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THERMAL INACTIVATION OF ENTEROHAEMORRHAGIC ESCHERICHIA COLI O157:H7 AND ITS SENSTIVITY TO NISIN AND LACTIC ACID CULTURES

Faheim, A. Shaltout; Mohamed, A.Hassan and Fatin,S.Hassanin
Food Control Department, Faculty of Veterinary Medicine, Moshtohor, Zagazig University, Benha Branch.

ABSTRACT

Thirty eight block samples of minced meat "Kobeba" were divided into three groups to study the effect of different cooking methods (18 samples), nisin (15 samples) and lactic acid cultures (5 samples) on survival of *E. coli*O_{157}:H_7 inoculated into these samples at dose 10^6 /CFU.The counts of the organism were sharply decreased to $1.25X10^2$ $\pm0.16X10^2$, $1.43X10^2$ $\pm0.20X10^2$ and $2.72X10^2\pm0.46X10^2$ /g. after 5 ,4 and 2 minutes of boiling, roasting and frying, respectively. Furthermore the frying method had a significant influence on viability of *E. coli*O_{157}:H_7 as compared with the other cooking methods. On the other side, addition of nisin at doses of 10, 25 and 40 ppm decreased the counts of *E. coli* O_{157}:H_7 to $3.54X10^4\pm0.45X10^4$, $1.83X10^3\pm0.14X10^3$ and $4.83X10^2\pm0.42X10^2$ /g. after 18 hours, respectively. Accurately, nisin at doses of 25 and 40 ppm destroyed *E. coli*O_{157}:H_7 after 30 and 24 hours, respectively, while nisin at dose 10 ppm did not destroy all numbers of the organism . Significant differences appeared in *E. coli*O_{157}:H_7 counts as a result of using of nisin at different doses ($P\leq0.05$). Concerning addition of lactic acid cultures, there is a direct relationship between the pH values of inoculated minced meat and the counts of *E. coli*O_{157}:H_7. The mean values of *E. coli*O_{157}:H_7 were $8.59X10^2\pm1.36X10^2$ and $1.20X10^2\pm0.11X10^2$/g. at pH values of 4.92±0.10 and 4.86±0.13 after 12 and 16 hours, respectively. However, complete destruction of *E. coli*O_{157}:H_7 was occurred after 20 hours at pH value 4.45±0.12. Generally, selection of the accurate time of each cooking method, the best dose of nisin and the effective pH value to control such serious pathogen were discussed.

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INTRODUCTION

Enterohaemorrhagic *E. coli*O_{157}:H_7 is a new emerging pathogen of low infective dose (10-50 cells/g).Recently, *E. coli*O_{157}:H_7 has received a considerable attention as it was frequently implicated in several outbreaks of gastroenteritis and certain syndromes including haemolytic ureamic syndrome"HUS", haemorrhagic colitis "HC"and thrombotic thrombocytopenic purpura "TTP " (5,9).

To be safe, the meat must be cooked to an internal temperature enough to destroy any harmful bacteria, particularly, *E. coli*O_{157}:H_7. Accordingly, any cooking method must produce an internal temperature at least 71.1ºC inside the meat to be safe for human consumption (12). Thus quantitative information on the thermal variability of *E. coli*O_{157}:H_7 during cooking of meat is certainly required for any cooking method.

Nisin is considered the only antibiotic allowed to be used in processed meat as recommended by most food agencies. Nisin has an effective action on different types of bacteria contaminating meat especially Gram negative bacteria (10). Thus, many meat processors use nisin at various doses during manufacture of meat products to control food poisoning bacteria but the exact dose of nisin required to destroy *E. coli*O_{157}:H_7 is still in question.

Lactic fermentation is a simple and inexpensive method to control the microorganisms in meat by lowering pH value. A low pH ranging 4-4.5 inhibits the growth of both spoilage and pathogenic bacteria (4). A recent study revealed that minced meat was kept for 36 hours at 37ºC when inoculated with lactic acid cultures (16).

Hence, the present study was carried out to study the effect of different cooking methods and addition of nisin as well as lactic acid cultures on survival of *E. coli*O_{157}:H_7.

MATERIAL AND METHODS

Preparation of Enterohaemorrhagic E. coliO_{157}:H_7 cultures:

Actually, Enterohaemorrhagic *E. coli* O_{157}:H_7 strain was obtained from Animal Health Research Institute, Zagazig. The strain was kept on tryptone soya agar slant at 4ºC. Before being used, it was grown twice in 1% peptone water at 37ºC for 24 hours. The inoculum was determined by dilutions and subsequent enumeration on plate count agar. The level of inocula $1X10^6$ CFU of *E. coli*O_{157}:H_7 /g. sample was used (6).

Collection of samples:

A total of 38 block samples of minced meat "Kobeba" were wrapped in polyethylene bags. Each block sample was divided into three portions to apply any test three times. The collected block samples were classified into three groups, the first (18 samples) to study the effect of cooking methods, the second (15 samples) to study the effect of nisin and the third one (5 samples) to study the effect of lactic acid cultures on viability of *E. coli*O_{157}:H_7. Before being inoculated, the minced meat samples were examined for naturally occurring *E. coli*O_{157}:H_7 by streaking of 0.1 ml from the prepared dilutions over sorbitol MacConkey agar (2). The meat samples were then inoculated with the prepared cultures of *E. coli*O_{157}:H_7 at dose of 10^6/g. sample. All inoculated minced meat samples were examined to determine the following:

1-Effect of different cooking methods on *E. coli*O_{157}:H_7(13):

Accurately, 18 block samples were used to study the effect of different cooking methods as boiling, roasting and frying (6 for each) on the viability of *E. coli*O_{157}:H_7 inoculated into these samples. The enumeration of the organism was carried out every minute for 6 successive minutes of boiling, roasting and frying.

2-Effect of nisin on Enterohaemorrhagic *E. coli*O_{157}:H_7 (25):

Nisin at doses of 10 , 25 and 40 ppm were added to 15 block samples of inoculated minced meat (5 for each dose) and the enumeration of *E. coli*O_{157}:H_7 was determined after 6, 12, 18, 24 and 30 hours.

3-Effect of lactic acid culture on Enterohaemorrhagic *E. coli*O_{157}:H_7 (3):

The last five block samples were inoculated with 0.02% lactic acid culture (Ezal My 087, Texel, 86220 Dange Saint, Romaine, France). Moreover, the *E. coli*O_{157}:H_7 was enumerated after 4, 8, 12, 16 and 20 hours.

Generally, enumeration of *E. coli*O_{157}:H_7 in all treated samples was applied by using sorbitol MacConkey agar plates incubated at 37ºC for 24 hours.

Statistical analysis was done using Analysis of Variance (ANOVA) test (21)

RESULTS

Table 1: Effect of different cooking methods on viability of *E. coli*O_{157}:H_7 (10^6/g.) inoculated into minced meat "Kobeba"

Cooking Methods / Time	Boiling	Roasting	Frying
	Mean ±S.E.	Mean ± S.E.	Mean ± S.E.
Zero time	$1X10^{6*}$	$1X10^6$	$1X10^6$
1 min.	$1.63X10^4 \pm 0.13X10^4$	$8.39X10^3 \pm 1.72X10^3$	$5.18X10^3 \pm 0.82X10^3$
2 min.	$9.87X10^2 \pm 1.51X10^2$	$1.57X10^3 \pm 0.65X10^3$	$2.72X10^2 \pm 0.46X10^2$
3 min.	$4.66X10^2 \pm 0.74X10^2$	$4.84X10^2 \pm 0.39X10^2$	-
4 min.	$2.19X10^2 \pm 0.33X10^2$	$1.43X10^2 \pm 0.20X10^2$	-
5 min.	$1.25X10^2 \pm 0.16X10^2$	-	-
6 min.	-	-	-

*Significant differences by ANOVA test ($p \leq 0.05$)

Table 2: Effect of nisin on viability of *E. coli*O_{157}:H_7 (10^6/g.) inoculated into minced meat "Kobeba"

Dose / Time	10 ppm	25 ppm	40 ppm
	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.
Zero time	$1X10^{6*}$	$1X10^6$	$1X10^6$
6 hours	$4.91X10^5 \pm 0.70X10^5$	$1.52X10^5 \pm 0.21X10^5$	$8.12X10^4 \pm 1.46X10^4$
12 hours	$8.28X10^4 \pm 1.67X10^4$	$7.69X10^3 \pm 1.32X10^3$	$2.94X10^3 \pm 0.31X10^3$
18 hours	$3.54X10^4 \pm 0.45X10^4$	$1.83X10^3 \pm 0.14X10^3$	$4.83X10^2 \pm 0.42X10^2$
24 hours	$1.79X10^3 \pm 0.26X10^3$	$2.06X10^2 \pm 0.29X10^2$	-
30 hours	$2.85X10^2 \pm 0.38X10^2$	-	-

*Significant differences by ANOVA test ($p \leq 0.05$)

Table 3: Effect of lactic acid cultures on viability of *E. coli*O_{157}:H_7 (10^6/g.) inoculated into minced meat "Kobeba"

PH value / Time	pH value	*E. coli* O_{157} :H_7 count
	Mean ±S.E	Mean ± S.E
Zero time	5.76	$1X10^{6*}$
4 hours	5.47±0.09	$3.47X10^5$±$0.41X10^5$
8 hours	5.11±0.08	$5.81X10^3$±$0.79X10^3$
12 hours	4.92±0.10	$8.59X10^2$±$1.36X10^2$
16 hours	4.86±0.13	$1.20X10^2$±$0.11X10^2$
20 hours	4.45±0.12	-

*Significant differences by ANOVA test ($p \leq 0.05$)

DISCUSSION

It is obvious from the results recorded in table 1 that the mean values of *E. coli*O_{157}:H_7 inoculated in meat (10^6/g.) were $1.63X10^4$ ± $0.13X10^4$, $8.39X10^3$±$1.72X10^3$ and $5.18X10^3$±$0.82X10^3$ / g. after one minute of boiling, roasting and frying, respectively. Such counts of *E. coli*O_{157}:H_7 were decreased to $1.25X10^2$±$0.16X10^2$ at 5th minute of boiling, $1.43X10^2$ ± $0.20X10^2$/ g. at 4th minute of roasting and $2.72X10^2$±$0.46X10^2$ / g. at 2nd minute of frying. Accordingly, complete destruction of *E. coli*O_{157}:H_7 was occurred after 5, 4, and 2 minutes of boiling, roasting and frying, respectively. Thus, the frying method had a great effect on the destruction of *E. coli*O_{157}:H_7 strains.

The differences associated with the counts of *E. coli*O_{157}:H_7 as a result of different cooking methods, boiling, roasting or frying were significant. Also, significant differences appeared due to the time of cooking (Table 1).

The current results come in accordance with those reported by some authors (1, 13). In this respect, the log.5 cycle reductions of the counts of *E. coli* inoculated into meat as a result of boiling for 5 minutes(8).

E. coli O_{157}:H_7 continues to be recognized as a foodborne pathogen of primary concern. The organism was detected in 0.09% of beef samples in United States of America (USA) in 1994 with an increase to 0.18% of 7400 beef samples in 1998(19), While, the incidence of *E. coli*O_{157}:H_7 in 2000 raw ground beef samples was greatly increased to reach 0.86 % (14).

Typically, *E. coli*O_{157}:H_7 contaminating meat can survive heating at 55ºC for 5 minutes and 60ºC for 10 seconds (13). Actually, *E. coli*O_{157}:H_7 is completely

destroyed when the temperature in the meat centre reaches 71.1ºC (12). Alternatively, over cooking may char and dry out the beef with a high loss in its texture (4).

In practice, there is difficulty to recommend the people to measure the temperature of meat center after cooking. Thus, determination of accurate time required for consumption is of great magnitude. Consequently the present study indicated that boiling, roasting and frying of meat for 6, 5 and 3 minutes respectively were sufficient to kill all Enterohaemorrhagic *E. coli*O_{157}:H_7.

Table 2 declared that the addition of nisin at levels of 10, 25 and 40 ppm to minced meat decreased the count of *E. coli*O_{157}:H_7 from 10^6 at zero time to $4.91X10^5 \pm 0.70X\ 10^5$, $1.52X10^5 \pm 0.21X10^5$ & $8.12\ X10^4 \pm 1.46X\ 10^4$ / g. after 6 hours , $8.28X\ 10^4 \pm 1.67X.10^4$, $7.69X10^3 \pm 1.32X10^3$ & $2.94X10^3 \pm 0.31X10^3$ / g. after 12 hours and $3.54X10^4 \pm 0.45X10^4$, $1.83X10^3 \pm 0.14X10^3$ and $4.83X10^2 \pm 0.42\ X10^2$ /g. after 18 hours, respectively . It is of interest to mention that addition of 10 ppm nisin to meat could not destroy all numbers of *E. coli*O_{157}:H_7 . While , nisin at doses of 25 and 40 ppm killed this organism after 30 and 24 hours , respectively .Such variations in *E. coli*O_{157}:H_7 counts in relation to different doses of nisin and time were significant ($p \leq 0.05$) .These findings agreed with those obtained by several authors (11,22). Nisin had lethal effect on most Gram negative bacteria, but they did not determine the effective dose at which complete destruction of *E. coli*O_{157}:H_7 could be occurred (18).

In general, addition of nisin to meat products has a broad spectrum activity against *E. coli* organisms, but its action is greatly affected by the initial bacterial count and pH of the meat product (22).

Unlike nitrite, the inhibition effect of nisin is not affected by high levels of iron content in meat. However, the action of nisin is enhanced when mixed with nitrite where nisin –nitrite combination appears to have a synergistic action (17).

Accordingly, addition of nisin to meat with a level at least 25 ppm is effective for complete destruction of Enterohaemorrhagic *E. coli*O_{157}:H_7 as indicated in the present work.

Results achieved in table 3 showed the effect of addition of lactic acid cultures on viability of *E. coli*O_{157}:H_7 inoculated into minced meat. There is direct relationship between the counts of *E. coli*O_{157}:H_7 and the pH values of inoculated minced meat. Respectively, the mean values of *E. coli* O_{157}:H_7 were $3.47X10^5 \pm 0.41X10^5$, $5.81X10^3 \pm 0.79X10^3$, $8.59X10^2 \pm 1.36X10^2$ and $1.20X10^2 \pm 0.11X10^2$ /g. at pH values of 5.47±0.09, 5.11±0.08, 4.92±0.10 and 4.86±0.13 after 4, 8, 12, and 16 hours of storage in refrigerator (4ºC). Complete disappearance of *E. coli* O_{157}:H_7 was attained at pH value of 4.45±0.12 after 20 hours of storage.

The differences in *E. coli* O_{157}:H_7 as a result of changes in pH values of meat during storage were significant ($P \leq 0.05$).Nearly similar results were obtained by several authors (15, 20, 23).

Simultaneously , the sharp decrease in pH value of meat from 5.76 up on adding of lactic acid culture to pH 4.45 at the end of experiment (20 hours)can explain why did such decline in *E. coli* O_{157}:H_7 counts has taken place.

The effect of pH on viability of *E. coli* is some what little where *E. coli* is capable to grow over the pH range of 4.6 to 9.0(10). However, *E. coli*O_{157}:H_7 can survive but do not grow during fermentation of some meat products by using lactic acid cultures (24).

Generally, using of lactic acid cultures in production of fermented meat products can limit efficiently the viability of pathogenic strains of *Escherichia coli*, particularly Enterohaemorrhagic strains (7).

The current study allow to conclude that the application of the selective cooking method with its accurate time and addition of nisin (at least 25ppm) or lactic acid culture (0.02 %) can control the Enterohaemorrhagic *E. coli* O_{157}:H_7 .

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تأثير الحرارة على الايشريشيا كولاى $O_{157}:H_7$ المسببة للنزيف المعوي وحساسيتها للنيسين وبكتريا التخمر

فهيم عزيز الدين شلتوت محمد أحمد حسن فاتن سيد حسانين

قسم مراقبة الأغذية - كلية الطب البيطري بمشتهر- جامعة الزقازيق- فرع بنها

أجريت هذه الدراسة على عدد ثمانية و ثلاثون (38) عينة رئيسية من اللحم المفروم " كبيبة " بعد التأكد من خلوها تماما من ميكروب الايشريشا كولاى $O_{157}:H_7$ المسببة للنزيف المعوي وذلك لمعرفة تأثير طرق الطهي المختلفة و كذا تأثير إضافة النيسين وبكتريا التخمر على نمو الميكروب الذي تم حقنة في العينات بمستوى 10^6/جرام وقد تم تقسيم العينات بواقع ثمانية عشرة (18) عينة لاختبارات الطهي متمثلة في الغليان و الشى و القلي (6لكل طريقة)، خمسة عشر (15) عينة لدراسة تأثير إضافة النيسين و خمس (5) عينات لدراسة تأثير بكتريا التخمر على الميكروب, وكل عينة رئيسية كانت مقسمة في الأساس إلى ثلاث (عينات) وذلك لإجراء أي اختبار ثلاث مرات للحصول على أدق النتائج0 و قد دلت نتائج الدراسة على أن العدد الكلى للميكروب قد تناقص بدرجة كبيرة إلى$10X1.25^2$ و 1.43 X 10^2 و 2.72 X 10 ^{2}/جرام بعد مرور زمن 5, 4 , 2 دقيقة من الغليان و الشي و القلي للكبيبة ،على التوالي0 كما تبين أن طريقة القلي كانت الأكثر فعالية على الميكروب عند مقارنتها بطريقتي الغليان والشى. و بالنسبة لإضافة النيسين إلى الكبيبة بمعدلات 10و 25 و 40 جزء في المليون، فقد أدى ذلك إلى انخفاض عدد الميكروب (10^6 /جرام) ليصل إلى 3.54 X 10^4 و 1.83 X 10^3 و 4.83 X 10 ^{2} /جرام بعد مرور 18 ساعة , على الترتيب 0 و قد تم التخلص من الميكروب نهائيا عند إضافة النيسين بمعدل 25 و40 جزء في المليون بعد 30 و24 ساعة ,على التوالي. لم يؤدى إضافة النيسين بمعدل 10 جزء في المليون إلى التدمير الكامل للميكروب . إضافة بكتريا التخمر إلى الكبيبة التي تم حقنها بميكروب الايشريشيا كولاى $O_{157}:H_7$ المسببة للنزيف المعوي (10^6 /جرام) قد أدى إلى نقصان العدد الكلى للميكروب إلى 8.59 X 10 ^{2} و 1, 20 X 10^2 عندما كانت قيمة الايون الهيدروجيني4.92 و 4.86 بعد مرور زمن 12 و16 ساعة من الإضافة ، على الترتيب 0 وقد تم القضاء نهائيا على الميكروب بعد 20 ساعة عندما كانت قيمة الايون الهيدروجيني 4.45 . كما اهتمت الدراسة باختيار الوقت الكافي لكل طريقة من طرق الطهي وكذا الجرعة الفعالة من النيسين وبكتريا التخمر للتحكم في هذا الميكروب الخطير .