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EFFECT OF *NIGELLA SATIVA* AND SALT ON THE GROWTH AND SURVIVAL OF *E.COLI* O157:H7 DURING MANUFACTURE AND STORAGE OF DAMIETTA CHEESE

(With 4 Tables)

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تأثير حبة البركة والملح على نمو وبقاء ميكروب الايشيريشيا كولاى O157:H7 أثناء تصنيع وتخزين الجبن الدمياطى

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يعتبر الجبن الدمياطى أحد منتجات الألبان المنشرة فى مصر والتى لها قيمة غذائية عالية نظرا لاحتوائه على نسبة عالية من البروتين والكالسيوم, ولما كان لحبة البركة(الحبة السوداء) من فضل كبير على سائر الأعشاب فى القضاء على العديد من الميكروبات المرضية. لذا قامت هذه الدراسة لمعرفة مدى تأثير الحبة السوداء والملح على ميكروب الايشيريشيا كولاى *E.coli* O157:H7 أثناء تصنيع وتخزين الجبن الدمياطى. تم استخدام لبن مبستر معملياً بتصنيع الجبن الدمياطى مضافا إليه (٢× ١٠^{٤}/مليلتر) من ميكروب *E.coli* O157:H7 مع إضافة الملح بتركيز ٥% , ١٠% وكذا حبة البركة بتركيز ١% , ٣% لكل منهما ثم تم تقسيم الجبن المصنع وتخزينه فى الشرش عند درجة حرارة الغرفة والثلاجة. تم فحص العينات أثناء وبعد التصنيع ثم اسبوعيا من حيث عدد الميكروب ونسبة الرطوبة والحموضة والملح , وقد وجد أن الميكروب *E.coli* O157:H7 تزايد فى العدد ووصل أقصى عدد له ٨× ١٠^{٥} /جرام مع نهاية الاسبوع الاول فى الجبن الخالى من حبة البركة والمحفوظ عند درجة حرارة الغرفة بينما وصل إلى ١،٦×١٠^{٤}/جرام فى الجبن الغير محتوى على حبة البركة والمحفوظ فى الثلاجة والغرفة على التوالى , أما بالنسبة للجبن المحتوى على حبة البركة فقد أوضحت نتائج البحث انخفاض أعداد الميكروب حتى تلاشى مع نهاية الاسبوع الثالث فى العينات التى تم تخزينها فى درجة حرارة الغرفة والاسبوع الثانى فى الجبن الذى تم تخزينه فى الثلاجة وكذلك لوحظ تزايد نسبة الحموضة ونسبة الملح أثناء التخزين بينما تناقصت نسبة الرطوبة. وقد اوصت الدراسة باستخدام تركيز ١% من حبة البركة مع ٥% من الملح لانتاج نوع جديد من الجبن .

SUMMARY

Damietta cheese was prepared from pasteurized milk inoculated with *E.coli* O157:H7 and contained 5% , 10% sodium chloride. *Nigella sativa* seeds (1%, 3%) were added. Cheeses were stored in their whey, at room (25 ± 2°c) and refrigerator (5 ± 2°c) temperatures. Cheese samples were examined periodically for *E.coli* O157:H7 count, acidity, salt and moisture content. The results indicated that counts of *E.coli* O157:H7 increased rapidly during storage of control cheese at room temperature reaching 8×10^5 cfu/g by the end of the first week, then decreased gradually till the fourth week. *E.coli* O157:H7 could survive until the end of the third week of storage in control cheese stored at refrigerator. However, the organism was not detectable after three and two weeks in cheeses containing *Nigella sativa* and stored at room and refrigerator temperatures respectively. There was a decrease in moisture content while, acidity and salt contents increased. *Nigella sativa* seeds were found to have antimicrobial activities against *E.coli* O157:H7. The results recommended the use of 5% salt and 1% *Nigella sativa* to produce a new type of Damietta cheese.

Key Words: *Nigella sativa, E.coli O157:H7, Damietta cheese.*

INTRODUCTION

The black seed or *Nigella sativa* (Habbet El Baraka) is a plant which has been used widely as a natural treatment for more than 2000 years, is also included in the list of natural drugs of Al-Tibb al-Nabawi, and, according to a tradition attributed to Prophet Mohammed (pbuh) in Sahih Bukhari "Hold onto the use of the black seed for it has a remedy for every illness except death". This prophetic reference in describing black seed as " having a remedy for all illnesses" may not be so exaggerated as it at first appears. Recent researches have provided evidences which indicate that black seeds contain an ability to significantly boost the human immune system- if taken over time. It stimulates bone marrow and immune cells and raises the interferon production, protects normal cells against cell destroying effect of viruses, destroys tumor cells and raises the number of antibodies producing blood cells. In Addition black seeds proved to have an antihistamine, antioxidant, antibiotic, antimycotic and broncho-dilating effect (Kgul, 1989; Hanafy and Hatem, 1991; Mahdi, 1993; Mahmoud,

1993 and Kahira Pharma. & Chem. Ind. Co., 1995). Moreover, it is a valuable source of protein, carbohydrates, essential fatty acids, vitamin A, B_1, B_2 , C and niacin as well as minerals such as calcium, potassium, iron, magnesium, selenium and zinc.

Recently, Enterohaemorrhagic *Escherchia coli* (EHEC O157:H7) has received a considerable attention because of its implication in several outbreaks. It was first recognized as a food pathogen in 1982 (Zheo and Doyle, 1994; Hudson *et al.*, 1997 and Economic Research Service (ERS), 1998). EHEC infection is associated with outbreaks of haemorrahgic colitis (HC), which occur most frequently in developed countries and may be associated with the consumption of raw milk and yoghurt (Martin *et al.*, 1986 and Keene *et al.*,1997). The rate of death may reach 36% in case of HC (Griffin, 1991). Subsequent development of serious complication including the hemolytic uremic syndrome (HUS) and thrombotic thrombocytopanic purpura (TTP) may happen. HUS occurs more in infants and young children as it is the major cause of renal failure in such age (Weagent *et al.*,1994; Allerbrger *et al.*,1997 and Koatkia *et al.*, 1997). Because of the very low infective dose of this organism (50 viable cells/ml or g) (Keene *et al.*,1994) bacteria entering the human food chain can still pose a health problem even after undergoing enormous dilutions (Abdulmawjood and Bulte, 2003).

It is well known that *Nigella sativa* have preservation qualities, besides their obvious functions in food production as flavour components (Furia, 1968). Moreover, the increasing use of natural preservatives to extend life of dairy products has raised concerns about the potential health risk for consumers by this organism.

Reports on efficaciousness of black seeds on microorganisms in dairy products are not available, however, several studies have been done to characterize survival of *E.coli* in Damietta cheese (Moustafa *et al.*, 1988; El-Gazzar, 1993 and Seham Farrag *et al.*, 1997).

Therefore, this work was planned to build up information on the effect of *Nigella sativa* and salt on growth and survival of *E.coli* O157:H7 in Damietta cheese during storage at room and refrigerator temperatures.

MATERIAL and METHODS

A-Culture preparation:

E.coli O157:H7 strain used in this study was previously isolated from raw milk samples and identified using PCR technique in Molecular Biology Center in Assiut University.

It was propagated in nutrient broth at 37°C for 16 h. Two consecutive transfers were made prior to inoculation, the number of microorganisms/g was determined by using Sorbitol MacConkey agar as a selective medium (APHA, 1992).

B-Preparation of Damietta cheese:

The procedure described by Fahmy and Sharara (1950) was used. Raw buffalo's milk was obtained from the experimental station of department of Animal production, Fac. Agric. Assiut Univ.

Raw milk was laboratory pasteurized at 63°C for 30 min. and inoculated with the organisms to yield a concentration of about 10^4 cfu/ml. A sample was taken after inoculation to determine the initial count and acidity. The inoculated milk was divided into equal parts, each was subjected to the following treatments.

- Addition of 5% salt and 1% and 3% *Nigella sativa*.
- Addition of 10% salt and 1% and 3% *Nigella sativa*.

Two control blocks of cheese were prepared. Samples from the curd and finished cheese were examined for *E.coli* O157:H7 count and acidity. Finished cheese with their control were stored at room temperature (25 ± 2°C) and refrigerator temp. (5 ± 2°C).

C-Cheese analysis:

Samples from the finished cheese with their controls were examined weekly for *E.coli* O157:H7 count according to (APHA, 1992), moisture content according to Marth, (1978), titratable acidity (AOAC, 1975) and salt concentration as described by Atherton and Newlander, 1977.

RESULTS

The obtained results are recorded in Tables (1-4).

DISCUSSION

The results presented in tables 1&2 showed that there was a gradual increase in salt contents of cheese during storage at room (25 ± 2°C) and refrigerator (5 ± 2°C) temperatures accompanied by a decrease in moisture content, these results agreed to that obtained by Saleem *et al.*, (1978) and Moustafa *et al.*, (1988). They noted that pH of the cheese appeared to have a greater effect on the counts than did moisture and salt.

Growth of *E.coli* O157:H7 during the manufacture of Damietta cheese resulted from a combination of such favorable conditions as high moisture, high temp. and low acidity (El-Gazzar, 1993 and Seham Farrag *et al.*,1997). They added that the rate of growth was faster in cheese made from 9 % salted milk than that made from 6% as it had a lower pH value.

Regarding, the counts of *E.coli* O157:H7, it increased during manufacture of cheese prepared from milk with 5% salt (control) with a maximum population of 6×10^4 cfu/g in the finished cheese and 8.0×10^5 cfu /g at the end of the 1st week. On storage at room temperature, gradual decrease in numbers occurred untill it reached a minimum count of 6 × 10 cfu/g by the end of the 4th week (Table 3).

From data in Table 4 it is evident that *E.coli* O157:H7 rapidly lost its viability in cheese made from milk containing 1%, 3% *Nigella sativa* with 5% salt and stored at refrigerator temperature to be undetectable by the end of the 2nd week. However, cheese samples stored at room temp. showed an increase in counts of *E.coli* O157:H7 to reach 3.2×10^4, 3.6×10^4 cfu/g respectively followed by a decrease in numbers until it could not be detected by the end of the 4th week of storage.

The corresponding results obtained from cheese prepared from milk with 5% salt (control) and stored at refrigerator pointed out that *E.coli* O157:H7 counts increased slowly until the end of the 1st week. A reduction in number occurred until the organism failed detection by the end of the 4th week of storage (Table 4).

As shown in Tables 3&4 cheeses prepared from milk with 10% salt and stored at room and refrigerator temperature (control), *E.coli* O157:H7 increased in numbers (4×10^5 /g and 1.6×10^6 /g) respectively until the 1st week of storage, then decreased as the storage period prolonged until reached undetectable level by the end of the 4th week of storage.

The results illustrated in Tables 3 & 4 verify that there was an increase in number of *E.coli* O157:H7 in cheeses with 10% salt and added 1% and 3% *Nigella sativa* seeds during its preparation as it reached 2.9×10^4, 2.7×10^4 /g, respectively.

The counts decreased during the prolonged shelf life of inoculated cheeses kept at both room and refrigerator temp. *E.coli* O157:H7 achieved its minimum counts on the 3rd and 2nd weeks of storage, respectively. The rate of decrease was faster than that of control

cheeses and it could not be detected by direct plating by the end of the 4th and the 3 rd weeks, respectively.

It was clear from the forementioned results the high counts of the organism in control Damietta cheese in contrast to the rapid reduction of *E.coli* O157:H7 counts in cheese containing *Nigella sativa* seeds added during preparation. Similar inhibitory influence induced by these seeds on other microorganisms was reported by Hanafy and Hatem (1991) and Sabreen (1996).

The present findings in Tables 1& 2 revealed that the acidity % increased gradually during storage of all cheeses at room and refrigerator temperatures. These results were similar to those obtained by El-Gazzar (1997) who studied fate of *E.coli* O157:H7 during manufacture of Damietta cheese.

Generally, it may be concluded that the use of 1% *Nigella sativa* seeds combined with 5% salt as well as refrigerator temp. are important factors influencing in the rate of growth and multiplication of *E.coli* O157:H7. These results agree to a certain extent with those reported by Naguib *et al.*,(1979) and Ahmed *et al.*, (1983).

The number of bacteria necessary to cause illness, is 50 viable cells/ml or g (Keene *et al.*, 1994). The present results indicated that *E.coli* O157:H7 may reach infective levels in fresh Damietta cheese during storage, so presence of *E.coli* O157:H7 in Damietta cheese must be regarded as a public health hazard. Thus, to ensure *E.coli* free food products, the organism must be kept out of any product during preparation and subsequent handling of the product. Use of adequate hygienic practices are needed to accomplish this goal.

REFERENCES

Abdulmawjood, A. and Bulte, M. (2003): Multicentre ring trials for validation of *E.coli* O157 PCR detection system. 2nd Int. Cong. Food Hyg. and Human Health Fac. Vet. Med. Assiut Univ.

Ahmed, A. A. H.; Moustafa, M. K. and Marth, E. H. (1983): Growth and survival of *Staphylococcus aureus* in Egyptian Domiati cheese. J. Food Prot. 46: 412-416.

Allerbrger, E.; Solder, B.; Capride, A. and Karch, H. (1997): Enterohaemorrhagic *E.coli* and haemolytic uremic syndrome. Wein-Klin. Wochenschr., 17: 669-677.

American Public Health Association (APHA) (1992): Compendium of Methods for the Microbiological Examinations of Foods. 2nd Ed. APHA, Washington, DC.USA.

A.O.A.C. (1975): Association of Official Agricultural Chemist, Official Methods of Analysis.

Atherton, H. V. and Newlander, J. A. (1977): Chemistry and Testing of Dairy Products. 4 th Ed. AVI Publishing Company Inc.

Economic Research Service "ERS" (1998): Total numbers of reported cases of *E.coli* O157:H7 disease 1996. Department of Agriculture.

El-Gazzar, F. E. (1993): Fate of *E.coli* O157:H7 during manufacture and storage of yoghurt and Domiati cheese. Assiut J. Agri. Sci., 24: 185-196.

El-Gazzar, F. E. (1997): Proceeding of the First Scientific Conference of Agricultural Sciences. Faculty of Agri. Assiut, December 13-14, 1997, Vol.II

Fahmy, A.H. and Sharara, H. A. (1950): Studies on Egyptian Domiati cheese. J. Dairy Res. 17: 312-327.

Furia, T. E. (1968): Hand book of food additives. The chemical Rubber company, Cleveland, OH.

Griffin, P. M. (1991): The epidemiology of infection caused by *E.coli* O157:H7, other Enterohaemorrhagic *E.coli* and the associated haemolytic uraemic syndrome. Epidemiol. Rev., 13: 60-98.

Hanafy, M. S and Hatem, M. E. (1991): Studies on the antimicrobial activity of *Nigella sativa* seed (black cummin). J. Ethno-Pharmacol. 34: 275-278.

Hudson, L. M.; Chen, J.; Hill, A. R. and Griffith, S. M. W. (1997): Bioluminescence: A rapid indicator of *E.coli* O157:H7 in selected yoghurt and cheese varities. J. Food. Prot., 60, 891-897.

Kahira Pharma & Chem. Ind. Co. (1995): Nigellar soft Gelatin capsules. Natural product from black seed (Habbet elbarakah) Cairo, Egypt.

Keene, W. E.; McAnulty, J. M.; Hoesly, F. C.; Williams, L. P.; Hedberg, K.; Oxman, G. L.; Barrett, T.J.; Pfallwer, M. A. and Fleming, D. W. (1994): A swimming-associated outbreak of haemorrhagic colities caused by *E.coli* O157:H7 and *Shigella sonnei.*N. Engl. J. Med. 331:579-584.

Keene, W. E.; Hedberge, D. E.; Herriot, D.; Hancack, D.; Machay, R. W.; Barrett, T. J. and Fleming, D. W. (1997): A prolonged outbreak of *E.coli O157:* H7 infection caused by commercially distributed raw milk. J. Infect. Dis. 176:815-818.

Kgul, A. (1989): Antimicrobial activity of black cummin (*Nigella sativa*) essential oil. Ataturk Univ-Eczacilik-Fac Derg. 6:63-68.

Koatkia, P.; Mylonakis, F. and Flanigon, T. (1997): EHEC O157:H7 an emerging pathogen. Am. Fam. Physician, 56 (3): 253-856, 859-861.

Mahdi, H.E.B. (1993): Effect of *Nigella sativa* (black seeds) on immune system in patients with liver cirrhosis, M.D. Thesis Fac. Med., Al-Azhar Univ., Egypt.

Mahmoud, H. M. (1993): Inhibitory action of black cummin (*Nigella sativa*) against *Listeria monocytogenes*. Alex. J. Agri. Res. 38: 123-134.

Marth, E. H. (1978): Standard Methods for the Examination of Dairy Products. 14 th Ed. American Public Healh Association, Washington, D.C.

Martin, M. L; Sphipman, L. D.; Potter, M. E.; Wachsmuth, I. K.; Wells, J. G.; Hedberg, K.; Tauxe, R. V.; Davis, J. P.; Arnoldi, J. and Tillell, J. (1986): Isolation of *E. coli* O157:H7 from dairy cattle associated two cases of haemolytic uremic syndrome. Lancet, II: 1043.

Moustafa, M. K.; Ahmed, A. A. H. and Saad, N. M. (1988): Fate of enteropathogenic *E.coli* during manufacture and storage of Domiati cheese. Assiut Vet. Med. J., 20: 104-109.

Naguib, M. M.; Naguib, Kh. and Erian, A. F. (1979): Effect of storage temperature on bacteriological and biochemical properties of liquid rennet. Egypt. J. Dairy Sci. 7: 209-214.

Sabreen, M. S. (1996): Incidence of staphylococci microorganisms in Domiati cheese and the effect of *Nigella sativa* on the growth of *Staphylococcus aureus*.7 th Sci. Cong. Fac. Vet. Med. Assiut Univ.

Saleem, R. A.; Abdel-Salam, M. H.; Nagmoush, M. R. and El-Abd, M. M. (1978): White pickled soft cheese from concentrated milk II. Effect of concentration of brine and calcium chloride added. Egyptian J. Dairy Sci., 6: 207-220.

Seham, A. Farrag; Abdul-Raouf, U. M. and El-Gazzar, F. E. (1997): Influence of salt concentration and initial count of *E.coli* O157:H7 on its survival in Domiati cheese. 1st Sci. Cong. Agri. Sci., Fac. Agric. Assiut Univ.

Weagent, S. D.; Bryint, J. L. and Brok, D. H. (1994): Survival of *E.coli* O157:H7 in mayonnise based sauciest room and referigerated temperatures. J. Food Prot. 57 (7), 659-661.

Zheo, T. and Doyle, M. P. (1994): Fate of enterohaemorrhagic *E.coli* O157:H7 in commercial mayonnaise. J. Food Prot. 57, 780-783.

Table 1: Changes in chemical composition of Damietta cheese during storage at room temperature

Storage period	Cheese with 5% salt			Cheese with 10% salt		
	Salt %	Moisture %	Acidity %	Salt %	Moisture %	Acidity %
Curd	3.3	60.2	0.16	4.6	65.3	0.16
Finished cheese	3.6	58.8	0.16	4.9	59.1	0.16
Ist week	5.6	55.5	0.36	6.8	55.0	0.36
2 nd week	6.6	54.8	0.64	7.4	52.9	0.64
3 rd week	6.9	51.7	0.52	7.6	50.1	0.46

Table 2: Changes in chemical composition of Damietta cheese during storage at refrigerator temperature

Storage period	Cheese with 5% salt			Cheese with 10% salt		
	Salt %	Moisture %	Acidity %	Salt %	Moisture %	Acidity %
Curd	3.1	60.2	0.16	4.6	64.3	0.16
Finished cheese	3.5	59.7	0.16	4.9	62.9	0.16
Ist week	4.3	57.2	0.24	5.6	61.4	0.24
2 nd week	4.8	56.8	0.20	5.7	59.3	0.18
3 rd week	4.7	55.1	0.12	6.2	58.4	0.16

Table 3: Effect of *Nigella sativa* on *E.coli* O157:H7 in Damietta cheese stored at room temp.

Storage period	Cheese with 5% salt			Cheese with 10% salt		
	1% *N.sativa*	3 % *N.sativa*	Control	1% *N.sativa*	3 % *N.sativa*	Control
Curd	2.0×10^4	2.0×10^4	2.0×10^4	2.0×10^4	2.0×10^4	2.0×10^4
Finished cheese	3.2×10^4	3.6×10^4	6.0×10^4	2.9×10^4	2.7×10^4	3.9×10^4
Ist week	6.0×10^3	1.8×10^3	8.0×10^5	8.0×10^3	1×10^3	4×10^5
2 nd week	3.3×10^2	1.3×10^2	9.0×10^3	3.5×10^2	3.2×10^2	3.1×10^3
3 rd week	2.2×10^2	1.0×10^2	6.7×10^2	2.8×10^2	2.6×10^2	7.1×10^2
4 th week	-	-	6.0×10	-	-	4.2×10

Table 4: Effect of *Nigella sativa* on *E.coli* O157:H7 in Damietta cheese stored at refrigerator temp.

Storage period	Cheese with 5% salt			Cheese with 10% salt		
	1% *N.sativa*	3 % *N.sativa*	Control	1% *N.sativa*	3 % *N.sativa*	Control
Curd	2.0×10^4	2.0×10^4	2.0×10^4	2.0×10^4	2.0×10^4	2.0×10^4
Finished cheese	3.4×10^4	3.5×10^4	6.0×10^4	2.7×10^4	2.7×10^4	2.9×10^4
I st week	2.1×10^4	1.1×10^4	3.2×10^4	5.0×10^3	5.0×10^3	1.6×10^4
2 nd week	5×10^2	7×10^2	6.7×10^3	2.2×10^3	2.2×10^3	7.4×10^3
3 rd week	-	-	2.0×10^2	-	-	5.0×10^2