hamburgers (n = 144), subjected to short microwave heating was determined. 50 g hamburger samples were contaminated by bacterial suspension injections (the initial contamination level was 10^6 cfu/g of product). Microwave heating for 30, 60, 90, 120 and 150 s was done using a microwave cooker (Dialog cook, Moulinex, at 480 and 760 W). 30 s heating at 480 W caused test bacteria cells partial reduction that was more pronounced in breaded hamburgers. In the case of *E. coli* O 157: B 032, the reduction was lower than for the other two strains. At 480 W, none of the *E. coli* O 157 test strains survived 60 s of heating that corresponded to the hamburgers' temperature ranging from 75.2 to 89.1°C. Increasing the power to 760 W, decreased the bacteria inactivation time to 30 s while the hamburgers' temperature ranged from 71.9 to 78.8°C. Indifferent of the cooker power for each of the heating times, breaded hamburgers achieved higher temperatures than the unbreaded ones.

Key words: *E. coli* O 157, inactivation, microwaves, beef hamburgers.

INTRODUCTION

Escherichia coli O 157 (STEC) that produced Shiga-toxin was detected for the first time in 1982 as the etiologic factor of haemorrhagic colitis (HC) in human patients (CDC, 1982). In 1983, Karmali et al. (1983) demonstrated that *E. coli* O 157:H7 and other STEC serologic groups were responsible for the occurrence of the haemolytic uremic syndrome (HUS).

Epidemiological studies carried out to investigate the above and similar cases of infection with Shiga-toxin producing strains of *E. coli* showed that the food products of animal origin (especially beef and milk), raw fruits and vegetables contaminated with animal excrements, fruit juices and water were the main sources of infection (Armstrong et al., 1996; Paton and Paton, 1998). Also, direct animal-human and human-human contacts play an important role in this epidemiological chain. Over 100 STEC serotypes, isolated from patients with HC and/or HUS symptoms, have been described so far. *E. coli* O 157:H7 seem to be the most pathogenic among them, as it causes both sporadic and epidemiological infections, leading even to lethal outcomes (Johnson et al., 1996).

The best-known cases of infections with *E. coli* O 157 in humans are the epidemic in Japan in 1996 (8576 infected children, 106 HUS cases, three lethal outcomes), and the epidemic in Scotland, also in 1996 (501 cases of infection, 20 lethal outcomes) (Watanabe et al., 1996). Vegetables (Japan) and meat (Scotland) were the sources of infection in those epidemics. Those and other similar cases indicate that *E. coli* O 157 is one of the most dangerous infectious agents in food poisonings in humans.

Consequently, *E. coli* O 157 presence in products of animal origin (particularly beef products) resulting from their contamination may pose a serious consumer health hazard. For those reasons the knowledge on resistance

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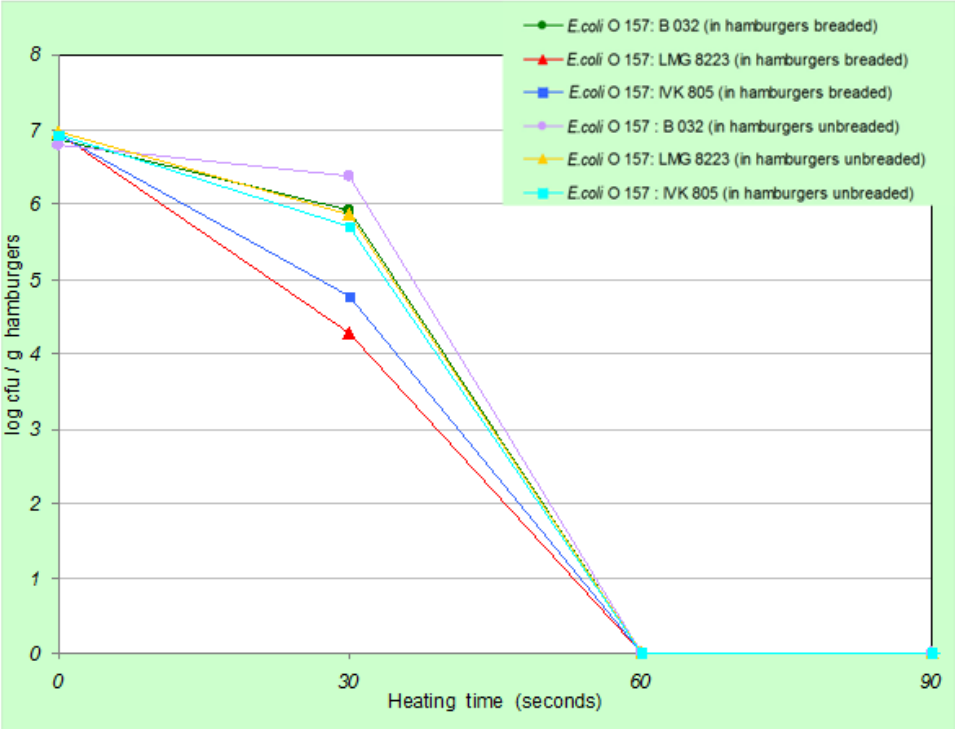

Figure 1. Survival of *Escherichia coli* O 157 after microwave heating (wattage of microwave oven - 480 Watt), according to bacterial strain, type of hamburgers and heating time.

of those bacteria to the environmental and technical factors and considering the wide range of products of animal origin that might be contaminated with *E. coli* O 157, plays an important role.

MATERIALS AND METHODS

Three strains: *E. coli* O 157*:* B 032, *E. coli* O 157*:* LMG 8223 and *E. coli* O 157: IVK 805, isolated from food infection cases in Europe were the test organisms.

50 g samples of breaded and unbreaded beef hamburgers were contaminated with the test strains. The contamination was conducted immediately prior to commencement of heating the hamburgers in the microwave cooker by injecting them with freshly prepared suspension of the specific bacterial strain (18 to 24 h culturing in a TSB - Tryptone Soya Broth by Oxoid), prepared according to the Mc Farland scale so that the initial contamination with the *E.coli* O 157 bacteria was at the level of 10^6 cfu/g of the product heated. The hamburgers temperature after contamination and before heating was at the level of ca. 6°C.

Hamburgers prepared in that way were microwave heated in the household microwave cooker (Dialog cook, Moulinex) applying two different power settings (480 and 760 W) and different heating times (30, 60, 90, 120 and 150 s). At the end of the heating process, the hamburgers were left in the closed cooker for another 30 s to equalise the temperature. Next, they were taken out recording at the same time their temperature (average temperature value form six different measurement points). During preparation of the hamburgers for quantitative microbiological tests, they were homogenised in a stomacher (Lab-blender 400, England) for 60 s. After preparation of consecutive decimal dilutions the culturing on the Eosin Methylene Blue Agar (Modified) (mEMB by Oxoid) was conducted. Following 48 h of incubation at 37°C, the *E. coli* O 157 colonies were counted on two parallel plates and the average values per 1 g of product were determined. At the same time, the test bacteria counts in the hamburgers that were not subjected to heating representing the controls were taken. Two series of tests were conducted for each bacterial strain applying all the specified variants of determination (n = 144). Statistical analysis was conducted by means of the univariate repeated-measures ANOVA test, t-Student and Mann–Whitney U Test, using the Microsoft Excel 2010 and Statistica 9 PL software package. The statistical significance level of α=0,05 was assumed.

RESULTS

The study results are presented in Figures 1 to 4. Figure 1 presents the survival rates of three *E. coli* O 157 strains in unbreaded and breaded hamburgers heated for different times in the microwave cooker at 480 W. The figure indicates that heating both types of hamburgers for 30 s caused reduction in the numbers of test bacteria cells that was more pronounced in breaded hamburgers, however the recorded differences were insignificant (univariate repeated-measures ANOVA test, p=0,131). In the case of *E. coli* O 157*:* B 032 strain, the reduction level was slightly lower than in the case of the other strains. Nevertheless, no statistically significant differences between the survival of individual strains of *E. coli* 0157 in both types of heated hamburgers in the 480 W

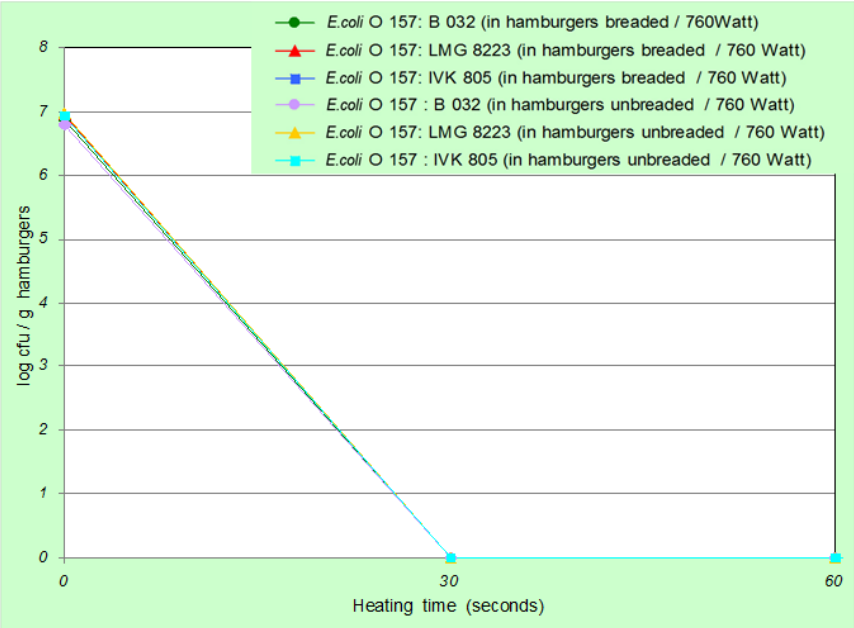

Figure 2. Survival of *Escherichia coli* O 157 after microwave heating (wattage of microwave oven – 760 Watt), according to bacterial strain, type of hamburgers and heating time.

microwave oven was found (Mann–Whitney U Test, $p > 0.05$). Extending the heating to 60 s at the same power setting (480 W), caused total destruction of all the test *E. coli* O 157 strains. Application of the higher cooker power (760 W), accelerated the effect of total inactivation of the test bacteria, which was achieved after 30 s heating already (Figure 2). That effect was achieved for all three test *E. coli* strains and for both hamburger types. Statistical analysis of results confirmed the existence of statistically significant differences in the survival of *E. coli* 0157 strains tested in the experiment regarding the power of the oven (480 and 760 W respectively) in both breaded and unbreaded hamburgers (univariate repeated-measures ANOVA test, $p=0.000$).

The presented reduction level results (Figures 1 to 2), represent average values from the two series of tests; no significant differences between the results from the individual test series were found.

To present the complete image of the results obtained, the time and hamburger's temperature after heating was determined (as the average value of measurements taken at six different points at the time of taking the hamburger out from the cooker). Figures 3 and 4 present the correlation between the microwave radiation influence and changes of temperature taking place in breaded and unbreaded hamburgers depending on the heating power applied. Figure 3 indicates that after heating the hamburgers in the cooker set at 480 W for 30 s, the temperature of the samples increased to 64.1 °C in unbreaded hamburgers and 72.2 °C in breaded hamburgers; with heating time increased to 60, 90, 120 and 150 s, the temperatures increased to 75.2 and 89.1, 90.7 °C and 94.1; 91 and 94.5 °C and finally to 93.1 and 96.2 °C respectively. In the case of heating in the cooker set at 760 W, the temperature of unbreaded and breaded hamburgers reached the values of 71.9 and 78.8 °C; 82.5 and 92.3 °C; 90.2 and 94.4 °C; 92.7 and 95.4 °C and 94.6 and 98.1 °C for the same heating times respectively (Figure 4). Indifferent of the cooker power (480 and 760 W), for every heating time applied, breaded hamburgers reached higher final temperatures than the unbreaded ones. The recorded differences were insignificant statistically (Mann–Whitney U Test, $p = 0.296$ and t-Student test $p=342$).

In the case of unbreaded hamburgers heated at 760 W for 30 s, the temperature of which was similar to the temperature of breaded hamburgers heated at 480 W also for 30 s (Figures 3 and 4) deserves attention. Despite similar temperatures (71.9 and 72.2 °C), the effect of test bacteria reduction in those products was surprisingly different. Almost identical temperature (ca. 72 °C), in one case resulted in total inactivation of the test bacteria (Figure 2), while in the other, it resulted in *E. coli* O 157 survival at the levels ranging from 4.28 to 5.92 log cfu/g of the product depending on the strain (Figure 1). That situation was recorded in both test series.

DISCUSSION

Use of microwave cookers shows continually increasing trend in households, gastronomy as well as food industry,

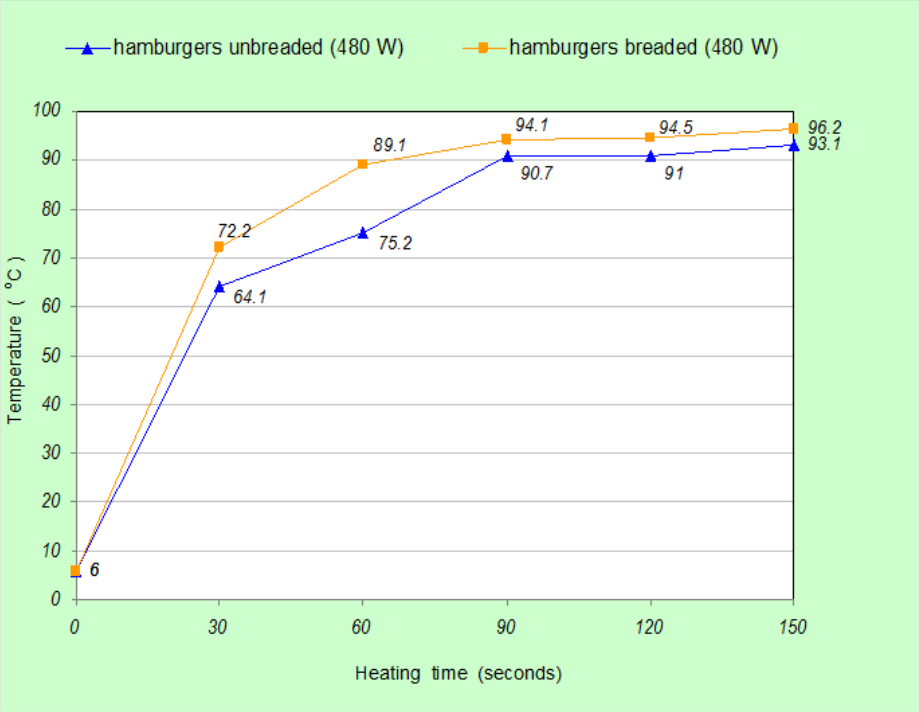

Figure 3. Temperature of hamburgers after microwave heating (wattage of microwave oven - 480 Watt) at different times.

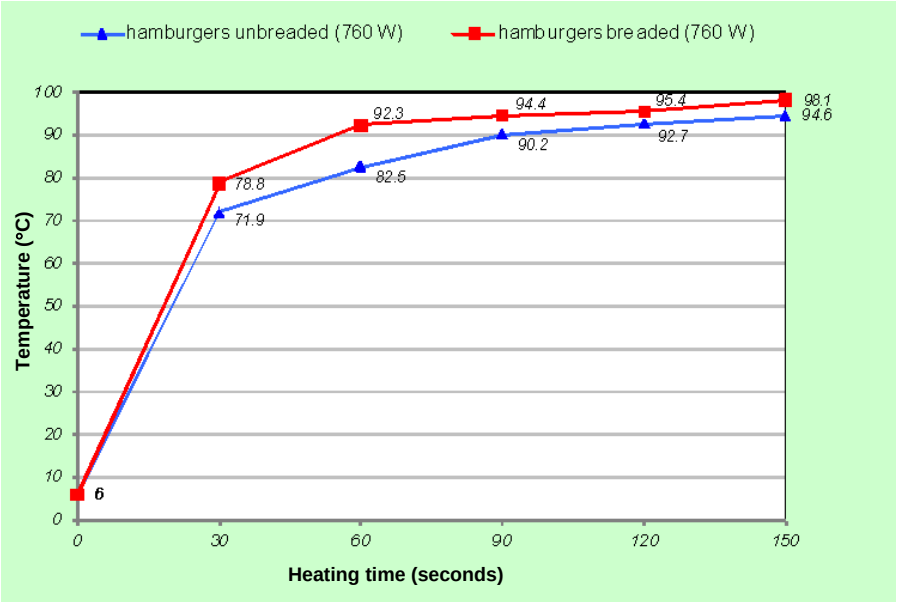

Figure 4. Temperature of hamburgers after microwave heating (wattage of microwave oven - 760 Watt) at different times.

which is related to both the simplicity of operating such devices and the purely practical aspect of offering a short meal preparation time. As indicated by numerous studies, the possibility of survival of various pathogens in food products subjected to microwave heating exists. That is possible as a consequence of differences in the

sensitivity of individual microorganisms present in meat and meat products heated by microwaving (Baker et al., 1983; Aleixo et al., 1985; Lindsay et al., 1986; Schnepf and Barbetu, 1989; Farber et al., 1998; Göksoy et al., 2000; Apostolou et al., 2005; Pucciarelli and Benassi, 2005; Dąbrowski et al., 2009; Shen, 2009; Jamshidi et al. 2009; Jamshidi et al. 2010).

This is also the case with *E. coli* O 157*,* which is present in slaughter animals (particularly cattle) and from the products of animal origin, can pose a serious threat for consumer health. The studies concerning the influence of microwave heating on *E. coli* O 157 survival indicates that the bacteria inactivation effectiveness is influenced by numerous factors. In own studies conducted, it was shown that the destruction of *E. coli* O 157 strains depends on the cooker power, hamburger type, temperature obtained and product heating time. Similar conclusions were reached by Quesada et al. (2003), who showed clearly during microwave heating of minced beef (for 60, 90 and 120 s) that the *E. coli* destruction level depends on the temperature and meat heating time. Jamshidi et al. (2010), investigating the influence of microwave heating on *E. coli* O 157:H7 survival on the surface of 200 g beef pieces, showed that the sample surface temperature increase was accompanied by gradual reduction in the test bacteria numbers. Total elimination of the pathogens was achieved after 30 s heating and exceeding the temperature of 70°C.

Czechowicz and Zottola (1996), heating beef rolls contaminated with *E. coli* O 157 in different types of microwave cookers, proved that the time necessary for inactivation of those bacteria was determined by cooker power and temperature obtained within the rolls. Korean researchers, Kim and Lee (2002), determined that beef immersion in 2% lactic acid solution for 2 or 4 s and next vacuum closing and microwave heating (after 70 s) was the most effective in the reduction of the *E. coli* O 157 count on the surface of samples tested. Entirely different conclusions concerning the possible reduction in the bacterial number under the influence of microwaves were reached by Göksoy et al. (2000). Those researchers, investigating the effects of short-time exposure to microwaves on selected bacteria determined that heating for 30 s and shorter did not cause changes in the number of bacteria present on the chicken breast surface and the number of *E. coli* and *Campylobacter jejuni* cells may even increase slightly.

Interesting results were obtained by Huang and Sites (2010), who placed beef samples contaminated with *E. coli* O 157:H7 (5,0±0,3 log cfu/g) in the heating chamber at different heights (0.03 m and 0.07 m), subjecting them next to heating in the high power microwave cooker (2,45 GHz; 1250 W). They showed that heating the samples positioned higher (applying the same heating parameters) was more effective in elimination of the pathogens investigated.

Woo et al. (2000), in their study on damages to bacteria at the cellular level caused under the influence of microwaves, exposed the suspension of bacteria (*E.coli* and *Bacillus subtilis)* in 0.9% NaCl to microwave radiation (in the 600 W cooker). Those observations showed that both the type and extent of damages on the surface of the bacteria depended to a large extent on the type of bacteria; those damages were much more extensive in the case of *E.coli*. On the other hand, Tsuji and Yokoigawa (2011), in their studies related to the influence of microwaves on microorganisms investigated the important issue of the microwaving influence on the ability of verotoxin production by *E. coli* O 157:H7.

The results they achieved indicate that both heating the suspension of bacterial cells at 65°C (60 s; 2.45 GHz, 100 W), and the influence of microwaves without a clear thermal effect (temperature increase to 37°C; 2.45 GHz, 0.6 W/ml), caused evident inhibition of verotoxin production ability. Consequently, Tsuji and Yokoigawa came to the conclusion that exposure of *E. coli* O 157:H7 bacterial cells may result not only in the reduction of their number but it can also limit their verotoxin production capacity. The present study showed higher temperature ranges and more expressed reduction of pathogens in breaded hamburgers. Thus, it is likely that coating batter - being a kind of "thermoisolator" - influenced both higher terminal temperature of heating hamburgers, as well as the slower rate of decrease hamburgers' temperature after heating. This created less favorable thermal conditions to the survival of bacteria in the product than in hamburger without batter. The special case recorded in our own studies where it was noticed that depending on the cooker power applied (480 or 760 W), 30 s of heating the hamburgers and the temperature achieved as a consequence of ca. 72°C, was accompanied by the effect of total inactivation of all the *E.coli* O 157 strains tested while in the other case, the survival of those bacteria at the level dangerous to human health was observed which deserves attention. The described case provides evidence that achieving the specific range of temperatures in the product heated is not always equivalent to achievement of the desired effect in reduction in the number or total inactivation of microorganisms in the product. The temperature is then an important but only one of many factors influencing the survival of microorganisms in the products heated in microwave cookers and as a consequence is correlated to their safety to health.

Conclusions

Own studies as well as the studies by the referenced authors indicate the need for considering the cooker power and duration of microwave influence, the type, size and shape of the product heated and its temperature during microwave heating of food products so that the

chances of inactivating the possible pathogens are maximised. Appropriately high temperature of the product heated by microwaving does not always offer the guaranty of a significant reduction in the number or total inactivation of pathogens possibly contained in the product. Both the mechanisms and the scope of changes taking place within the bacterial cells, on their surface or in their life functions resulting from the exposure of the bacteria to microwaving are not fully known yet. That is why undertaking further studies concerning the widely understood influence of microwaves on microorganisms (particularly the bacteria the presence of which in food has direct influence on food safety) seems still fully justified.

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