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GROWTH AND SURVIVAL OF *LISTERIA MONOCYTOGENES* DURING MANUFACTURE AND STORAGE OF DAMIETTA CHEESE

(With One Table & Two Figs.)

By

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مدى نمو وبقاء ميكروب الليستيريا (*L. monocytogenes*)
أثناء تصنيع وتخزين الجبن الدمياطى

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يعتبر الجبن الدمياطى من أكثر أنواع الجبن إنتشاراً بين المستهلكين لما له من قيمة غذائية عالية ، ومع ذلك فإن له دور خطير فى نقل كثير من الأمراض إلى الإنسان . من هذه الأمراض مرض الـ *Listeriosis* والذي يسببه ميكروب الـ *L.monocytogenes* . ولما لهذا الميكروب من خطورة كبيرة على صحة الإنسان أجريت هذه الدراسة لمعرفة مدى نمو وبقاء هذا الميكروب أثناء تصنيع وتخزين الجبن الدمياطى . لذلك قمنا بتصنيع الجبن الدمياطى من لبن ميستر معلباً مضاف إليه 5% ملح وآخر مضاف إليه 10% ملح . وتم إضافة ميكروب الليستيريا (10^8 /مل) وذلك قبل إضافة الملح والمنفحة . ثم قسماً بعد الميكروب المتواجد الرقم الهيدروجينى ، نسبة الرطوبة ونسبة الملح دورياً أثناء التصنيع وكذلك أثناء التخزين وقد وجد أن ميكروب الليستيريا تزايد فى العدد أثناء التصنيع فى كلا النوعين من الجبن مع تفاوت فى نسبة الزيادة . وقد وصل إلى أقصى عدد له (10^{10}) / جرام مع نهاية تصنيع الجبن المعد من لبن مضاف إليه 5% ملح . بينما وصل عدد الميكروب فى النوع الآخر من الجبن إلى (10^5) / جرام مع نهاية التصنيع . وقد بدأ عدد ميكروب الليستيريا يتناقص أثناء فترة التخزين ، حيث لم يمكن إكتشافه مع نهاية الأسبوع الثالث . كذلك تناقص الرقم الهيدروجينى للجبن أثناء فترة التخزين حتى وصل إلى (ر) ، ه فى كلا النوعين على التوالى مع نهاية الأسبوع الثالث . بينما تناقصت نسبة الرطوبة وتزايدت نسبة الملح أثناء فترة التخزين . وقد تم ذكر الخطورة الصحية لهذا الميكروب والشروط الواجب إتباعها لمنع تلوث الجبن الدمياطى بميكروب الليستيريا .

SUMMARY

Damietta cheese was prepared from pasteurized milks containing 5 or 10% added sod. chloride. Pasteurized milk was inoculated with *L.monocytogenes* strain V7 (milk isolate-serotype 1), before addition of salt and rennet. Cheese samples were stored in their whey at 30°C and examined periodically for *L.monocytogenes* count, pH value, salt and moisture contents. There was a rapid

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A.A-H. AHMED, et al.

increase in the numbers of L.monocytogenes during the manufacture of both types of cheeses. L.monocytogenes could survive until the end of the third week of storage, and reached a minimum of $\frac{1}{100}$ cells/g. which could not be recovered on the plates. There was a decrease in pH value and moisture content of cheeses, while the salt contents (water phase) increased gradually during the storage period. The public health hazards, and the suggestive measures were mentioned.

INTRODUCTION

Listeria monocytogenes is a potential foodborne pathogen and has become of considerable concern to food industry in different countries. L.monocytogenes can cause abortion in pregnant women and meningitis or meningioencephalitis in immunocompromised men and women (SEELIGER, 1961, GRAY and KILLINGER, 1966 and RALOVICH, 1984).

Excretion of L.monocytogenes in milk as a result of Listeria mastitis has been documented (SPIKA, et al. 1973 and GITTER, et al. 1980), while the isolation of Listeria from milk of clinically normal cow has been reported by GITTER, et al. (1980). Furthermore, HIRD (1987) stated that L.monocytogenes was recovered from a sour cream like product and cottage cheese by-products.

A large outbreak of listeriosis occurred in Germany due to ingestion of raw milk (SEELIGER, 1961). Moreover, pasteurized milk and fresh high moisture Mexican-style cheese have been implicated in two recent outbreaks of listeriosis in U.S.A. FLEMING, et al. 1985 and USPHS, 1985).

The ability of L.monocytogenes to survive during manufacture and storage of cheeses has been studied by several investigators. IKONOMOV & TODOROV (1964) recorded that L.monocytogenes did not disappear from kachkaval cheese until after 50 to 70 days of storage at 18 to 22°C. SPIKA, et al. (1974) found that L.monocytogenes survived the manufacture of white brined cheese, and increased in numbers from 240 to over 10^6 cells/g after 14 days of ripening. The organism can survive in unsalted cheese prepared from infected skim milk through 7 days of storage at 3 to 5°C (STAJNER, et al. 1979).

This work was planned to determine the growth and multiplication of Listeria monocytogenes during manufacture and storage of Damietta cheese.

MATERIAL and METHODS

Strain of L. monocytogenes:

L.monocytogenes strain V7 (milk isolate-serotype 1) was obtained from the Dept. of food science, Univ. of Wisconsin, Madison, USA. A culture of L.monocytogenes was prepared using Tryptose broth as described by RYSER, et al. (1985).

Assiut Vet.Med.J. Vol. 22, No. 43, 1989.

L. MONOCYTOGENES IN DAMIETTA CHEESE

Preparation of cheese:

Raw milk obtained from the dairy farm of the faculty of Agriculture, Assiut Univ., was pasteurized at 63°C for 30 min. Broth culture of L.monocytogenes was added to the warmed (40°C) pasteurized milk to provide 3×10^6 cells/ml. A sample was taken after inoculation to determine L.monocytogenes count and pH value. The inoculated pasteurized milk was divided into two portions, which were salted by addition of sod. chloride to give concentration of 5 and 10%. The procedure described by FAHMY & SHARARA (1950) was used to manufacture Damietta cheese. Two control blocks of Damietta cheese were prepared from pasteurized milk containing the same percentages of salt. Samples from the curd and finished product were tested for L. monocytogenes count and pH value. Finished cheeses were examined for moisture, and salt content. Cheeses with their controls were stored at $30 \pm 1^\circ\text{C}$ and tested weekly for Listeria count, pH value, moisture content and salt percentage.

Preparation of cheese samples for examination:

Cheese samples were prepared for examination according to Standard methods (MARTH, 1978).

Enumeration of L. monocytogenes:

Surface plating technique for each sample and its dilutions was done using Tryptose Agar supplemented with 40 Ug/ml. Nalidixic acid and 30 u/ml Polymyxin B sulphate as described by HOFER (1983). The plates were incubated at 37°C for 24h., and typical colonies presumed to be L.monocytogenes were counted. Confirmatory tests were done on each isolate suspected to be L.monocytogenes as described by SEELIGER (1961).

pH values:

The pH value of cheese was determined using a pH meter (an orion Model 701) equipped with standard electrode.

Moisture and salt content:

Moisture was determined according to the Standard methods (MARTH, 1978). The salt content in cheese was determined by the method of ATHERTON and NEWLANDER (1977).

RESULTS

The obtained results were recorded in Table 1 and Fig. (1 & 2).

DISCUSSION

The results in (Table 1) reveal that, there was a gradual increase in salt contents (water phase) of cheeses during storage, while a decrease in moisture contents occurred during this period. These changes were noted by SALEEM, *et al.* (1978); AHMED, *et al.* (1983) and MOUSTAFA, *et al.* (1988).

A substantial multiplication and rapid increase in the number of L.monocytogenes occurred during manufacture of cheese prepared from milk with 5% added salt (Fig.

1). L.monocytogenes achieved its maximum population (4×10^{10} cells/g) by the end of cheese making, and rapidly lost its viability during storage period which could not be recovered from cheese by the third week. The pH of cheese after preparation was 6.5, and slowly decreased during storage. A low value of 4.1 was reached by the end of the third week.

Concerning the results obtained from cheese made from milk with 10% added salt (Fig. 2), an increase in the number of L.monocytogenes up to 5×10^{10} cells/g was achieved by the end of cheese manufacture. The organism rapidly decreased in number during storage and could not be detected by the third week. The pH value was 6.4 when curd was made and decreased to 5.0 at the third week of storage.

From the aforementioned results, it is evident that both types of cheese had a similar pH value during their manufacture. Such pH was favorable for growth and multiplication of L.monocytogenes. Similar findings were reported by SPIKA (1974); RYSER, et al. (1985) and RYSER & MARIH (1986). The increased amount of salt had an inhibitory effect on growth and multiplication of L.monocytogenes during manufacture of cheese prepared from milk with 10% added salt (Fig. 2). Similar results were reported by SHAHAMAT, et al. (1980). However, LARSEN (1969) stated that L.monocytogenes could survive high concentration of salt.

As shown in Fig. (1 & 2), L.monocytogenes lost its viability during storage period of cheeses, and could not be recovered from cheeses by the end of their third week. This probably resulted from lactic acid production and consequent decrease of pH value of cheeses during the storage, which had a negative effect on survival of L.monocytogenes. The obtained results are in agreement with the conclusion of IRVIN (1968), that L.monocytogenes lost its viability and decreased in number at pH value below 5.5. Similar findings were obtained by RYSER, et al. (1985), while different results were recorded by IKONOMOV & TODOROV (1964) and STAJNER, et al. (1979). These differences can be attributed to the type of cheese used, techniques of cheese preparations and the variation in storage temperatures.

On conclusion, L.monocytogenes grew to a high level during cheese manufacture, and survived for at least 15 days. The increased amount of salt served to retard growth of such organism during cheese making, while at pH value lower than 5.5, L.monocytogenes lost its viability and decreased in number. Therefore, Damietta cheese could become of public health hazard, if the milk is contaminated with L.monocytogenes, and if growth of such organism occurs during curdling of milk and preparation of cheese. Strict hygienic measures should be followed during milk production, handling and cheese processing to prevent the contamination by L.monocytogenes.

REFERENCES

- Ahmed, A.A-H.; M.K. Moustafa and E.H. Marth (1983): Growth and survival of S.aureus in Egyptian Domiati cheese. J. food prot. 46: 412-415.

L. MONOCYTOGENES IN DAMIETTA CHEESE

- Atherton, H.V. and J.A. New Lander (1978): Chemistry and testing of dairy products 4th ed. AVI publishing company Inc., Westport Connecticut.
- Fahmy, A.H. and H.A. Sharara (1950): Studies on Egyptian Domiati cheese. J. Dairy Res. 17: 312-327.
- Fleming, D.W.; S.L. Cochi, K.L. Macdonald, J. Brondum; P.S. Hayes; B.D. Plikaytis; M.B. Holmes; A. Audurier; C.V. Broom and A.L. Reingold (1985): Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 212: 7: 404-407 (Cited after Ryser, et al. 1985).
- Gitter, M.; R. Bradley and P.H. Blampid (1980): Listeria monocytogenes infection in bovine mastitis. Vet. Rec. 107: 390-393.
- Gray, M.L. and H. Killinger (1966): Listeria monocytogenes and listeric infections. Bacteriol Rev. 30: 309-382 (Cited after Ryser, et al. 1985).
- Hird, D.W. (1987): Review of Evidence for Zoonotic listeriosis. J. Food Prot. 50: 5-429.
- Hofer, E. (1983): Bacteriologic and Epidemiologic studies on the occurrence of L.monocytogenes in healthy cattle. Zbl. Bakt. Hyg. A 256, 175-183.
- Ikonomov, L. and D. Todorov (1964): Studies of viability of L.monocytogenes in ewe's milk and dairy products. Vet. Med. Nauki, Sofia 1:7 23-29 (Dairy Sci. Abstr. 27: 2, 1965).
- Irvin, A.D. (1968): The effect of pH on the multiplication of L.monocytogenes in grass silage media Vet. Rec. 82: 115-116.
- Larsen, H.E. (1969): L.monocytogenes Carl, F.R. Mortensen, copenhagen (Cited after Shahamat, et al. 1980).
- Marth, E.H. (ed) (1978): Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington D.C.
- Moustafa, K.M.; A.A-H. Ahmed and Nagah, M.S. (1988): Fate of Enteropathogenic Escherichia coli (EEC) during manufacture and storage of Domiati cheese. Ast. Vet. Med. J. 20: 39, 104-109.
- Ralovich, B. (1984): Listeria research. Present situation and prespective. Akademiai Kiado, Bodabest (Cited after Hird, 1987).
- Ryser, E.T.; E.H. Marth and M.P. Doyle (1985): Survival of L.monocytogenes during manufacture and storage of cottage cheese. J. Food. Prot. 48: 746-750.
- Ryser, E.T. and E.H. Marth (1986): Fate of L.monocytogenes during manufacture and ripening of camembert cheese J. Food. Prot. 49: 818.
- Saleem, R.A.; M.H. Abdel-Salam; M.R. Nagmouh and M.M. El-Abd (1978): White pickled soft cheese from concentrated milk 11. Effect of concentration of brine and calcium chloride added. Egyptian J. Dairy Sci. 6: 207-220.
- Seeliger, H.P.R. (1961): Listeriosis. Hafner Publishing Co., New York (Cited after Ryser, et al. 1985).
- Shahamat, M.A. Seaman and M. Woodbin (1980): Survival of L.monocytogenes in high salt concentration Zbl. Bakt. Hyg., L. Abst. orig. A 240, 50-511.
- Spika, M.; B. Stajner and S. Zakula (1973): Detection of Listeria in milk. Wiener tierarztlich Monatsschrift 60: 50-52. (Dairy Sci. Abst. 11: 35, 1973).

A.A-H. AHMED, et al.

- Spika, M.; S. Zakula, I. Kovincic, and B. Stajner (1974): Secretion of L.monocytogenes in cow's milk and its survival in white brined cheese. XIX Intern. Dairy Congr. 1E: 157 (Cited after Hird, 1987).
- Stajner, B.; S. Zakula; I. Kovincic and M. Galic (1979): Heat resistance of L.monocytogenes and its survival in raw milk products. Veterinaski Glasnik 33: 109-112. (Dairy Sci. Abstr. 43: 5, 1981).
- USPHS (United States Public Health Service Centers for Disease Control (1985): Listeriosis outbreak associated with Myxican-style cheese. California, Morbid, Mort. Weekly Rep. 34, 357-359. (Cited after Hird, 1987).

Table (1)

Changes in chemical composition of Damietta cheese during storage

Time of storage (weeks)	Cheese prepared from milk with 5% salt			Cheese prepared from milk with 10% salt		
	T.S. %	Moisture %	Salt ^a %	T.S. %	Moisture %	Salt %
0 Finished product	35.7	64.3	4.7	34.3	65.7	8.6
1	38.3	61.7	5.8	35.3	64.7	9.8
2	41.1	58.9	6.4	38.2	61.8	10.0
3	42	58	6.5	40	60	10.3

^a : Salt content in water phase of cheese.

Count of *Listeria monocytogenes* / g.

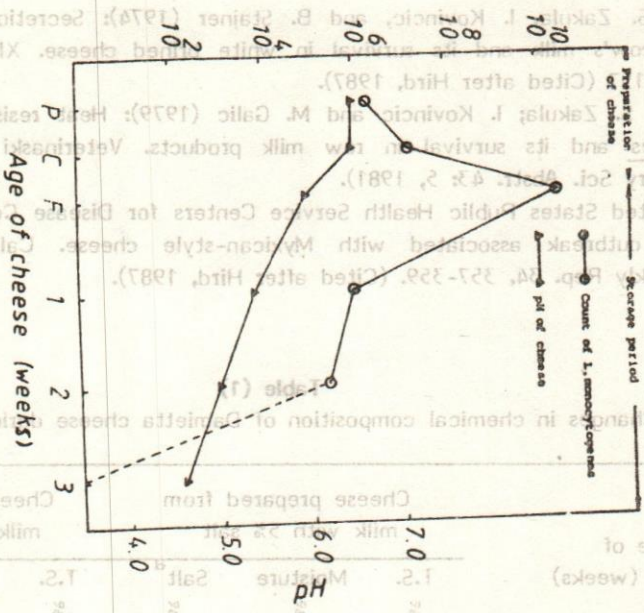


Fig. (1): Count of *Listeria monocytogenes* strain V7 in Damietta cheese prepared from milk containing 5% salt and stored at 30±1 C.
 P = Inoculated pasteurized milk
 C = Curd
 F = Finished cheese

Count of *Listeria monocytogenes* / gm.

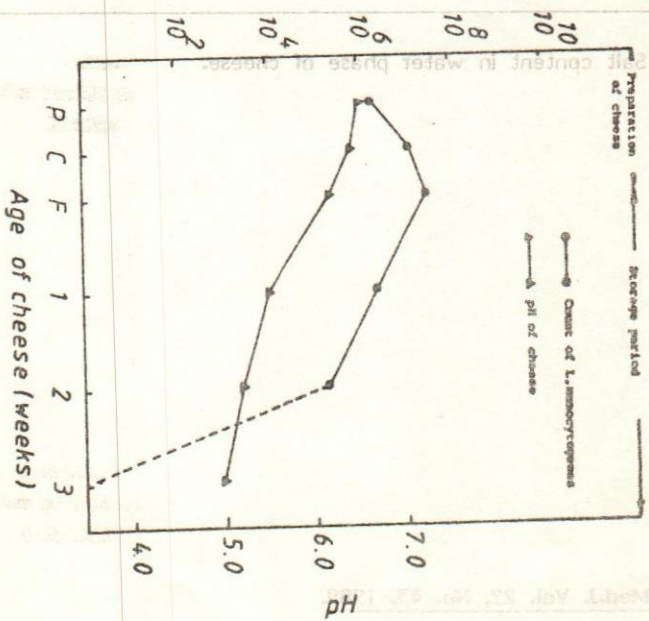


Fig. (2): Count of *Listeria monocytogenes* strain V7 in Damietta cheese prepared from milk containing 10% salt and stored at 30±1 C.
 P = Inoculated pasteurized milk
 C = Curd
 F = Finished cheese