VIABILITY OF LISTERIA. MONOCYTOGENES IN RAW GROUND BEEF

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ABSTRACT

The effect of some stress factors on the viability of isolated L. monocytogenes serotype 1 inoculated in raw ground beef was studied.

The temperature of 70°C for more than 20 minutes in the center of the product will ensure a total kill of L. monocytogenes, even in a heavily contaminated one.

Freezing and storage of the inoculated raw ground beef at - 18°C decreased the counts from 8.041 to 2.00 log CFU/g after 8 weeks. L. monocytogenes significantly decreased from 7.033 to 6 log CFU/g by the week 3 and from 4.5 to 2.566 log CFU/g by the week 7.

Nisin at concentration of 1600 IU/g of raw ground beef stored at 4°C may be useful in controlling of L. monocytogenes.

The treatment of the inoculated ground beef with potasium sorbate at 0.26% exhibited significant inhibitory properties against L. monocyfogenes from 7.952 to 3.996 log CFU/g by the day 21 of refrigerated storage.

The treatment of the inoculated ground beef with potassium nitrite (125 ppm) significantly decreased L. monocyotgenes counts from 7.65 to 3.139 by the day 21.

Combination of nisin, potassium nitrite and potassium ascorbate produced enhanced listericidal effect more than the use of each preservative alone.

INTRODUCTION

In recent years, listeriosis has come to prominence as a leading cause of death from food borne illness. Although the responsible organism, *Listeria monocytogenes* mainly infects immunocompromised and otherwise susceptible individuals estimates indicate that in the United

States alone. 1,092 cases and 248 deaths occurred in 1993 as a result of infection with this pathogen **(Tappero et al. 1995).**

Control of *L. monocytogenes* is difficult for two reasons. The first is its ubiquitous nature, it is commonly found in plant, soil, and surface water samples and has also been isolated from the feces of livestock, in slaughterhouses, food - proeessing environments, and at home. It has been reported to contaminate 6.2 to more than 60% of fresh meat and poultry and about one - third of processed, ready - to - eat meat products. Meanwhile its ability to tolerate environmental stresses The second reason is its ability to tolerate envizonmental stresses **(Farbar and Peterkin 1991).**

The heat resistanee of bacteria may be affected by a number of factors other the heating mestrum, such as environmental eonditions, example the composition and temperature of culture medium, age of baeterial culture studied **(Farber and Brown, 1990)**, rate of thermal treatment **(Quintavalla and Campanini, 1991)** and the procedure used in a study for the recovery of heat treated cells may be also influence the obtained heat resistance values **(Sorquist, 1994)**.

Listeria was not detected in meat products (minced meat and sausage) which had been heat treated to reach a eore temperature of 71°C **(Radistic and paunovic, 1992)**. Furthermore **L. monocytogencs** was not detected in the hot smoked sausage heated to an internal temperature of 70-75°C afte: the smoking process **(Buncic, 1991)**.

Freezing and storage of *L. monocytogenes* Scott A for one month in phosphate buffer at - 18°C lead to 87 % death and 79% injury. furthermore repeated freezing and thawing caused more death and injury than did a single freeze thawing cycle **(El-kest and Marth, 1992)**.

Nisin is a small antimicrobial polypeptide produced by some strains of *lactococcus lactis* **(Marrick and Hirsch, 1994)**. It inhibits the growth of a broad spectrum of gram positive microorganisms including *L. monocytogenes* **(Okereke and Montville, 1991)**. Nisin is approved by the World Health Organization and has enjoyed international for approximately 45 years in a wide variety of food products **(Delves, 1990)**. Furthermore nisin was also recommended for use as a food preservative in the food and dairy industries.

The concentration of 0.25 or 3% potassium sorbate completely inhibited the growth and caused complete inaetivation of the pathogens, whereas presence of less than or equal 0.15% potassium sorbate allowed growth of the pathogens, higher concentration caused either complete inhihitory or inhibition plus partial or complete inactivation of *L. monocytogenes* **(Unda et al., 1991)**.

The inhihitory effect of sodium nitrite upon *L. monocytog.* ene was found to depend greatly

upon pH and concentration of nitrite as high as 25000 ppm were insufficient to prevent growth at pH 7.4. The bacteriostatic effects were enhanced by low pH and by low temperature **(Sahamat et al., 1980)**.

So, the aim of the present study is to throw a light on the effect of stress factor including heating, freezing and addition of preservatives on the survival of *L. monocytogenes*. Beside that the effect of storage temperatur on the growth rate of *L. monocytogenes* inoculated in minced meat.

MATERIAL AND METHODS

Preparation of inocula :

L. monocytogenes serotype 1 previously isolated from frozen beef according to **Hanan El-Lawendy (2001)** were maintained as stock cultures with monthly transfers of trypticase soya agar supplemented with 0.6 % yeast extract (TSA-YE) agar slants. Cultures from TSA-YE agar were subcultured overnight in TSB-supplemented with 0.6%/O YE to obtain working cultures for each experiment. The cultures were harvested using centrifugation, washed twice in sterile phosphate buffered saline (PBS) then serial dilutions were made in PBS and triplicate 0.1 ml volumes of the dilutions were surfaec - plated on Listeria isolation Palcam agar. The colonies were counted after incubation at 30°C for 48 hours. The cultures were inoculated in ground beef samples to reach the final inoculum levels 10^8 CFU ml / g of raw ground beef. These samples were subjected to study the effect of stress factors on the survival of isolated *L. monocytogenes* in raw ground beef as follows:

1. Thermal treatment of L. monoeytogenes at 55, 60, 70°C, for zero, 10, 20, 40, 50, and 60 minutes according to **Dorsa et al. (1992)**.
2. Effect of freezing and storage at - 1 8°C for 2 months. Triplicate inoculated samples were analyzed at 1, 2, 3, 4, 5, 6, 7, and 8 weeks of freezing.
3. Effect of nisin (800 and 1600 IU) according to **Nassar and Farrag (1995)**, potassium sorbate (0.26%) according to *Wederquist et al. (1994)*, potassium nitrite (125 ppm) according to **Grau and Vanderlinde (1992)**, potassium ascorbate (550 ppm) according to **Unda et al. (1991)**.
4. Effect of interaction of nisin (800 IU), potassium sorbate (0.26%), potassium nitrite (125 ppm) and potassium ascorbate (550 ppm).

Triplicate inoculated treated and untreated samples were analyzed at 0, 7 14, 21 days of stor-

age. Mean values of *L. monocytogenes* were reported and it's number was expressed as Log_{10} CFU/g.

Statistical analysis :

F - test for complete randomized design. The analysis of variance (ANOVA) was carried out following the method explained by **Snedecor and Cochran (1989).**

RESULTS AND DISCUSSION

Listeria monocytogenes is widespread in the environment and among one of the heat resistant and osmotolerant non sporing food pathogen **(Marth, 1993).** The use of heat for control of microorganisms is fundamental in food preservation **(Toledo, 1991).**

The thermal treatment of inoculated ground beef at 60°C for 60 minutes decreased the counts of *L. monocytogenes* from initial mean log (8.0) to 1.997 log CFU/g. Thermal treatment at 60°C for 10 minutes decreased not significantly *L. monocytogenes* count from 8 to 7.682 log CFU/g. After 10 minutes of thermal treatment at 60°C *L. monocytogenes* count significantly decreased (LSD at 0.01 = 0.401) from 7.682 to a final count of 1.997 log CFU/g (Table 1).

Results presented in Table 1 showed that the heating of inoculated ground beef at 70°C for 20 minutes significantly decreased (LSD at 0.01=0.169) *L. monocytogenes* counts from 8 to 2.586 log CFU/g. on viable population were recorded after 20 minutes of thermal treatment at 70°C.

From the above mentioned results we ean conclude that the treatment at 70°C for 20 minutes was more efficient than the heat treatment at 55°C and 60°C for 60 minutes. However, **Kwiatek and wojton (1993)** concluded that a temperature greater than 68.9°C in the center of the product will ensure kill of *L. monocytogenes*, even in heavily contaminated products.

Results obtained in Table (2) showed that *L. monocytogenes*, decreased from 8.041 to 2 log CFU/g after 8 weeks of frozen storage at - 1 8°C. After two weeks of frozen storage at - 18°C *L. monocytogenes*, counts significantly decreased (LSD at 0.01=0.574) when were compared to control.

The obtained data revealed that *L. monocytogenes* significantly decreased (P<0.01) from 7.033 to 6.00 Log CFU/g by the week 3. Nearly simtlar findings after 14 days of frozen storage were published by **Golden et al. (1988).**

When the temperature is lowered rapidly, the frozen cells of microorganisms will be injured mechanically by intra and extracellular-ice crystals. As water is removed there is a concentration

of cell solutes lead to dissoeiation of cellular lipoprotein. thawing of frozen mieroorganisms lead to changes in cellular morphology, release and denaturation of macro-molecules **(El kest and Marth, 1992)**.

The results reported in table (3) revealed that there was no significant difference between the inhibitory effect of two concentrations of nisin (800 and 1600 IU/g) on *L. monocytogenes* counts at the day O and the day 7 when comparing the counts of *L. monocytogenes* in treated samples at two coneentrations.

On the other hand the obtained results were compared with the control on the day O. nisin treatment at two different concentrations showed signifieant decreases (LSD at 0.01=0.912) in *L. monocytogenes* counts yielding a count of 6.985 CFU/g and counts of 7.10 CFU/g at 800 and 1600 IU/g of raw ground beef stored at 4°C respectively.

In conclusion, the antilisterial action of nisin at two concentrations occurred immediately (day 0) after treatment with this baeterocin. Nisin treatment at 1600 IU/g ground beef may be useful in controlling *L. monocytogenes*.

Potassium sorbate is an antimicrobial agent with a broad spectrum activity and is used to extend the shelf life of many foods **(EL - Shenawy and Marth, 1991)**.

The inhibitory effect of potassium sorbate was summarized in table (4). Inoculated untreated ground beef by potassium sorbate (control) showed significantly (P<0.01) increase in *L. monocytogenes* counts from 8.04 to 9.553 log CFU/g by the 7 day. *L. monocytogenes* counts decreased not significantly from 9.553 to 9.393 log CFU/g by the day 21. potassium sorbate significantly (P<0.01) decreased *L monocytogenes* counts from 7.952 to 3.996 log units by the day 21 (LSD=0.554).

The maximum levels of the preservative allowed in food were sufficient to inhibit the growth of *L. monocytogenes* in beef mince **(Hammad, 1996)**.

It was observed in table (5) that the inoculated raw ground beef untreated with potassium nitrite showed significant (p<0.01) increase in counts from 8.076 to 9.553 log CFU/g by the day 7. *L. monocytogenes* decreased not significantly from 9.553 to 9.44 log CFU/g by the day 21. potassium nitrite at concentration of 125 ppm exhibited significant (p<0.01) inhibitory properties against *L. monocytogenes* from 7.65 to 3.159 log units by the day 21 (LSD = 0.509)

Sodium nitrite has a significant bacteriostaic activity against *L. monocytogenes* and provided cured meat with a degree of protection against this microorganism particularly if imployed in conjunction with a combination of acidic pH, vacuum packaging, high salt concentration and adequate refrigeration **(Bunchanan et al., 1989)**.

The data presented in table (6) indicated that *L. monocytogenes* counts were significantly ($P<0.01$) increased from 8.01 to 9.54 log CFU by the day 7 and the counts decreased not significantly from 9.54 to 9.363 log units by the day 21. The treatment of inoculated raw ground samples with potassium ascorbate at 550 ppm showed significant decreases in *L. monocytogenes* counts ($P<0.01$) from 7.893 to 3.576 log CFU/g by the day 21 (LSD = 0.251).

Compared to the control on the day 0, *L. monocytogenes* counts decreased not significantly from 8.01 to 7.893 log CFU/g. Significant differences (LSD at 0.01 = 0.267) between *L. monocytogenes* counts in treated and untreated samples were detected at 7, 14, and 21 day of refrigerated storage.

Duffy et al., (1994) reported that sodium nitrite reduced the growth rate and increased log titer. The effectiveness of nitrite was significantly increased by sodium ascorbate (0.042 %). In the absence of nitrite, ascorbate had no detectable effect on growth.

It was observed from table (7) that the treatment of inoculated raw ground beef with coupling of nisin with potassium nitrite, potassium sorbate and potassium ascorbate showed an immediate significant decrease in *L. monocytogenes* counts and a maximum significant reduction to 2.257 log CFU/g was observed at the day 14. The treatment of inoculated raw ground beef with this combination resulted in a larger reduction in *L. monocytogenes* counts comparing to that obtained by other treatment in this study except with nisin treatment at 1600 IU/g at the day 14.

The antilisterial action of nitrite was predominately bacteriostatic. Sorbate alone had a bacteriostatic effect, where sorbate acting in the presence of nitrite produced a marked listericidal effect. Nisin acting alone produced an almost immediate 90% of listericidal response but initial numbers were restored within 14 days. Sorbate and nisin acting in combination produced enhanced listericidal effect which was also seen in the presence of curing salts, but was delayed **(Buncic et al., 1998)**.

Table (1) : Thermal treatment of isolated *L. monocytogenes* serotype 1 (10^8 CFU/g) in the inoculated raw ground beef .

Elapsed time in minutes	* Mean log CFU/g ± SD		
	At 55°C	At 60°C	At 70°C
Zero	8.000±0.00a†	8.000±0.000a	8.000±0.000a
10	7.735±0.126^{ab}	7.682±0.100^{a}	5.576±0.89^{b}
20	7.367±0.180^{b}	5.259±0.146^{b}	2.586±0.086^{c}
30	5.291±0.111^{c}	4.651±0.156^{c}	-
40	5.111±0.111^{cd}	3.938±0.228^{d}	-
50	4.725±0.123^{d}	3.269±0.142^{e}	-
60	3.726±0.069^{e}	1.997±0.305^{f}	-
** LSD at 0.01	0.519	0.401	0.169

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column are significantly different ($p<0.01$).

** LSD = Least significant difference .

Table (2) : Effect of frozen storage at-18°C on the survival of isolated *L. monocytogenes* serotype 1 (10^8 CFU/g) in the inoculated raw ground beef .

Weeks of Storage	* Mean log CFU/g ± SD
0	8.041± 0.040a†
1	7.541±0.75^{ab}
2	7.033±0.80^{b}
3	6.000±0.301^{c}
4	5.440±0.125^{cd}
5	4.999±0.301^{de}
6	4.500±0.100^{e}
7	2.566±0.305^{f}
8	2.000±0.300^{f}
** LSD at 0.01	0.574

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column are significantly different ($p<0.01$)

** LSD = Least significant difference .

Table (3) : Effect of 800 and 1600 IU nisin/g on the survival of isolated *L. monocytogenes* serotype 1 (10^8 CFU/g) in the inoculated raw ground beef stored at 4°C.

Treatment	* Mean log CFU/g ± SD			
	Day 0	Day 7	Day 14	Day 21
Lm only (10^8 CFU/g)	8.08±0.05^{b}†	9.090±0.107^{a}	9.777±0.075^{a}	9.327±0.031^{a}
Lm + 800 IU nisin/g	6.985±0.157^{c}	5.87±0.088^{d}	3.249±0.066^{e}	2.958±0.242^{ef}
Lm + 1500 IU nisin/g	7.10±0.173^{c}	5.593±0.107^{d}	2.088±0.08^{fg}	1.993±0.0969

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column are significantly different ($p<0.01$).

• For comparing inoculated treated with untreated (control) samples, at the same period, the LSD at 0.01 was = 0.912.

• For comparing periods with inoculated treated or untreated (control) samples, LSD at 0.01 was = 0.963.

▪ Lm. Listeria monocytogenes.

Table (4) : Effect of potassium sorbate 0.26% on the survival of isolated *L. monocytogenes* serotype 1 (10^8 CFU/g) in the inoculated raw ground beef stored at 4°C.

Treatment	* Mean log CFU/g ± SD			
	Day 0	Day 7	Day 14	Day 21
Lm only (10^8 CFU/g)	8.04±0.04^{b}†	9.553±0.064^{a}	9.513±0.278^{a}	9.393±0.090^{a}
Lm + potassium sorbate	7.952±0.04^{b}	7.127±0.151^{c}	5.259±0.451^{d}	3.996±0.306^{e}

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column are significantly different ($p<0.01$).

• For comparing inoculated treated with untreated (control) samples, at the same period, the LSD at 0.01 was = 0.619.

• For comparing periods with inoculated treated or untreated (control) samples, LSD at 0.01 was = 0.554.

• Lm. Listeria monocytogenes.

Table (5) : Effect of potassium nitrite (125 ppm) on the survival of isolated *L. monocytogenes* serolype 1 (10^8 CFU/g) in the inoculated raw ground beef stored at 4^oC.

Treatment	* Mean log CFU/g ± SD			
	Day 0	Day 7	Day 14	Day 21
Lm only (10^8 CFU/g)	8.076 ± 0.125^{b}†	9.553 ± 0.064^{a}	9.537 ± 0.278^{a}	9.44 ± 0.135^{a}
Lm + potassium nitrite	7.65 ± 0.156^{b}	6.976 ± 0.720^{c}	3.919 ± 0.208^{d}	3.153 ± 0.204^{e}

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column are signilicantly different ($p<0.01$).

- For comparing inoculated treated with untreated (control) samples, at the same period, the LSD at 0.01 was = 0.529.
- For comparing periods with inoculated treated or untreated (control) samples, LSD at 0.01 was = 0.509.
- Lm, Listeria monocytogenes.

Table (6) : Effect of potassium ascorbate (550 ppm) on the survival of isolated *L. monocytogenes* serotype 1 (10^8 CFU/g) in the inoculated raw ground beef stored at $4\ ^oC$.

Treatment	* Mean log CFU/g ± SD			
	Day 0	Day 7	Day 14	Day 21
Lm only (10^8 CFU/g)	8.01 ± 0.065^{b}†	9.54 ± 0.062^{a}	9.43 ± 0.125^{a}	9.363 ± 0.065^{b}
Lm + potassium ascorbate	7.893 ± 0.111^{b}	6.963 ± 0.169^{c}	4.44 ± 0.126^{d}	3.576 ± 0.089^{e}

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column are significantly different ($p<0.01$).

- For comparing inoculated treated with untreated (control) samples, at the same period, the LSD at 0.01 was = 0.267
- For comparing periods with inoculated treated or untreated (control) samples, LSD at 0.01 was = 0.251.
- Lm, Listeria monocytogenes.

Table (7) : Effect of combination of nisin, potassium sorbate, potassium nitrite, and potassium ascorbate (800 IU/g, 0.28%, 125 ppm and 550 ppm raspectively) on the survival of isolated *L. monocytogenes* serotype 1 (10^8 CFU/g) in the inoculated raw ground beef stored at 4 ^{o}C.

Treatment	* Mean log CFU/g ± SD			
	Day 0	Day 7	Day 14	Day 21
Lm only (10^8 CFU/g)	8.088 ± 0.044^{b}†	9.605 ± 0.058^{a}	9.557 ± 0.068^{a}	9.447 ± 0.133^{a}
Lm + combination of n, ps, pn, and pa	6.938 ± 0.242^{c}	6.280 ± 0.043^{c}	2.257 ± 0.238^{d}	1.958 ± 0.242^{d}

n : nisin ps : Potassium sorbata pn : potassium nitrite pa : potassium ascorbate .

* Means of triplicate samples analyzed in duplicate and expressed by log CFU/g.

† Means with different superscript with same column or raw are significantly different ($p<0.01$).

- For comparing inoculated treated with untreated (control) samples, at the same period, the LSD at 0.01 was = 0.693.
- For comparing periods with inoculated treated or untreated (control) samples, LSD at 0.01 was = 1.015.
- Lm. Listeria monocytogenes.

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الملخص العربى

بقاء عترات الليستيريا مونوسيتوجينز فى اللحم البقرى المفروم الطازج

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تم دراسة العوامل المؤثرة على نمو وتكاثر ميكروب الليستيريا مونوسيتوجينز من النوع السيرلوچى الفردى والمحقونة فى اللحم البقرى المفروم الطازج بنسبة ٨١٠ ميكروب لكل جم من اللحم المفروم والمخزن عند 4 درجة مئوية مثل تأثير الحرارة والحفظ بالتجميد وكذلك إضافة بعض المواد الحافظة عليه.

أولاً : أظهرت الدراسة أن تسخين الميكروب عند ٧٠ درجة مئوية لمدة ٢٠ دقيقة أدت إلى نقص معنوى فى العدد الكلى للميكروب من ٨ إلى ٢٫٥٨٦ لوغاريتم ميكروب لكل جم من اللحم المفروم، ولم يكتشف أى نمو للعترة المختبرة عند درجة حرارة ٧٠ درجة مئوية داخل اللحم كافية لقتل الميكروب خصوصاً فى المنتجات الملوثة بدرجة كبيرة.

ثانياً : أظهرت النتائج أن البرودة عند - ١٨ درجة مئوية قد أدى إلى نقص فى العدد الكلى للميكروب من ٨٫٠٤١ إلى ٢٫٠٠٠ لوغاريتم ميكروب لكل جرام لمدة شهرين وقد أسفرت النتائج ميكروب الليستيريا مونوسيتوجينز قد نقص نقصاً معنوياً من ٧٫٠٣٣ إلى ٦٫٠٠٠ لوغاريتم ميكروب لكل جرام عند الإسبوع الثالث من التجميد ومن ٤٫٥٠٠ إلى 2.566 لوغاريتم ميكروب لكل جرام عند الإسبوع السابع من الحفظ بالتجميد.

ثالثاً : أثبتت هذه الدراسة أن النيسين عند ١٦٠٠ وحدة دولية لكل جم من اللحم أدت إلي التحكم فى الميكروب بدرجة كبيرة.

رابعاً : أثبتت الدراسة أن معالجة اللحم المحقون بسوربات البوتاسيوم (٠٫٢٦ ٪) قد أدى إلى تثبيط معنوى فى الميكروب من (٧٫٩٥٤) إلى (٣٫٩٩٦) لوغاريتم ميكروب لكل جم خلال يوم من التخزين عند درجة

مئوية مما يؤكد أن سوربات البوتاسيوم لها تأثير واسع المدى على ميكروب الليستيريا مونوسيتوجينز وضمان سلامة المنتج.

خامساً : أسفرت النتائج أن نيتريت البوتاسيوم عند تركيز ١٢٥ جزء لكل مليون جزء من اللحم أدى إلى نقص معنوى فى العدد الكلى للميكروب من ٧.٦٥ إلى ٣.١٥٩ لوغاريتم ميكروب لكل جم بينما قد زاد العدد الكلى للميكروب فى اللحم المفروم الغير معالج بنيتريت البوتاسيوم من ٨.٠٧٦ إلى ٩.٤٤ لوغاريتم ميكروب ميكروب لكل جم خلال يوم من التخزين عند درجة مئوية.

سادساً : تمت دراسة تأثير اسكيوم عند تركيز ٥٥٠ جزء لكل مليون جزء من اللحم على العترة المختبرة وأوضحت النتائج أن هذا التركيز قد أدى إلى نقص معنوى فى العدد الكلى لميكروب الليستيريا مونوسيتوجينز من ٧.٨٩٣ إلى ٣٫٥٧٦ لوغاريتم ميكروب لكل جم خلال ٢١ يوم على العكس من عينات اللحم المفروم الغير معالج باسكوربات البرتاسيوم حيث زاد العدد الكلى للميكروب من ٨.٠١ إلى ٩.٣٦٣ لوغاريتم ميكروب لكل جم خلال ٢١ يوم.

سابعاً : تم دراسة تأثير مركب من كل من النيسين ونيتريت البوتاسيوم وسوربات البوتاسيوم واسكوربات البوتاسيوم عند تركيز ٨٠٠ وحدة دولية لكل جم و ١٢٥ جزء لكل مليون جزء من اللحم و ٠.٢٦ ٪ من اللحم المفروم و ٥٥٠ جزء لكل مليون جزء من اللحم على التوالى وذلك على العترة المختبرة لميكروب الليستيريا مونوسيتوجينز من النوع السيرلوچى الفردى والمحقونة فى اللحم المفروم والطازج بنسبة ٨١٠ ميكروب لكل جم من اللحم المفروم والمخزن عند ٤ درجة مئوية، وقد أوضحت النتائج أن المعالجة بهذا المركب قد أدى إلى نقص معنوى فى العدد الكلى للميكروب من ٨٫٠٨٨ إلى ٦.٩٣٨ بمجرد حقنه فى اللحم وقد أوضحت الدراسة أن أعلى نقص معنوى للميكروب كان عند اليوم ١٤ حيث وصل العدد الكلى للميكروب إلى ٢.٢٥٧ لوغاريتم ميكروب لكل جم، تم دراسة الأهمية الصحية للميكروب فى هذا البحث.