J. Food and Dairy Sci., Mansoura Univ., Vol. 5 (2):153 - 168, 2014

EFFECT OF MICROWAVE EXPOSURE COMPARED WITH CONVENTIONAL HEAT TREATMENTS ON *Escherichia coli* O157:H7 AND *Salmonella typhimurium* IN CHICKEN BURGER AND MEAT SAUSAGE

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ABSTRACT

Escherichia coli O157:H7 *and Salmonella typhimurium* are two of the most important foodborne pathogens causing gastroenteritis all around the world . Two samples were taken from chicken burger and meat sausage samples ,which prepared in a manner similar to commercial one . Then, samples inoculated with approximately 10^6-10^7cfu/g of *E. coli* O157:H7 and10^7-10^8cfu/g of *S. typhimurium* then, cooked by microwaving , grilling , frying and boiling . Following exposures to heat treatments, viable counts and temperature measurements were immediately performed.

Elimination of *E. coli*O157:H7 inoculated onto chicken burger was observed after microwaving, grilling, frying and boiling exposure for 40 sec., 7 min., 2.5 min. and 1.5min. , respectively and after 35 sec., 8 min., 2 min., and 2 min., for meat sausage samples, respectively.

S. typhimurium inoculated onto chicken burger was undetectable after microwaving, grilling, frying and boiling for 35 sec., 7 min., 1.5 min. and 1.2 min., respectively, and after 30 sec., 6 min., 1.5 min., and 1min., for meat sausage samples, respectively . This study showed that the lowest temperature degree at which elimination of *E. coli* O157:H7 was realized by microwave heating at 74°C for 40sec. and 66.6°C for 35sec. for both chicken burger and meat sausage. As for *S. typhimurium,* it was 65°C for 35 and 30 sec. for both chicken burger and meat sausage samples, respectively.

This study also showed that *E. coli*O157:H7 was more heat resistant than *S. typhimurium* under the same conditions.

INTRODUCTION

Sausages and burgers are common meat products in the market. The two products differ with regard to factors such as product component and cooking procedure, but they also have much in common: the main ingredients in both are meat and fat as raw materials, with salt and water added (Andersson *et al* ., 2000). Factors that may affect the growth or survival of foodborne pathogens in sausage products including water activity(aw),pH and temperature(cooking)(Hagmeer *et al*., 2011) *.* Exposure to high temperatures is one of the most common stresses experienced by food borne pathogens (Audia *et al*., 2001).As a consequence, undercooked meat is one of the main factors causing food borne illness (Zhao et al., 1998; Oldfild, 2001and Lianou and Koutsoumanis, 2009) and a large part (40–60%) of food borne illness cases are expected to originate from private households(Cogan *et al*.,2002).Consumption of beef products contaminated

with pathogens by susceptible individuals including children and patients may lead to severe symptoms such as watery or bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Bacon and Sofos, 2003).

The thermal processing of convenience foods is normally performed by typical, conventional methods, such as: cooking, frying, stewing and baking, but microwave heating is becoming an increasingly popular alternative (Dabrowski *et al.*, 2009). In a microwave oven, heating of food results from molecular friction between water molecules under an oscillating electric field of specific frequency (Pucciarelli and Benassi, 2005). Heating by microwave energy is used for several purposes, e.g., cooking, pasteurization, sterilization and blanching of foods (Giese, 1992; Datta and Davidson, 2001and Dabrowski *et al*., 2009) .

Meat and meat products have been implicated in the transmission of several kinds of human pathogens such as *E. coli* and *Salmonella spp* (Sanchez *et al*., 2002; Schlisselberg *et al*., 2013). There is no exact infective dose of Salmonella, but as few as 100 cells / 100 g sample of food have been reported to make people sick (Jay, 1996).Whilst *E. coli* O157:H7can cause an infectious dose which may be as low as 10 / 100 g organisms (Doyle *et al.,* 1997and Coia, 1998**)** .

The present study aims to investigate the effect of microwave heating compared with other heat treatments on the fate of *E. coli* O157:H7 and *S. typhimurium* inoculated onto both chicken burger and meat sausage samples .

MATERIALS AND METHODS

MATERIALS

Imported frozen boneless beef steer from the shoulder cut , frozen boneless chicken breasts ,fat tissues (fresh from different parts of the bufflo carcass,then minced), salted mutton and spices (salt , suger , blackpepper , cardamon , cubeb , cloves and nutmeg) were purchased from local market at El-Mansoura city, Egypt.

E.coli O157:H7 was obtained from Microbiol.,Dept.,Pharmacy.Fac. ,Mans. ,Univ., Egypt.

Salmonella typhimurium (ATCC-14028) was secured from Dairy Science Dept., Agric., Fac., Mans.,Univ. ,Egypt.

A stock culture of each strains was prepared in Tryptone Soya Agar.

METHODS

Preparation of samples

Chicken burger samples were prepared by mixing hens meat(68 %), fat tissues (10 %) , water (20 %),Na CL (1.4 %) , black pepper (0.4%) and nutmeg(0.2%) .Then ,were formed to 8 cm diameter and 1 cm thick patties, frozen at -18 °C and analysis were carried out (Gerred ,1969).

Sausage samples were prepared by mixing ingredients (meat 66% , fat tissues 15%,water 17 %, Na CL 1.79 % , sugar 0.008 % ,black pepper 0.056 %, nutmeg 0.033 %, cardamon 0.033 %, cubeb 0.04% and cloves

0.04%) using a blender , put in natural mutton casings, frozen at -18 °C then analysis were carried out (El-Dashlouty ,1978).

Preparation of *E .coli* O157:H7 and *Salmonella typhimurium* inocula

E .coli O157:H7 and *S. typhimurium* (ATCC-14028) were used for inoculating onto two samples of chicken burger and sausage meat .Stock culture of strains were prepared in Tryptone Soya Agar (TSA) at 4°C (Oxoid , Basingstoke,UK). Prior to each use , *E .coli* O157:H7 strain was inoculated at 1 % (v/v) into tryptone soya broth (TSB)(Oxoid), followed by incubation at 37° C for 18 to 24 h and *S. typhimurium* was grown in TSB at 37°C for 18 to 24 h .The concentration of the resulting culture of *E.coli* O157:H7 on MacConky agar(MA) and *S. typhimurium on* Salmonella and Shigella agar(SSagar)(Oxoid) were determined. This culture media were used for samples inoculating.

Inoculation procedure and microbiological analysis

Samples were immersed in 200 ml of the cultured *E.coli* O157:H7 *and S. typhimurium* in TSB for 30 min , separately . They were placed in sterile glass Petri dishes . One sample of each was reserved for estimating the concentration of each bacteria .

Heat treatments:

Various methods of heat treatment were used in this study such as microwaving , grilling , frying and boiling .

Microwave irradiation was performed in a household microwave oven (MW(Eco), NGM-120E) with a rotating glass plate, a frequency of 2450 MHz, and power of 1200 W) . The microwave was used at its full power at for heating inoculated samples for 15, 20 , 25 , 30 , 35,and 40sec. for both samples .Other samples were grilled at 180°C for 2,4,5,6,7 and 8min .Third samples were fried at the original frying pan temperature of 170 ° C for 20, 40, 60, 90 and 120 sec . Fourth samples were boiled at 100 °C in water base for 20, 40, 60, 90, and 120 sec .

Surface temperatures were measured immediately after exposures , using a thermometer(MINOLTA) .

Assessment of the survival *E.coli* O157:H7 and *S. typhimurium*

Different Heated samples were homogenized by homogenizer (MPW-120) in distilled water for 2 min. Decimal dilutions from each sample was prepared and viable count was carried out by surface plating on selective agar media followed by incubation at 37°C for 24 h .

RESULTS AND DISCUSSION

Effect of different heat treatments on *Escherichia coli* O157:H7 inoculated onto chicken burger and meat sausage

Results presented in Fig. 1and 2 show the effect of microwave treatment on *E .coli* O157:H7 in chicken burger and meat sausage samples

It could be seen that the increasing of heat temperature after heating chicken burger under microwave oven from 44 to 65°C , the decreasing of viable count of *E. coli* O157:H7 was 6.06 and 3.30 log cfu/g, meaning a drop of 0.81 and 3.57 log cycle for 15 and 35 sec., respectively .

A rapid decrease in viability from 5.07 to 3.39 log cfu/g realizing 1.62 and 3.3 log drop after microwaving sausage samples , when surface temperature was 48 ,60°C for 15 ,30 sec., respectively Fig. 1 and 2 .

The full elimination of *E .coli* O157:H7 in chicken burger was achieved after 40sec. ,when the surface temperature was increased to 74°C ,while it was 35 sec. for meat sausage, when the surface temperature was increased to 66.6°C Fig.1and 2 .

These results were consistent with those of Jamshidi *et al.*(2010), who found that enhancing the surface temperature more than 70°C, can eliminate *E. coli* O157:H7 of cattle beef slices.

In another study, *E. coli* O157:H7 in chicken breast portions was undetectable after 35 sec. of microwave exposure at 73.7°C (Apostolou *et al.,* 2005**).**

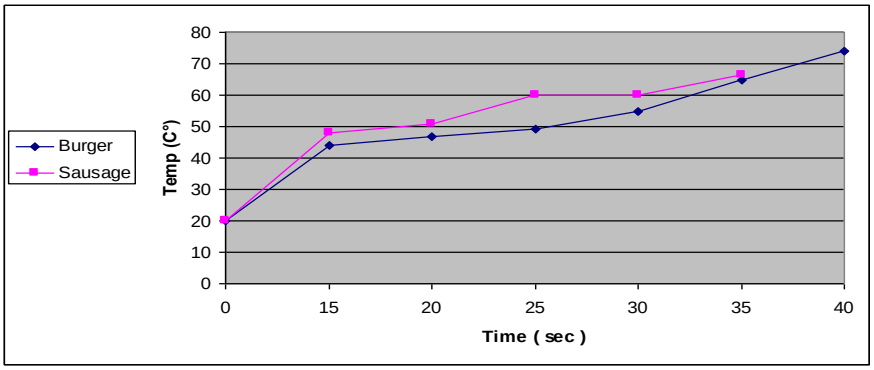


Figure1:-Final surface temperature of chicken burger and meat sausage inoculated with *E .coli* O157:H7 at different times of microwave exposure.

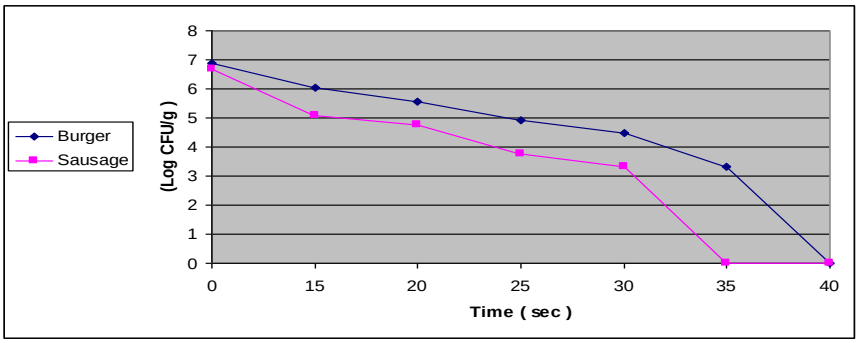


Figure2:- Survival of *E .coli* O157:H7 in chicken burger and meat sausage after microwave heating.

Data in Fig. 3and 4 showed that , when heat temperature during grilling chicken burger samples increased from 40 to 72.1°C, the viable count of *E .coli* O157:H7 decreased from 7.69 to 3 log cfu/g for120sec.and 360sec., respectively .It was observed that the drop in the viable count of bacteria was 0.52 and 4.69 log cycle .

As for meat sausage samples , the temperature degree increased during grilling from 53 to 67.1°C for 2 and 6min., the viable count of bacteria decreased from 6.60 to 3.39 log cfu/g, respectively. The log drop of bacteria were 0.42 and 3.63 log cycle Fig. 3 and 4 .

Elimination of *E .coli* O157:H7 was observed after 420 sec. exposure time ,when the surface temperature was increased to 75°C in chicken burger . When the surface temperature was increased to 72.2°C in meat sausage this bacteria was undetectable after the end of 480 sec. exposure time as showed in Fig. 3 and 4 .

These results are in agreement with those of Sporing (1999) ,who realized a reduction in viable number of *Escherichia coli* from 4.8 log to 6.5 log cycle using oven-broiled for cooking thick steaks on internal temperature of 54.4 °C and 76.7 °C, respectively .

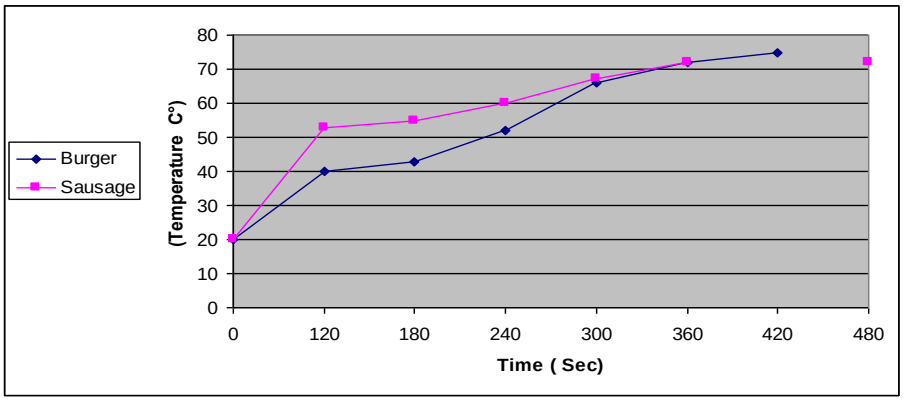


Figure3:-Final surface temperature of chicken burger and meat sausage inoculated with *E .coli*O157:H7 at different times of grilling.

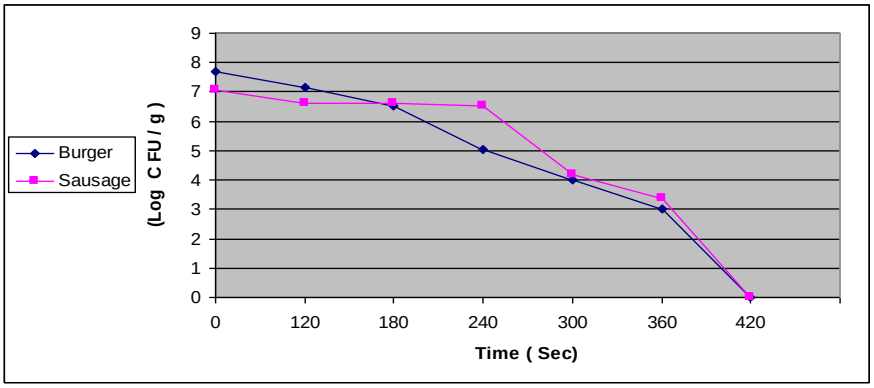


Figure 4:-Survival of *E .coli* O157:H7 in chicken burger and meat sausage after grilling.

The tolerance of *E .coli* O157:H7 to frying at different times was expressed by calculating the log of cfu /g at each examined sample . It

could be seen in Fig. 5and 6 that there was a change in the viable numbers of *E .coli* O157:H7 after 20 and 120 sec. at 41and 68 °C, respectively ,which were 5 log cfu/g and 2.69 log CFU/g for chicken burger (a reduction number of bacteria was 2.17 and 4.57 log cycle) .

There was 6.47 and 2 log cfu/g decrease in the viable number of *E .coli* O157:H7 after frying meat sausage samples for 20 and 100 sec. at 52.1 and 70°C , respectively. The viable number of bacteria decreased to 0.7 and 5.17 log cycle after frying samples Fig. 5and6.

Elimination of *E .coli* O157:H7 was observed after frying chicken burger for 150sec., when the surface temperature reached to 78°C.After frying meat sausage for 120 sec,the heat temperature was75°CFig. 5and 6.

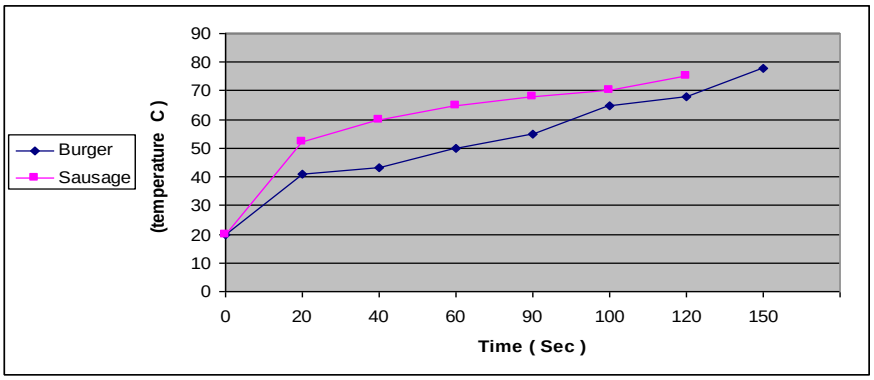


Figure 5:-Final surface temperature of chicken burger and meat sausage inoculated with *E .coli* O157:H7 at different times of frying.

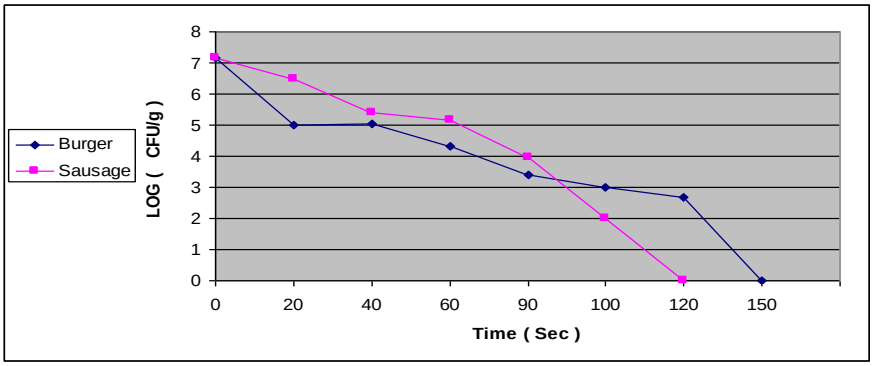


Figure 6:-Survival of *E .coli* O157:H7 in chicken burger and meat sausage after frying.

With regard to boiling treatment for chicken burger, increasing heat temperature from 45 to 61°C, resulted in a decrease of viable count number of *E .coli* O157:H7 (6 and 3.6 log cfu / g) after 20 and 90 sec. of boiling , respectively . This means that reduction number of bacteria is 1.39 and

3.79 log drop. The viable count of *E .coli* O157:H7 of meat sausage samples were 6.16 and 4.04 log cfu/g at 20 and 90 sec. ,for 50.4 and 60 °C ,respectively(The drop log was 0.83 and 2.96 log cycle) Fig. 7and 8 .

The full elimination of bacteria was achieved after boiling chicken burger at 72 °C for 90 sec. and meat sausage at 73 ° C for 120 sec. Fig. 7and 8.

These results were in agreement with those of **De Jong *et al*. (2012),** who determined the decimal reduction times of bacteria present on chicken fillet in boiling water, reported that whole chicken breast fillets were inoculated with *E .coli* O157:H7 . Extremely high decimal reduction times of *E .coli* O157:H7 of 1.97 min. were obtained when the surface temperature reached 85°C .

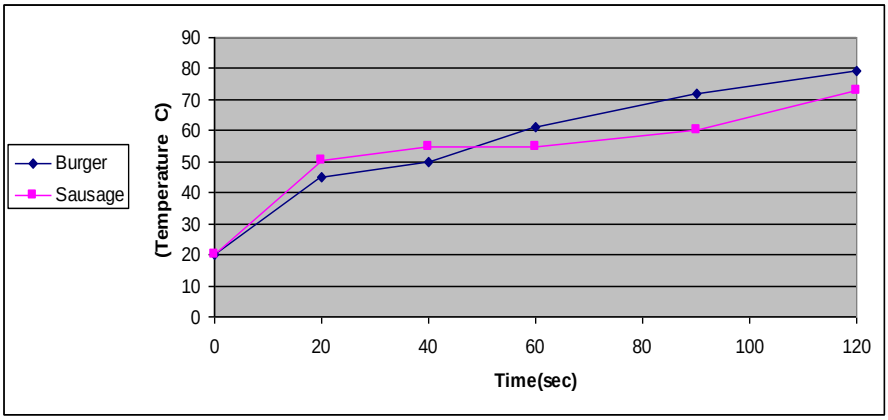


Figure 7:-Final surface temperature of chicken burger and meat sausage inoculated with *E .coli* O157:H7 at different times of boiling.

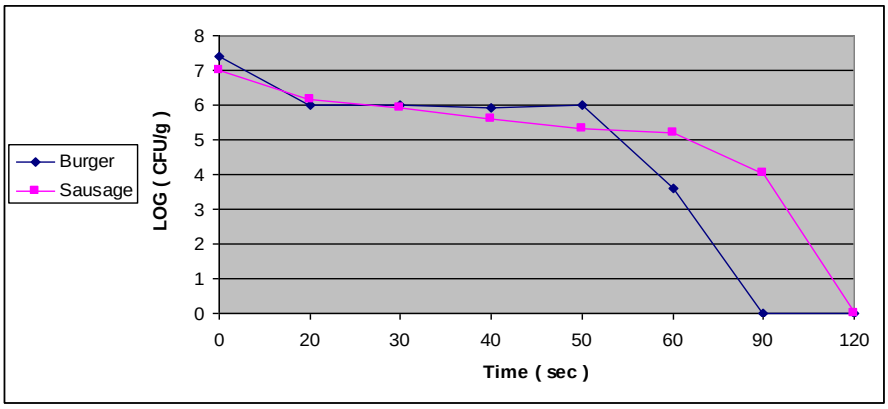


Figure 8:- Survival of *E .coli* O157:H7 in chicken burger and meat sausage after boiling.

Finally, it could be concluded that microwave heating is the best methods of inactivating *E .coli* O157:H7. Then, came boiling followed by frying. Grilling was the latest one as shown in Fig. (9).

A cooking study conducted by Sporing (1999) showed that the inactivation of *E .coli* by cooking decreased in order of : broiling > grilling > frying .

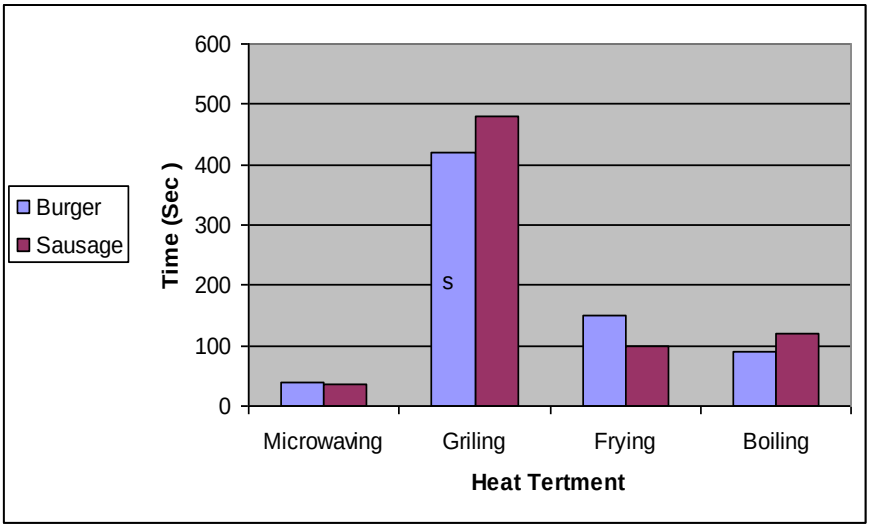


Figure 9:-The effect of microwave heating and conventional cooking methods on the inactivation of *E .coli* O157:H7.

Effect of different heat treatments on *S. typhimurium* inoculated onto chicken burger and meat sausage

Results obtained in Fig. 10 and 11 showed a rapid decrease in viability of *S. typhimurium* (from 6.87 to 3.81 log cfu/g) at 43 and 61.2°C, respectively, after microwaving chicken burger .The log drop in viability recorded 1.13 and 4.19 log cycle .

A rapid decrease in the viable number of bacteria was 6.17 and 3.30 log cfu/g at 45 and 61.1°C, respectively, after microwaving meat sausage samples .The log drop in viability recorded 1.22 and 4.09log cycle .

The full elimination of *S. typhimurium* was achieved after 35 and 30 sec. at 65°C for chicken burger and meat sausage samples, respectively Fig. 10 and 11.

These results agreed with of Jamshidi *et al*. (2009) who found that enhancing the surface temperature more than 72°C, can eliminate *Salmonella typhimurium* of chicken meat samples .

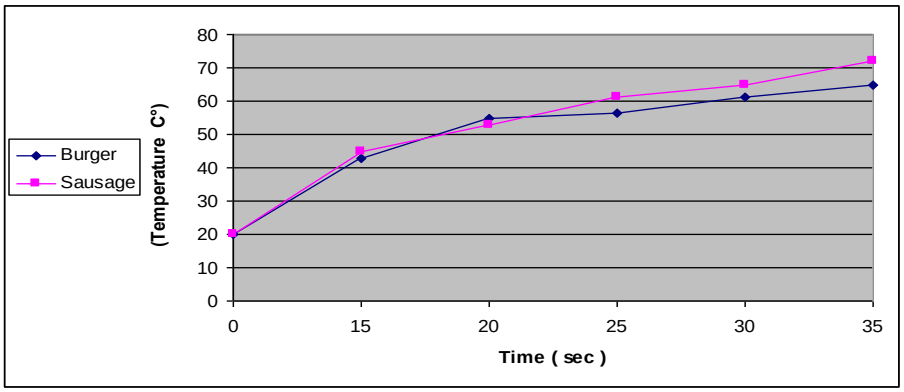


Figure 10:- Final surface temperature of chicken burger and meat sausage inoculated with *Salmonella typhimurium* at different times of microwave exposure.

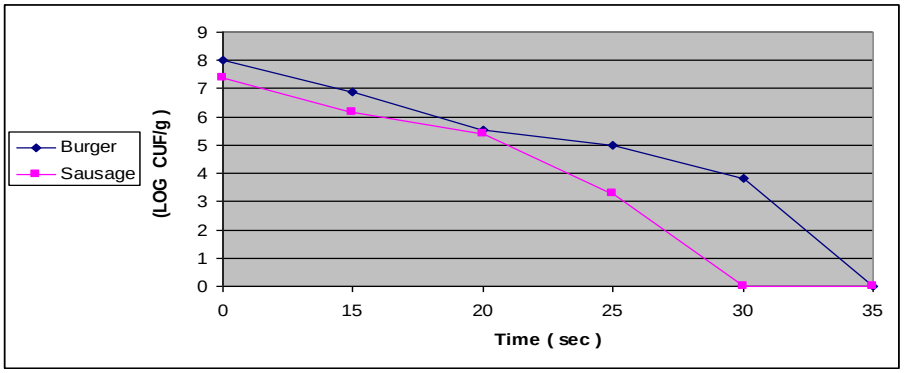


Figure11:- Survival of *Salmonella typhimurium* in chicken burger and meat sausage after microwaving

At temperature degree (45 and 61.1° C) for (120and 360 sec.), the viable number of *Salmonella typhimurium* dropped from 6.87 to 3.81 log cfu/g , after grilling chicken burger samples Fig.12 and 13 .

In the same manner , at temperature degree ranged from 46 to 60° C for 120 and 300 sec. , the viable number decreased from 5.69 to 3.65 log cfu/g for meat sausage samples .

The log drops were reduction in 0.49 and 3.7 log cycle, 1.38 and 3.42 log cycle, for chicken burger and meat sausage samples, respectively, Fig. 12 and 13.

Elimination of *Salmonella typhimurium* was observed after the end of 420 sec. exposure time,when the surface temperature was increased to 71°C in chicken burger . The strain was undetectable after the end of 360 sec. exposure time,when the surface temperature was increased to 67°C in meat sausage as showed in Fig. 12 and 13 .

These results were consistent with those of Doyle and Mazzotta, (2000)who recorded that Salmonella are usually killed by temperatures > 50° C.

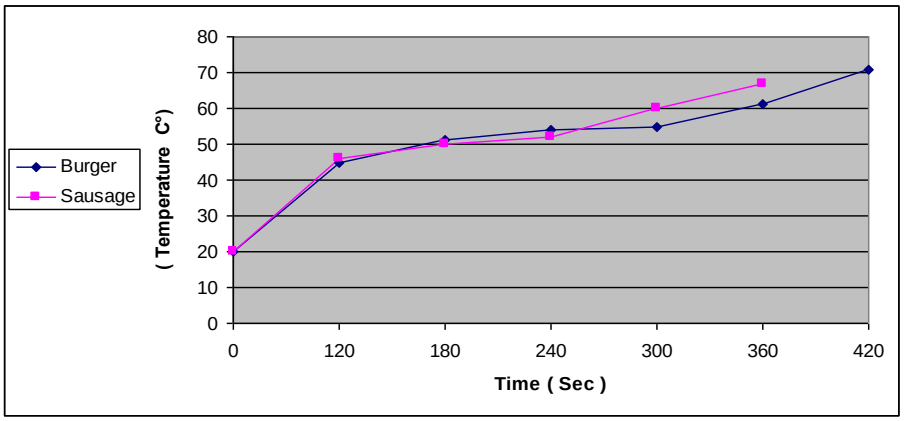


Figure12:-Final surface temperature of chicken burger and meat sausage inoculated with *Salmonella typhimurium* at different times of grilling.

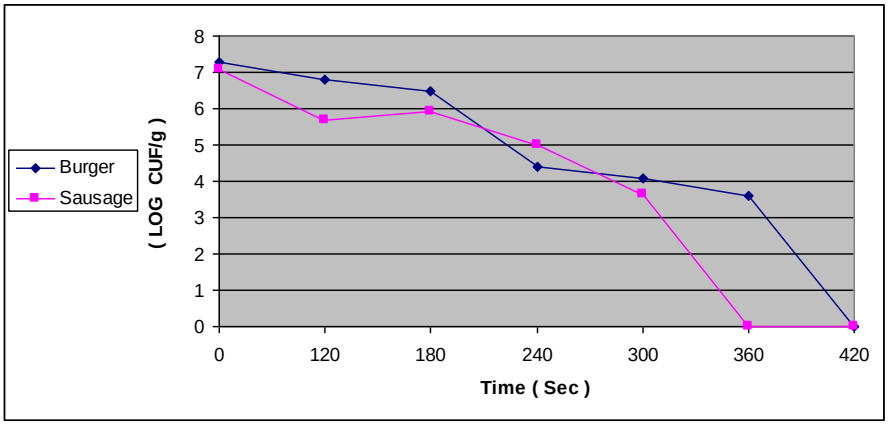


Figure 13:- Survival of *Salmonella typhimurium* in chicken burger and meat sausage after grilling.

Fig. 14 and 15 showed that the tolerance of *S. typhimurium* to frying at different times. There was a change in the viable numbers of *S. typhimurium* after 20 and 60 sec. at 45 and 60°C exposure for chicken burger :4.69 and 2.69 log cfu/g. The log drop was 2 and 4 log cycle.

The viable number in *S. typhimurium* in meat sausage samples dropped from 5.69 to 3.69 log cfu/g after 20 and 60 sec at 40 and 60°C, respectively. The log drop was 1.48 and 3.48 log cycle Fig. 14 and 15 .

Elimination of *S. typhimurium* was achieved at 69°C for 90 sec in chicken burger samples. Whereas, the strain was undetectable at 72°C after 90 sec exposure time too Fig. 14 and 15 .

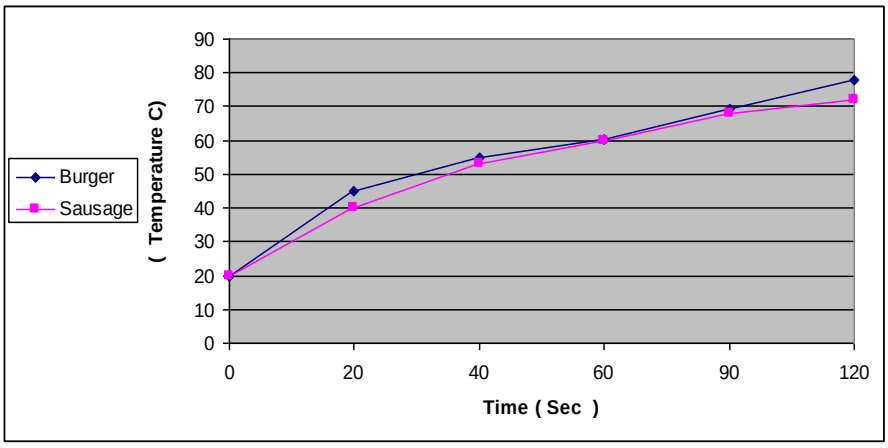


Figure 14:- Final surface temperature of chicken burger and meat sausage inoculated with *Salmonella typhimurium* at different times of frying.

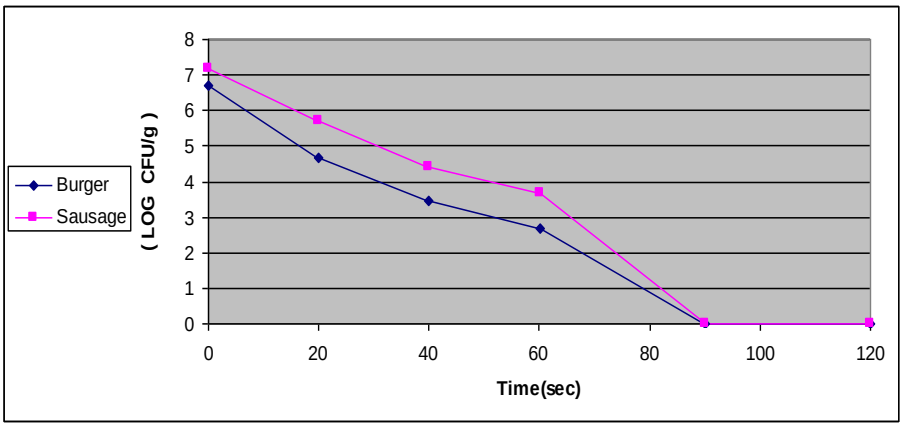


Figure 15:- Survival of *Salmonella typhimurium* in chicken burger and meat sausage after frying.

Fig. 16 and 17 showed the results obtained after boiling treatment for both chicken burger and meat sausage samples . Viable number of *S. typhimurium* after 20 and 60 sec. at 48 and 62°C was 5 and 3 log cfu/g, respectively, for chicken burger .The reduction number of bacteria was 2.54 and 4.54 log cycle.

For meat sausage samples a viable number of bacteria dropped from 6.17 to 3.6 log cfu/g at 45 and 62°C after boiling for 20 and 50 sec.The reduction number was 1.13 and 3.7 log cycle.

The full elimination of *S. typhimurium* of chicken burger was achieved after boiling for 80 sec. at 65°C and 60sec. at 65°C for meat sausage samples .

These results were consistent with those of Marcy *et al.* (2004) who found that treatment at 75°C for 30 sec. significantly reduced the numbers of mesophilic aerobes in different foods including pork, turkey, poultry ,meat, eggs and corn flour, by use of water bath and dry heat treatments.

These results were in agreement with De Jong *et al*. (2012) who studied the decimal reduction times of bacteria present on chicken fillet in boiling water. Whole chicken breast fillets were inoculated with *S. typhimurium*. Extremely high decimal reduction times of *S. typhimurium* of 2.20 min. were obtained when the surface temperature reached 85°C .

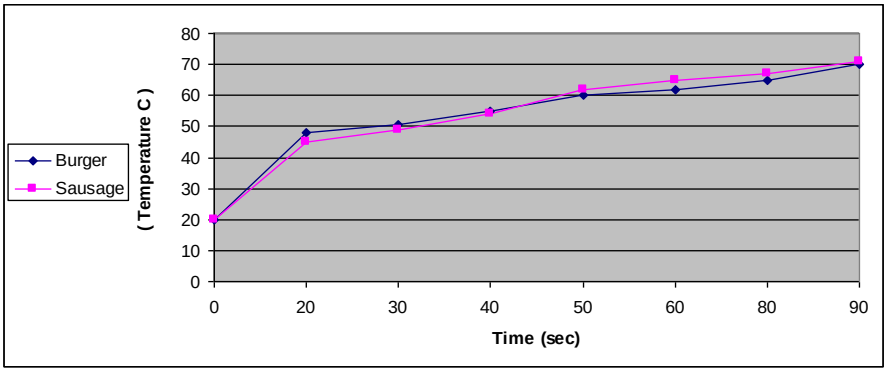


Figure 16:-Final surface temperature of chicken burger and meat sausage inoculated with *Salmonella typhimurium* at different times of boiling.

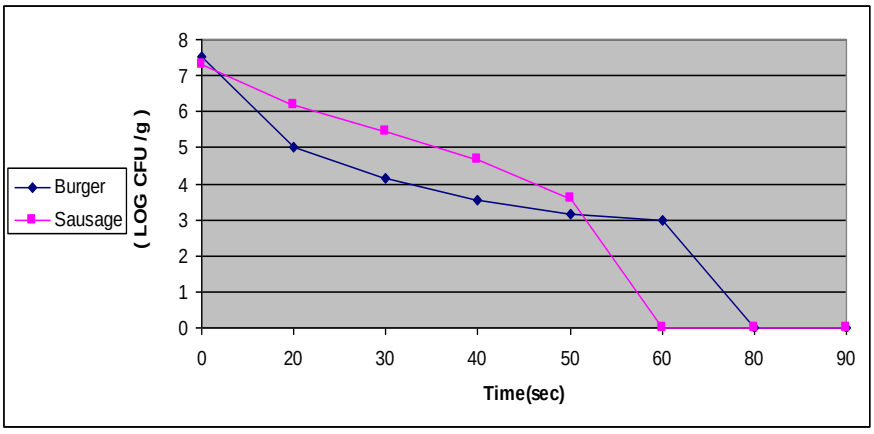
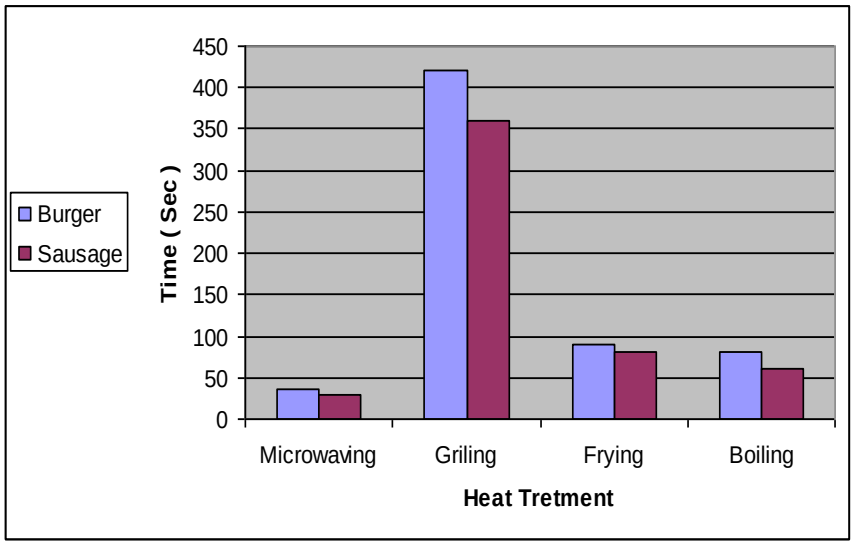


Figure17:- Survival of *Salmonella typhimurium* in chicken burger and meat sausage after boiling.

Fig.(18), show that the microwave heating was the shortest time for both chicken burger and meat sausage samples followed by boiling , frying ,then grilling .



Figure(18): the effect of microwave cooking and conventional cooking methods on the inactivation of *Salmonella typhimurium*

Conclusion

Microwave heat treatment may be considered as a cost-effective, practical, fast, easy, and safe method of decontaminating foods than any other heat treatment . Furthermore , pathogens inactivation by cooking increased in order of : microwaving >boiling > frying>grilling.

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تأثير الميكروويف والمعاملات الحرارية التقليدية على كلا من *Escherichia coli* O157: H7 و *Salmonella typhimurium* فى برجر الدجاج وسجق اللحم

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تعتبر *Escherichia coli* O157: H7 و *Salmonella typhimurium* إثنان من أهم البكتريا الممرضة الناشئة عن الغذاء المسببه للإضطرابات الهضمية فى العالم. تم أخذ عينتين من برجر الدجاج وسجق اللحم بطريقة مماثله للعينات التجارية.

بعد ذلك لقحت العينات بحوالى ١٠ ^{٦}-١٠ ^{٧} / جم من *Escherichia coli* O157:H7 و ١٠ ^{٧}-١٠ ^{٨} /cfu جم من *Salmonella typhimurium* و تم تعريضها للمعاملات الحرارية بواسطة الميكروويف و الشى والتحمير والغليان .تم تقديرالأعداد الحية ودرجات الحرارة فى العينات مباشرة.

لوحظ إزالة *Escherichia coli* O157:H7 تماما من عينات برجر الدجاج المعاملة بالميكروويف , الشى ,التحمير والغليان بعد ٤٠ ث,٧ د, ٢.٥د و دقيقتين على التوالى و بعد ٣٥ ث , ٨ د, ٢د ودقيقتين لعينات سجق اللحم على التوالى أيضا .

بالنسبة ل *Salmonella typhimurium* لم تظهر فى عينات برجر الدجاج بعد المعاملة بالميكروويف , الشى ,القلى والغليان لمدة ٣٥ ث, ٧ د , ١.٥د و ١.٢ د على التوالى و بعد ٣٠ ث , ٦د,١.٥د و دقيقة لعينات سجق اللحم على التوالى أيضا .

هذه الدراسة أظهرت أن أقل درجة حرارة تم إزالة *Escherichia coli* O157:H7عندها كانت ٧٤ ° م لمدة ٤٠ ث و٦٦ ° م لمدة ٣٥ ث لكلا من برجر الدجاج وسجق

اللحم على التوالى و ٦٥° م لمدة ٣٥ ث و ٣٠ ث لكلا من برجر الدجاج وسجق اللحم بالنسبة ل *Salmonella typhimurium*كانت بإستخدام الميكروويف .

كما أظهرت هذه الدراسة أن*Escherichia coli* O157:H7 أكثر مقاومة من *Salmonella typhimurium* لتأثير المعاملات الحرارية المختلفة تحت نفس الظروف .

بكتريـــا الإيكـــولاى (*E.coli* O157:H7) و بكتريـــا الســـالمونيلا (*S. typhimurium*) هما إحدى الميكروبات الممرضة المهمة المرتبطة بالأمراض المنقولة عبر الغذاء والتى تسبب إلتهاب المعدة والأمعاء فى جميع أنحاء العالم . تم تجهيز عينات برجر الدجاج وسجق اللحم بالطريقة التجارية المماثلة حيث يزن كلا من برجر الدجاج وسجق اللحم ٢٠ جرام , ١٥ جرام على التوالى . تلقح العينات بحوالى١٠ ^{٦} -١٠ ^{٧} CFU/g ايكولاي و ١٠^{٨}-١٠ ^{٩} CFU/g سالمونيلا ثم يتم طبخ العينات عن طريق الميكروويف و الشى والقلى والغليان .كانت الأعداد الحية من الميكروبات الممرضة و درجة حرارة العينات تقدر بعد كل معاملة حرارية

لوحظ اختزال الأعداد الحية من الإيكولاي أثناء معاملة برجر الدجاج بالميكروويف و الشى والقلى والغليان بعد ٤٠ ث,٧ د, ٢.٥د, ٢ د, على التوالى و بعد ٣٥ ث , ٨ د, ٢د,٢دأثناء معاملة سجق اللحم على التوالى . تتحمل السالمونيلا الملقحة فى عينات برجر الدجاج المعاملات الحرارية .هذه البكتريا لم يتم التعرف عليها بعد المعاملة بالميكروويف و الشى والقلى والغليان ل ٣٥ ث, ٧ د , ١.٥د , ١.٢ د, علـى التـوالى و بعـد ٣٠ ث , ٦د,١.٥د ,١ د, ل عينـات سـجق الدجاج, على التوالى .

أظهرت الدراسة الحالية أن اقل درجة حرارة حققت اختزال للأعداد الحية للإيكولاي كانت ٧٤ درجة مئوية ,٦٦.٦ درجة مئوية لكلا من برجر الدجاج وسجق اللحم عن طريق معاملة الميكروويف . اما بالنسبة للسالمونيلا كانت ٦٥ درجه مئوية لكلا من برجر الدجاج وسجق اللحم عن طريق معاملة الميكروويف.

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