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**A PRELIMINARY DETECTION OF INHIBITORY
SUBSTANCES IN MILK SOLD
IN ASSIUT GOVERNORATE**
(With 2 Tables)

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الكشف المبدئي عن المواد المثبطة في اللبن المباع بمحافظة أسيوط

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يشكل تواجد المواد المثبطة في اللبن خطورة كبيرة على صحة المستهلك. والتي يستخدم بعضها كمواد حافظة لتزويد فترة صلاحية اللبن. شملت هذه الدراسة فحص ١٦٠ عينة لبن تم جمعها عشوائياً من الباعة الجائلين، بيوت الفلاحين، محلات بيع الألبان ومن المزارع المنتجة للألبان (٤٠ عينة من كل نوعية إنتاج لبن). وقد خضعت هذه العينات لاختبارات تحديد تواجد المواد المثبطة بطريقتي Wynther Blyth test و Qualitative disc assay method. والاختبارات الكيميائية لتحديد تواجد الفورمالين، فوق أكسيد الهيدروجين، حامض البوريك وحامض السالسيك. أوضحت الدراسة أن كل العينات المختبرة كانت خالية من حامض السالسيك. كما أسفرت النتائج عن خلو عينات ألبان المزارع من المواد الحافظة الكيميائية تماماً في حين أن عينات ألبان الباعة الجائلين كانت الأسوأ حيث أظهرت نتائجها أن ٣٥% منها يحتوي على مواد مثبطة باستخدام Wynther Blyth test. وفوق ذلك ١٠، ١٥، ٢٠، ٥% من هذه العينات كان يحتوي على فورمالين، حمض البوريك وفوق أكسيد الهيدروجين على الترتيب. وبالكشف عن المواد المثبطة باستخدام Qualitative disc assay method أثبتت تواجدها بنسب ٣٠، ١٠، ١٥، ٥% في عينات ألبان الباعة الجائلين، بيوت الفلاحين، محلات بيع الألبان وعينات المزارع على التوالي. وبدراسة العلاقة بين نتيجة الاختبارات الكيميائية للمواد الحافظة في العينات الإيجابية لها و Wynther Blyth test و Qualitative disc assay method وجد أن ٩، ٩٠، ٢٥، ٦٦، ٦% من عينات الباعة الجائلين وبيوت الفلاحين ومحلات بيع الألبان بالترتيب كانت تحتوي على مواد حافظة كيميائية تم الكشف عنها باستخدام الاختبارين معاً. كما وجدت أفضلية في استخدام Wynther Blyth test عن Qualitative disc assay method في الكشف عن المواد الحافظة للكيميائية. خلصت الدراسة إلى أن تواجد هذه المثبطات في الألبان يشكل خطورة على صحة المستهلك لذا ينصح بتناول ألبان المزارع تليها ألبان بيوت الفلاحين مع ضرورة النوعية المستمرة للباعة الجائلين ومحلات بيع الألبان حول خطورة هذه المواد.

SUMMARY

The presence of inhibitory substances in milk arises in consumers public health hazard. Some certain chemical substances are used as preservatives to extend the shelf life of milk. In the present study, 160 milk samples were collected from street vendors, farmer's houses, shop keepers and dairy farms (40 samples of each). All investigated milk samples were subjected to inhibitory substance detection using Wynther Blyth test and the qualitative *B. subtilis* disc assay method. Chemically, the present samples were subjected to Hehner's test for detection of formalin, modified Piens test for hydrogen peroxide, glycerin test for boric acid and salicylic acid test. All examined milk samples were free from salicylic acid. Dairy farm milk samples proved to be free from chemical preservatives while, street vendors milk was the worst. 35% of their samples contained inhibitory substances using the Wynther Blyth test. Moreover, 10, 15 and 2.5% of these samples contained formalin, boric acid and hydrogen peroxide, respectively. Qualitative *B. subtilis* disc assay method concluded that 30, 10, 15 and 5% of street vendors, farmer's houses, shop keepers and farm milk samples contained inhibitory substances, respectively. Correlation between the results of specific tests for chemical preservatives and that obtained by the Wynther Blyth test and the disc assay method indicated that chemical preservatives could be detected by both tests in 90.9, 25, 66.6% of street vendors, farmer's houses, shop keepers milk samples, respectively. Moreover, the Wynther Blyth test proved to be more sensitive for detection of chemical preservatives than the qualitative *B. subtilis* disc assay method. It was concluded that these inhibitory substances are very objectionable and illegal substances, so they should be screened randomly and periodically. Moreover, intensification of the education among street vendors and shop keepers would greatly reduce the occurrence of these substances in milk.

Key words: Inhibitory substances, wynther blyth test, qualitative disc assay method

INTRODUCTION

In recent years, there is a widespread concern about the presence of inhibitory substances in milk. Inhibitors are considered the undesirable substances added to milk, it classified to 3 main categories which are: naturally occurring inhibitors (immunoglobulins and lactoferrin); drug residues (antibiotics and sulfonamides); preservatives

and residues of cleansing agents or disinfectants. Out of several studies on microbiological quality of milk, very little has been dealt with the presence of inhibitory substances (Deeb, 1996 and Karima Galal, 2002). The growth of the dairy industry in our country depending mainly upon fresh milk production, while powdered milk was still imported to satisfy demand. So, such an investigation is important as fresh milk quality has an impact on the dairy industry and human public health.

The health risks arising from such substances include hypersensitivity reactions (Sundloff, 1994 & Fein *et al.*, 1995), alteration of human intestinal microfloral ecology (Paige *et al.*, 1997) and antimicrobial resistance (Dowling 1997 and WHO, 1997). In addition, these substances inhibit the growth of lactic acid bacteria which may have a detrimental effect on cheese or yoghurt manufacture (Prato, 1997).

In the dairy industry, therapeutic drugs are administered to animal for treatment and prophylaxis of disease and secreted in milk. Treatment of mastitis is the usual source of antibiotics in milk. Sometimes, these drugs were used without supervision of a licensed veterinarians. Many authors studied the incidence of antibiotic residues in milk (Baynes, 1999; Abdel-Hakeim and El-Kosi, 2000; Van Bruijnsvoort *et al.*, 2004 and Shitandi and Sternesjo, 2004). The FAO/WHO convened to evaluate the safety of residues of certain veterinary drugs to recommend maximum levels for such residues in milk (WHO, 2004). The United States Milk Quality Program Post 1975 Stated antibiotics must be negative in high quality milk (Barbano, 1992). Elsewhere, monitoring antimicrobial agents include: The different types of antibiotics found in milk, their detection methods either microbiological or immunoreceptor tests and the importance of controlling antibiotic residues in milk (Samarzija and Antunac, 2002).

This preliminary study was conducted to throw out a light on the incidence of these inhibitory substances in milk sold in Assiut. Also, to compare between two methods used for detection of inhibitory substances in milk.

MATERIALS and METHODS

A – Collection of samples:

A total of 160 random samples of milk were collected in clean, dry and sterile containers from different sources in Assiut Governorate, including street vendors, farmer's houses, shop keepers and farm milk (40 samples of each).

B – Detection of inhibitory substances:

- 1- Wynther Blyth test (Wynther Blyth and Meredith, 1927).
- 2- Qualitative B. subtilis disc assay method (APHA, 1992).

Each milk sample was heated at 80°C for 5 minutes to inactivate the naturally occurring inhibitory substances in milk and to eliminate the possibility of false-positive results. After cooling, one tenth ml from each milk sample was applied in a circular well in Bacto-PM indicator agar inoculated with B. subtilis organism (Ginn *et al.*, 1978).

The plates were examined for violet-colored inhibition zones after 2.5-3 hours incubation at 65°C±1°C. Presence of zone of inhibition was recorded as a positive result.

- 3- Specific tests for detection of preservatives (Ling, 1963).
 - * Formalin test (Hehner's test).
 - * Salicylic acid test.
 - * Hydrogen peroxide test (Modified Pien's method).
 - * Boric acid and borax test (glycerin test).

RESULTS

Table 1: Detection of inhibitory substance in the examined milk samples.

Test	Street vendor milk (No. 40)		Farmer's houses milk (No. 40)		Shop keepers milk (No. 40)		Farm milk (No. 40)		Total (No. 160)	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
A- Wynther Blyth test	14	35	7	17.5	8	20	2	5	31	19.4
B- Qualitative B. subtilis disc assay method	12	30	4	10	6	15	2	5	22	13.8
C- Specific tests										
Formalin test	4	10	-	-	-	-	-	-	4	2.5
Salicylic acid test	-	-	-	-	-	-	-	-	-	-
Boric acid test	6	15	2	5	5	12.5	-	-	3	8.1
Hydrogen peroxide test	1	2.5	2	5	3	7.5	-	-	6	3.8

Table 2: Correlation between results of specific tests for chemical preservatives and Wynther Blyth & qualitative disc assay methods.

Types of examined milk	+ve samples for * Wynther blyth test * disc assay method *chemical preservatives		+ve samples for * Wynther blyth test *chemical preservatives		+ve samples for * disc assay method *chemical preservatives		+ve samples for * Wynther blyth test * disc assay method and -ve for chemical preservatives	
	N0.	%	N0.	%	N0.	%	N0.	%
Street vendors milk (11)	10	90.9	1	9.1	-	-	3	27.3
Farmer's houses milk (4)	1	25	3	75	-	-	3	75
Shop keepers milk (6)	4	66.6	2	33.3	-	-	2	33.3

DISCUSSION

In milk industry, a preservative means a substance which when be added to milk, will retard sourness or decomposition. The object of adding these preservatives being: (1) to prolong the period of sweetness of milk, (2) to inhibit and to destroy bacteria, and (3) to neutralize acids formed by bacteria and to delay curdling.

Using the Wynther Blyth test, the present study indicated that street vendors milk showed high incidence (35%), while farmer's houses and shop keepers milk samples have nearly similar incidence (17.5 and 20%), respectively. Only 2 samples (5%) from farm milk showed a positive result (Table 1). The qualitative disc assay method demonstrated the presence of inhibitory substances in 30, 10, 15 and 5% of street vendors, farmer's houses, shop keeper and farm milk samples, respectively (Table 1). In Quena Governorate, Karima Galal (2002) detected nearly similar results but no inhibitory substances were detected in farm milk samples. All market milk samples at Kafr El-Sheikh Governorate were negative for Wynther Blyth test (Deeb, 1996).

Hot weather and lack of cooling equipment intended some unscrupulous dairy producers to add these chemical preservatives to milk in order to face the problem of milk spoilage during storage and transportation. The problem is further compounded by the fact that many of these substances are readily available.

Through the present investigation, the four chemical preservatives could not be detected in farm milk, while in the contrary, street vendors samples are the worst, as they contained formalin, boric acid and hydrogen peroxide resembling 10, 15 & 2.5%, respectively, (Table 1). Many studies recorded nearly similar results, (Erdelyi and Bekei, 1997; Kamel, 2000 and Karima Galal, 2002). Formalin as a chemical inhibitory substance was detected in street vendor milk samples all over the present investigation. Formalin is a famous preservative for milk because it has the property of being in a liquid form, only street vendors milk contained formalin (10%). Boric acid and borax were detected in 15, 5, 12.5% from street vendors, farmer's houses and shop keepers milk samples, respectively, (Table 1). Owing to the fact that boric acid and borax have oxidizing power, 1 part of these substances in 1 to 2 thousand parts of milk is sufficient to preserve milk and to delay curdling for several days (Aggarwala and Sharma, 1967).

Salicylic acid is rarely employed to preserve milk because it is sparingly soluble in it. From the present findings all examined milk samples were free from salicylic acid. Only 6 samples (3.8%) all over the study contained hydrogen peroxide (Table 1). This substance is not much used as a preservative as it soon splits up into water and free oxygen, moreover, if larger amount are used, milk obtains a bitter taste (Aggarwala and Sharma, 1967).

Correlation between results of specific tests for chemical preservatives and that obtained by the Wynther Blyth & qualitative disc assay methods indicated that chemical preservatives could be detected by both testes in 90.9, 25, 66.6% of street vendors, farmer's houses, shop keepers milk samples, respectively, (Table 2). The Wynther Blyth test proved to be more sensitive for detection of chemical preservatives as 1, 3, 2 samples of street vendors, farmer's houses, shop keepers milk contained chemical preservatives detected by this test only. On the other hand, no samples were positive for chemical preservatives and the qualitative disc assay method only (Table 2). These results can be explained as the Wynther Blyth test may detect a minimum concentration of chemical preservatives that can not be appeared in the zone of the qualitative disc assay method.

Chemical preservatives could not be detected in farm milk, at the same time they were positive to the Wynther Blyth test and the qualitative disc assay method (Table 2). These samples may contain inhibitory substances other than those searched for in this study.

2 out of 4 samples of farmer's house milk proved to be free from chemical preservatives and drug residues while, they are positive for Wynther Blyth test (Table 2). These samples may contain residues of cleaning or disinfectants so, adequate rinsing of milk utensils and equipments was essential.

This preliminary study concluded that the high incidence of inhibitory substances was found in street vendors and shop keeper's milk samples. Those persons are mainly uneducated, don't having professional training and need more information about preservation of milk during transport and storage and the most serious adverse effect of these inhibitory substances in humans.

Finally, the practice of adding preservatives to milk is very objectionable, illegal and should be forbidden. So, immediate and rigourous measures ought to be put into effect by authorities to right this alarming situation.

REFERENCES

- A.P.H.A. (1992):* Standard Methods for the Examination of Dairy Products. American Public Health Association 16th ed. Washington, D.C.
- Abdel-Hakeim, E.H. and El-Kosi, O.H.R. (2000):* Occurrence of antibiotics and sulfa drug residues in raw milk. 9th Sci. Conj. Fac. Vet. Med. Assiut Univ.
- Aggarwala, A.C. and Sharma, R.M. (1967):* Laboratory Manual of Milk Inspection 4th ed. Asia Pub. New York.
- Barbano, D. (1992):* Raw milk quality improvement in the United States. Aust. J. Dairy Tech. 47: 89.
- Baynes, R.E.; Lyman, R.; Anderson, K.L. and Brownie, C.F. (1999):* A Preliminary Survey of antibiotic residues and viable bacteria in milk from three Caribbean Basin countries. J. Food Prot. 62; 177-180.
- Deeb, A.M.M. (1996):* Microbiological and chemical monitoring of raw milk and some dairy products as indices for sanitary quality. MVSc Thesis, Fac. Vet. Med. Kafr El-Shiekh, Tanta Univ.
- Dowling, P. (1997):* Antimicrobial resistance. Bov. Vet. November, December: 14-17.
- Erdelyi, J. and Bekei, A. (1997):* Inhibitor content of raw milk in Hungary with particular reference to new quality requirement. Tejgazdzsag (1997), No. Special, 122-133.
- Fein, S.B.; Jordan, L.C.T. and Levy, A.S. (1995):* Food borne illness: perception experience, and preventive behavior in the United States. J. Food Protect 58: 1405-1411.
- Ginn, R.E.; Tatini, S. and Packard, V.S.Jr. (1978):* Comparative evaluation of the Difco Disc assay method for detecting Penicillin in milk and milk products. J. Food Prot. 41: 361-363.
- Kamel, A.M. (2000):* Studies on raw milk quality in Kafr El-Shiekh. MVSc. Thesis, Fac. Vet. Med. Tanta Univ.
- Karima Galal, A. (2002):* Studies on the sanitary condition of raw milk in Qena Governorate. MVSc thesis, Fac. Vet. Med. Assiut Univ.
- Ling, T.R. (1963):* Textbook of Dairy Chemistry. Vol. II 3rd Ed., P.I. 30, Chapman and Hall, London.
- Paige, J.C.; Tollefson, L. and Miller, M. (1997):* Public health impact on drug residues in animal tissues. Vet. Hum. Toxicol. 39: 162-168.

- Prato, O.S. (1997):* Inhibitors in milk and their effect on milk processing. *Latte* 22: 40-48. in *D.S. Abst.* 60: 246, 1998.
- Samarzija, D. and Antunac, N. (2002):* The importance of antibiotic residues presence detection in milk. *Mljekarstvo* 52: 61-70 In *Food Sci. Tech. Abst.* 34: 1263.
- Shitandi, A. and Sternesjo, A. (2004):* Factors contributing to the occurrence of antimicrobial drug residues in Kenyan milk. *J. Food Prot.* 67: 399-402.
- Sundloff, S.F. (1994):* Human risk associated with drug residues in animal derived foods. *J. Agromed.* 1: 5-22.
- Van Bruijnsvoort, M.; Ottink, S.J.; Jonker, K.M. and de Boer, E. (2004):* Determination of streptomycin and dihydrostreptomycin in milk and honey by liquid chromatography with tandem mass spectrometry. *J. Chromatogr. A.* 1058 (1-2): 137-42.
- WHO (1997):* The medical impact of the use of the antimicrobial in food animals. Report of a WHO Meeting, Berlin, Germany, 13-17 October, 1997.
- WHO (2004):* Evaluation of certain veterinary drug residues in food. *World Health Organ Tech. Rep. Ser.* 925: 1-72.
- Wynter Blyth, A. and Meredith, W.B. (1927):* *Foods: Their composition and analysed.* 7th Ed., Charles, Griffin and Company, Ltd., London.