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

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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 O157:H7 inoculated into these samples at dose 10^6 /CFU. The counts of the organism were sharply decreased to 1.25X10^2 ±0.16X10^2, 1.43X10^2 ±0.20X10^2 and 2.72X10^2±0.46X10^2 /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 O157:H7 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 O157:H7 to 3.54X10^4±0.45X10^4, 1.83X10^3±0.14X10^3 and 4.83X10^2±0.42X10^2 /g. after 18 hours, respectively. Accurately, nisin at doses of 25 and 40 ppm destroyed E. coli O157:H7 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 O157:H7 counts as a result of using of nisin at different doses (P≤0.05). 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 O157:H7. The mean values of E. coli O157:H7 were 8.59X10^2±1.36X10^2 and 1.20X10^2±0.11X10^2 /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 O157:H7 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.
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 uremic 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 71.1\(^\circ\)C 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 37\(^\circ\)C 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. coli O\(_{157}:H_7\) 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 4\(^\circ\)C. Before being used, it was grown twice in 1% peptone water at 37\(^\circ\)C for 24 hours. The inoculum was determined by dilutions and subsequent enumeration on plate count agar. The level of inocula 1X10\(^8\) 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*O157:H7. Before being inoculated, the minced meat samples were examined for naturally occurring *E. coli*O157:H7 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*O157:H7 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*O157:H7 (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*O157:H7 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*O157:H7 (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*O157:H7 was determined after 6, 12, 18, 24 and 30 hours.

3-Effect of lactic acid culture on Enterohaemorrhagic *E. coli*O157:H7 (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*O157:H7 was enumerated after 4, 8, 12, 16 and 20 hours.

   Generally, enumeration of *E. coli*O157:H7 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*O₁₅₇:H₇ (10⁶/g.) inoculated into minced meat "Kobeba"

<table>
<thead>
<tr>
<th>Cooking Methods</th>
<th>Boiling</th>
<th>Roasting</th>
<th>Frying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Zero time</td>
<td>1X10⁶</td>
<td>1X10⁶</td>
<td>1X10⁶</td>
</tr>
<tr>
<td>1 min.</td>
<td>1.63X10⁶±0.13X10⁶</td>
<td>8.39X10⁶±1.72X10⁶</td>
<td>5.18X10⁶±0.82X10⁶</td>
</tr>
<tr>
<td>2 min.</td>
<td>9.87X10⁶±1.51X10⁶</td>
<td>1.57X10⁷±0.65X10⁷</td>
<td>2.72X10⁷±0.46X10⁷</td>
</tr>
<tr>
<td>3 min.</td>
<td>4.66X10⁶±0.74X10⁷</td>
<td>4.84X10⁷±0.39X10⁷</td>
<td>-</td>
</tr>
<tr>
<td>4 min.</td>
<td>2.19X10⁶±0.33X10⁷</td>
<td>1.43X10⁷±0.20X10⁷</td>
<td>-</td>
</tr>
<tr>
<td>5 min.</td>
<td>1.25X10⁶±0.16X10⁷</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 min.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Significant differences by ANOVA test (p≤0.05)

**Table 2**: Effect of nisin on viability of *E. coli*O₁₅₇:H₇ (10⁶/g.) inoculated into minced meat "Kobeba"

<table>
<thead>
<tr>
<th>Dose</th>
<th>10 ppm</th>
<th>25 ppm</th>
<th>40 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Zero time</td>
<td>1X10⁶</td>
<td>1X10⁶</td>
<td>1X10⁶</td>
</tr>
<tr>
<td>6 hours</td>
<td>4.91X10⁷±0.70X10⁷</td>
<td>1.52X10⁷±0.21X10⁷</td>
<td>8.12X10⁷±1.46X10⁷</td>
</tr>
<tr>
<td>12 hours</td>
<td>8.28X10⁷±1.67X10⁷</td>
<td>7.69X10⁷±1.32X10⁷</td>
<td>2.94X10⁷±0.31X10⁷</td>
</tr>
<tr>
<td>18 hours</td>
<td>3.54X10⁸±0.45X10⁸</td>
<td>1.83X10⁸±0.14X10⁸</td>
<td>4.83X10⁸±0.42X10⁸</td>
</tr>
<tr>
<td>24 hours</td>
<td>1.79X10⁸±0.26X10⁸</td>
<td>2.06X10⁸±0.29X10⁸</td>
<td>-</td>
</tr>
<tr>
<td>30 hours</td>
<td>2.85X10⁸±0.38X10⁸</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Significant differences by ANOVA test (p≤0.05)
Table 3: Effect of lactic acid cultures on viability of *E. coli*O₁₅₇:H₇ (10⁶/g.) inoculated into minced meat "Kobeba"

<table>
<thead>
<tr>
<th>Time</th>
<th>pH value</th>
<th>E. coli O₁₅₇ :H₇ count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E</td>
<td>Mean ± S.E</td>
</tr>
<tr>
<td>Zero time</td>
<td>5.76 1X10⁶</td>
<td></td>
</tr>
<tr>
<td>4 hours</td>
<td>5.47±0.09 3.47X10⁵±0.41X10⁵</td>
<td></td>
</tr>
<tr>
<td>8 hours</td>
<td>5.11±0.08 5.81X10¹±0.79X10¹</td>
<td></td>
</tr>
<tr>
<td>12 hours</td>
<td>4.92±0.10 8.59X10²±1.36X10²</td>
<td></td>
</tr>
<tr>
<td>16 hours</td>
<td>4.86±0.13 1.20X10³±0.11X10³</td>
<td></td>
</tr>
<tr>
<td>20 hours</td>
<td>4.45±0.12 -</td>
<td></td>
</tr>
</tbody>
</table>

*Significant differences by ANOVA test (p≤0.05)

**DISCUSSION**

It is obvious from the results recorded in table 1 that the mean values of *E. coli*O₁₅₇:H₇ inoculated in meat (10⁶/g.) were 1.63X10⁶ ± 0.13X10⁶, 8.39X10³±1.72X10³ and 5.18X10³±0.82X10³ / g. after one minute of boiling, roasting and frying, respectively. Such counts of *E. coli*O₁₅₇:H₇ were decreased to 1.25X10²±0.16X10² at 5th minute of boiling, 1.43X10² ± 0.20X10² / g. at 4th minute of roasting and 2.72X10²±0.46X10² / g. at 2nd minute of frying. Accordingly, complete destruction of *E. coli*O₁₅₇:H₇ 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*O₁₅₇:H₇ strains.

The differences associated with the counts of *E. coli*O₁₅₇:H₇ 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* O₁₅₇:H₇ 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*O₁₅₇:H₇ in 2000 raw ground beef samples was greatly increased to reach 0.86% (14).

Typically, *E. coli*O₁₅₇:H₇ contaminating meat can survive heating at 55°C for 5 minutes and 60°C for 10 seconds (13). Actually, *E. coli*O₁₅₇:H₇ 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*<sub>O157</sub>:H<sub>7</sub>.

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*<sub>O157</sub>:H<sub>7</sub> from 10<sup>6</sup> at zero time to 4.91X10<sup>5</sup>±0.70X 10<sup>5</sup>, 1.52X10<sup>5</sup>±0.21X10<sup>5</sup> & 8.12X10<sup>4</sup>±1.46X10<sup>4</sup> / g. after 6 hours , 8.28X10<sup>4</sup>±1.67X.10<sup>4</sup>, 7.69X10<sup>3</sup>±1.32X10<sup>3</sup> & 2.94X10<sup>3</sup>±0.31X10<sup>3</sup> / g. after 12 hours and 3.54X10<sup>4</sup>±0.45X10<sup>3</sup>, 1.83X10<sup>3</sup>±0.14X10<sup>3</sup> and 4.83X10<sup>2</sup>±0.42 X10<sup>2</sup> /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*<sub>O157</sub>:H<sub>7</sub>. While , nisin at doses of 25 and 40 ppm killed this organism after 30 and 24 hours , respectively .Such variations in *E. coli*<sub>O157</sub>:H<sub>7</sub> counts in relation to different doses of nisin and time were significant ( p≤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*<sub>O157</sub>:H<sub>7</sub> 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*<sub>O157</sub>:H<sub>7</sub> 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*<sub>O157</sub>:H<sub>7</sub> inoculated into minced meat. There is direct relationship between the counts of *E. coli*<sub>O157</sub>:H<sub>7</sub> and the pH values of inoculated minced meat. Respectively, the mean values of *E. coli* O<sub>O157</sub>:H<sub>7</sub> were 3.47X10<sup>5</sup>±0.41X10<sup>5</sup>, 5.81X10<sup>6</sup>±0.79X10<sup>5</sup>, 8.59X10<sup>2</sup>±1.36X10<sup>2</sup> and 1.20X10<sup>2</sup>±0.11X10<sup>2</sup> /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<sub>O157</sub>:H<sub>7</sub> was attained at pH value of 4.45±0.12 after 20 hours of storage.
The differences in *E. coli* O\textsubscript{157}:H\textsubscript{7} as a result of changes in pH values of meat during storage were significant (P≤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\textsubscript{157}:H\textsubscript{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\textsubscript{157}:H\textsubscript{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\textsubscript{157}:H\textsubscript{7}.

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تأثر الحرارة على الإشريشيا كولاي O157:H7 المسببة للنزيف المعوي وحساسيتها للنيسين وبكتريا التخمر

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أجريت هذه الدراسة على عدد ثمانية و ثلاثون (38 ) عينة رئيسية من اللحم المفروم.１ كبيبةة بةدةد الكددةد مةن الوهةا كمامةا من ميكروب الإشريشيا كولاي O157:H7 ويكرييا التخمر على نمو الميكروبات الذي تم تحصيلة في العينات بعظام 10/1جرام وقد تم تقسيم العينات بواقع ثمانية عشرة (18) عينة لإختبارات الطلبة. حيث تم نقل العينة إلى الغليان و الشيء والقلي ( 6 جرام طريقة )، خمسة عشرة (15) عينة تم دراسة تأثير إضافة التنسين و خمس عينة تم دراسة تأثير التكسير على الميكروبات، وكل عينة رئيسية كانت مقسمة في الأساس إلى ثلاث (عينات) وذلك لإجراء أي اختبار مرتين للحصول على أدق النتائج. وقد دلت النتائج على أن العدد الكلي للميكروبات قد تناقص بدرجة كبيرة إلى 10^2(جرام). بعد مرور زمن 4.5، 4.3، و 2.72 دقيقة من الغليان والشي و القلي للكبيبة، و على التوالي. كما تبين أن طريقة المائي كانت الأكثر فعالية على الميكروبات عند مقارنتها بطريقة الغليان والشي، و بالنسبة لإضافة التنسين إلى الكبيبة بمعدلات 10، 25 و 40 جء في المليون، فقد أدى ذلك إلى انخفاض عدد الميكروبات (10^9/جرام) ليصل إلى 3.54، 10^5، 10^4، 10^3، 10^2، 10^1، 1.83، و 1.43 عدد مور في المليون. أما الدراسة على التكسير 0 و 0.5 رقمي و 12 لاعادة التكسير، فبعد مرور زمن 12 و 16 ساعة من الإضافة، على التوالي. لم يؤدي إضافة التنسين بمعدل 10 جء في المليون إلى انخفاض كلامي للميكروبات، حيث تبين أن التكسير إلى الكبيبة التي تم حقنها بميكروب الإشريشيا كولاي O157:H7 المسببة للنزيف المعوي (10^6/جرام) أدى إلى انخفاض العدد الكلي للميكروبات إلى 8.59 ب عدد مور مم. و5.28. و10^1، 10^2، 10^3، 10^4، 10^5. عندما كانت قيمة الأيون الهيدروجيني 4.92، 4.86 و 4.78 بعد مرور زمن 12 و 16 ساعة من الإضافة. على التوالي. قد تم الفحص نهائيا على الميكروبات بعد 20 ساعة عندما كانت قيمة الأيون الهيدروجيني 4.45. كما أثبتت الدراسة باختبار الوقت الكافي لكل طريقة من طرق الطهي وكذا الجرعة العاملة من التنسين و بكتريا التخمر للتحكم في هذا الميكروبات الخطر.