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INCIDENCE OF AEROBIC AND ANAEROBIC SPOREFORMERS AND THERMOPHILIC FUNGI IN CONDENSED MILK IN ASSIUT CITY

(With 5 Tables)

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مدى تواجد الميكروبات المتحوصلة الهوائية واللاهوائية والفطريات والخمائر المقاومة لدرجة الحرارة العالية في اللبن المكثف المحلى بمدينة أسيوط

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تم جمع خمسين علبة من اللبن المكثف المحلى كامل الدسم من مختلف السوبرماركت الموجودة بمدينة أسيوط لفحصهم ميكروبيولوجيا . وقد أظهرت النتائج أن البكتريا المقاومة للحرارة موجودة بنسبة ٦٢% في العينات المفحوصة وبمتوسط قدره ٧,٦ × ١٠^{٣} لكل جرام. كما تم تصنيف البكتريا المتحوصلة الهوائية وعزل الباسيلس سيريس، الباسيلس كواجيولانز، الباسيلس أستيروسيرموفليس، الباسيلس سابتلس، والباسيلس ميجاتيريوم من العينات المفحوصة بالنسب المئوية التالية : ٢٨ ، ١٨ ، ١٢ ، ٢٢ ، ٦% على الترتيب . وقد دلت النتائج على أن ميكروب الكلوستريديم بيرفرنجنز كان موجوداً بنسبة ٦% في العينات المفحوصة وذلك باستخدام طريقة MPN بمتوسط مقداره ٢٩ ميكروب / جم، أما باستخدام طريقة الفرد السطحي على مستنبت SPS فقد كانت نسبة تواجد هذا الميكروب ٤% بمتوسط قدره ١٥٠/ جم . كما أسفرت النتائج عن تلوث العينات المفحوصة بالفطريات والخمائر المقاومة لدرجة الحرارة العالية بنسبة ٦% وبمتوسط مقداره ١,٦ × ١٠^{٣} لكل جرام. ولقد نوقشت الأهمية الاقتصادية والخطورة الصحية لتلك الميكروبات في اللبن المكثف المحلى.

SUMMARY

Fifty random samples of full cream sweetened condensed milk cans were collected from different supermarkets in Assiut City for microbiological evaluation. Thermoduric bacteria could be detected in 62% of the condensed milk samples, with an average count of 7.6 x 10^3/g. B. cereus, B. coagulans, B. stearothermophilus, B. subtilis and B. megaterium could be isolated from the examined samples with incidence percentages of 28, 18, 12, 22 and 6%, respectively. Cl. perfringens could be detected

of 28, 18, 12, 22 and 6%, respectively. Cl. perfringens could be detected in 6% of the condensed milk samples using MPN technique, with an average value of 2.9 x 10^1/g, while on SPS agar this organism was present in 4% of the examined samples, with an average of 1.5 x 10^2/g. On the other hand, 3 (6%) of condensed milk samples were positive for thermophilic fungi, with an average number of 1.6 x 10^3/g. The economic significance and the public health importance of these organisms were discussed.

Key words: *Condensed milk, Aerobic & Anaerobic Sporeformers, Fungi.*

INTRODUCTION

Condensed milk is a concentrated milk product resulting from the evaporation of a considerable portion of the water content of milk. Sweetened condensed milk is preserved by the addition of sugar to prevent or retard spoilage. Major advantages lie in a considerable reduction in transport and storage costs as a result of the reduction in bulk and convenience of use during formulation, there is also a significant extension of the ambient temperature storage life. Full cream sweetened condensed milk contains not less than 28% of milk solids and not less than 8.5% of milk fat. It is nourishing, delicious, easily digestible and a good source of energy. Condensed milk may be used undiluted in coffee, tea and other beverages, as a topping for fruits or ice cream, or just straight from the tin. It is also used for the preparation of fortified low-fat milks, confectionery, bakery products, infant formulas and other food items.

On the other hand, the initial microflora are those of the raw milks from which the condensed milks are made. Sugar used in such product may contain sporeforming bacteria, and yeasts and moulds (Lampert, 1987 and Milner, 1995). In general, sweetened condensed milk is not a sterile product, and the various methods of heat treatment used are not adequate to kill sporeforming bacteria, and further processing and handling usually contribute a variety of microorganisms, besides the sugar levels employed permit some types to grow if other conditions are favourable. Enough oxygen may be present in the head space of an incompletely filled, or poorly sealed container, to permit the growth of organisms able to tolerate the high osmotic pressure of the product.

These organisms may be associated with defects in condensed milk such as thickening, acid production, proteolysis and lipolysis. As well as, moulds, which form hard, coloured bodies or buttons, are sometimes found on the surface of the product, and such milk often is bitter or rancid. Occasionally, gas forms, usually caused by sugar-fermenting yeasts (ICMSF, 1980; Lampert, 1987; Tudor and Board, 1993 and Varnam and Sutherland, 1994). Furthermore, Bacillus cereus and Clostridium perfringens are widely recognized as a principal causes of human food poisoning of bacterial origin (Iversen et al., 1982; Anon, 1990 and Granum et al., 1993). In addition, some moulds are capable of producing toxic metabolites known as mycotoxins, such as aflatoxins which are known as carcinogens (Massey et al., 1995; Markaki and Melissari, 1997 and Li et al., 2000).

Therefore, the purpose of this investigation is to study the incidence and level of contamination of aerobic and anaerobic sporeformers and thermophilic fungi in condensed milk sold in Assiut City.

MATERIAL and METHODS

Fifty random samples of full cream sweetened condensed milk cans were collected from different supermarkets in Assiut City for microbiological evaluation. Preparation and handling of collected samples were done according to standard methods (A.P.H.A., 1992). The prepared samples were examined for the following:

1 - ***Thermoduric bacterial count:*** The technique recommended by A.P.H.A. (1992) using standard plate count agar was made. Separate colonies were identified according to Kramer et al. (1982) and Parry et al. (1983).

2 - ***Enumeration of Cl. perfringens:*** Was performed by using two techniques, the first, MPN technique using lactose sulphite (I.S) broth as described by Beerens et al. (1982). The second, direct plating technique using sulphite polymyxin sulfadiazine (SPS) agar as recommended by Angelotti et al. (1962). Moreover, suspected colonies were picked up for further confirmation according to Mead et al. (1981)

3 - ***Thermophilic fungi count:*** Malt extract agar with antibiotic {chlorotetracyclin Hcl and chloramphinicol} (Speck, 1976) was employed. The inoculated plates were incubated for 7-10 days at 55°C, and thermophilic yeasts and moulds were counted.

RESULTS

The results were recorded in Tables 1 - 5.

Table 1: Statistical analytical results of thermoduric bacterial count/g of the examined condensed milk samples.

No. of examined Samples	Positive samples		Count/g		
	No.	%	Min.	Max.	Average
50	31	62	$1.0x10^2$	$6.0x10^4$	$7.6x10^3$

Table 2: Occurrence of aerobic sporeforming bacteria isolated from the examined condensed milk samples.

Bacillus species	Positive samples	
	No./50	%
B. cereus	14	28
B. coagulans	9	18
B. stearothermophilus	6	12
B. subtilis	11	22
B. megaterium	3	6

Table 3: Statistical analytical results of Cl. Perfringens count/g of the examined condensed milk samples using MPN technique.

No. of examined samples	Positive samples		Count/g		
	No.	%	Min.	Max.	Average
50	3	6	3.0	$7.5x10^1$	$2.9x10^1$

Table 4: Statistical analytical results of Cl. perfringens count/g of the examined condensed milk samples using direct plating technique.

No. of examined samples	Positive samples		Count/g		
	No.	%	Min.	Max.	Average
50	2	4	$1.0x10^2$	$2.0x10^2$	$1.5x10^2$

Table 5: Statistical analytical results of thermophilic fungi count/g of the examined condensed milk samples.

No. of examined samples	Positive samples		Count/g		
	No.	%	Min.	Max.	Average
50	3	6	$1.0x10^2$	$4.0x10^3$	$1.6x10^3$

DISCUSSION

Results reported in Table 1 revealed that 62% of the examined condensed milk samples contained thermoduric bacteria, with an average count of 7.6 x 10^3/g. Likewise, B. cereus, B. coagulans, B. stearothermophilus, B. subtilis and B. megaterium could be isolated from the examined condensed milk samples with incidence percentages of 28, 18, 12, 22 and 6%, respectively (Table 2). On the other hand, Ahmed *et al.* (1988) could not detect B. cereus from the examined samples of condensed milk. Sweetened condensed milk is not a sterile product and the sugar acts as a preservative. Heat-resistant organisms, such as aerobic sporeforming bacteria may at times survive the heat treatment (Crossley and Craham, 1966 and Lampert, 1987). The main source of contamination of condensed milk by these microorganisms may be from imperfectly cleaned machinery and incompletely sterilized tins (Garcia, 1959). A general risk due to post-processing contamination, of course, exist with non-sterile products, while sterilized concentrated milk is subjected to the same risks as all canned foods with respect to underprocessing and seam leakage (Varnam and Sutherland, 1994). It is worthwhile to state that B. cereus was capable to cause off flavours of milk products such as unclean, fruity, bitter, putrid, rancid and yeasty (Stadhouders, 1993). Also, other aerobic sporeforming bacteria such as B. coagulans and B. stearothermophilus can cause an acid coagulation with a slight cheesy odour and flavour. B. subtilis causes a non-acid curd, which can result in a brownish liquid with a bitter taste. The coagulum formed by B. megaterium is accompanied by some gas and a cheesy odour (ICMSF, 1980; Nelson, 1990 and Milner, 1995). Furthermore, B. cereus is recognized as one of the potential organisms for human food poisoning (Iversen et al., 1982; Christiansson, 1992 and Granum et al., 1993).

The results outlined in Table 3 indicated that the incidence of Cl. perfringens in the condensed milk samples was 6% by applying lactose sulphite (LS) broth, with an average of 2.9 x 10^1/g. The LS broth gives a good selectivity which allows the detection of low numbers of Cl. perfringens in the presence of other sulphite-reducing bacteria. Confirmatory tests not required and the senstivity of the medium is the most important advantage of this procedure. Also, by plating on SPS agar, the occurrence percentage of Cl. perfringens was low (4%) in the examined condensed milk samples, with an average value of 1.5 x 10^2/g (Table 4). The thermal processing of condensed milk had an effect on lowering the incidence % and the count of Cl. perfringens. The obtained results agree to a certain extent with those reported by Ahmed et al. (1988). The incidence of Cl. perfringens in sweetened condensed milk has also been studied by Bhale et al. (1989) and Milner (1995). This work indicates the potential microbiological problems associated with this type of canned milk. Likewise, Fujisawa et al. (2000) suggested that the use of enrichment methods for detection of clostridia is essential. Moreover, Cl. perfringens is responsible for a considerable number of food poisoning outbreaks annually. From 1985 to 1989, Cl. perfringens food poisoning constituted 21.2, 18, 12.2, 9.1, 8, 7, 7 and 5.4% of the total food poisoning outbreaks in Finland, Switzerland, Denemark, Netherland, United Kingdom, France, Iceland and Germany, respectively (Anon, 1990).

The achieved results recorded in Table 5 show that thermophilic fungi could be detected in 6% of the examined condensed milk samples with an average number of 1.6 x 10^3/g. A higher incidence and lower counts of fungi were observed in condensed milk examined by Ahmed et al. (1988). Yeasts and moulds could be isolated from such product by Rao and Ranganathan (1970) and Sallam (1979). Generally, condensed milks are favourable media for the growth of a wide range of environmental contaminants. Canned sweetened condensed milk with its high sugar content may swell occasionally owing to growth of yeasts (Tudor and Board, 1993). If the can is underfilled, however, the large head space may allow enough oxygen for mould growth to occur on the product surface. Moulds may also have entered the product between the pasteurizer and the can-closing machine through a defective seam or pinhole, or contamination can arise from filling equipment and non-sterile cans. Mould growth often occurs as buttons i.e. small areas of mycelial gorwth on the surface of the stored product. The moulds most

likely to be present are Aspergillus and Penicillium (ICMSF, 1980; Varnam and Sutherland, 1994 and Milner, 1995). Concerning, the public health significance and the pathogenesis of mycotoxins, it is well known that in many cases potential problems involve the possibility of cancer or delayed organ damage due to repeated ingestion of subacute levels. Certain foodborne yeasts and moulds may be hazardous because of their ability to elicit allergic reactions. Aflatoxin M_1 is highly toxic, mutagenic, teratogenic and carcinogenic compound that have been implicated as causative agent in human hepatic and extrahepatic carcinogens (Mislivec et al., 1992; Massey et al., 1995; Markaki and Melissari, 1997 and Li et al., 2000).

In conclusion, using high quality raw milk and sugar in condensed milk manufacture, good sanitation and hygiene during production, handling and storage of such product are important to prevent the condensed milk from spoilage and to protect the consumers from infection. Also, a high standard of plant hygiene is needed to avoid post-processing contamination. However, the heat treatments used in production of condensed milk are insufficient to sterilize the product, so strict hygienic measures are still required. In addition, the viscous nature of this product needs to be taken into account for the cleaning procedures. Finally, employment of experienced staff is necessary at all times.

REFERENCES

A.P.H.A. (American Public Health Association) (1992): Compendium of methods for the microbiological examination of foods. 3rd Ed. American Public Health Association, Washington, D.C., USA.

Ahmed, A.A-H.; El-Bassiony, T.A. and Moustafa, M.K. (1988): Microbiological evaluation of condensed milk. Assiut Vet. Med. J. 20: 98-102.

Angelotti, R.; Hall, H.E.; Foster, M.J. and Lewis, K.H. (1962): Quantitation of Clostridium perfringens in foods. Appl. Microbiol. 10: 193-199.

Anon (1990): FAO / WHO, News letter, Robert Von Ostertag Institute, Berlin.

Beerens, H.; Romond, C.; Lepage, C. and Griquelion, J. (1982): A liquid medium for the enumeration of Clostridium perfringens in food and faeces. In: Isolation and identification methods for food poisoning organisms, edited by J.E. Corry; D. Roberts and F.A. Skinner, Academic Press, London, PP. 137-149.

Bhale, P.; Sharma, S. and Sinha, R.N. (1989): Clostridia in sweetened condensed milk and their associated deteriorative changes. J. Food Sci. Technol. 26: 46-48.

Christiansson, A. (1992): The toxicology of Bacillus cereus in milk and milk products. Bulletin IDF. 275: 30-35.

Crossley, E.L. and Craham, P.A. (1966): Microbiological changes during the storage of sweetened condensed milk. XVII International Dairy Congress, Sec. E/3, 167, Munich.

Fujisawa, T.; Aikawa, K.; Furukawa, I. and Takahashi, T. (2000): Occurrence of clostridia in glass bottled foods. Int. J. Food Microbiol. 54: 213-217.

Garcia, F.R. (1959): Microbiological control of the manufacture of condensed milk. Dairy Sci. Abs. 24, 256 (1962).

Granum, P.E.; Brynestad, S.; Sulivan, K.O. and Nissen, H. (1993): Enterotoxin from Bacillus cereus production and characterization. Nether. Milk and Dairy J. 47: 63-70.

I.C.M.S.F. (International Commission on Microbiological Specifications for Food) (1980): Microbial ecology of foods. Vol. 2: Food commodities. London: Academic Press.

Iversen, H.; Rosef, O. and Selas, P. (1982): Outbreak of food poisoning caused by Bacillus cereus at a geriatric and health care centre. Norsk-Veterinaertidskrift. 94: 337-340.

Kramer, J.M.; Turnbull, P.C.; Munshi, G. and Gilbert, R.J. (1982): Identification and characterization of Bacillus cereus and other Bacillus species associated with food poisoning. In: Isolation and Identification Methods for Food Poisoning Organisms, Corry, J.E.; Roberts, D. and Skinner, F.A. (Eds). Academic Press. London and New York.

Lampert, L.M. (1987): Modern Dairy Products. Eurasia Publishing House (Pvt) LTD.

Li, S.; Marquardt, R.R. and Abramson, D. (2000): Immunochemical detection of molds: A review. J. Food Prot. 63: 281-291.

Markaki, P. and Melissari, E. (1997): Occurrence of aflatoxin M_1 in commercial pasteurized milk determined with ELISA and HPLC. Food Addit. Contam. 5: 451-456.

Massey, T.E.; Stewart, R.K.; Daniel, J.M. and Ling, L. (1995): Biochemimammalian susceptability to aflatoxin B_1 carcinogenicity. Proceed. Soc. Exp. Biol. Med. 208: 213-227.

Mead, G.C.; Paezdeleon, L. and Adams, B.W. (1981): A study of rapid and simplified confirmatory tests for Clostridium perfringens. J. Appl. Bacteriol. 51: 355-361.

Milner, J. (1995): Microbiology Handbook. Leatherhead Food RA. Randalls Road, Leatherhead, Surrey KT 22 7RY.

Mislivec, P.B.; Beuchat, L.R. and Cousin, M.A. (1992): Yeasts and Molds. Chapter 16. Compendium of Methods for Microbiological Examination of Foods. 3rd Ed. American Public Health Association, Washington, DC.

Nelson, F.E. (1990): The microbiology of concentrated milks. Dairy microbiology. Vol. 1: The microbiology of milk products. Ed. R.K. Robinson. London: Elsevier Applied Science Publishers.

Parry, J.M.; Turnbull, P.C. and Gibson, J.R. (1983): A Colour Atlas of Bacillus Species. Wolfe Medical Publications Ltd. London.

Rao, V.J. and Ranganathan (1970): Studies on the microbiology of sweetened condensed milk. Indian J. Dairy Sci. 23: 205.

Sallam, S.S. (1979): Microbiological investigation of powdered and condensed milk. M.V.Sc. Thesis. Fac. Vet. Med., Cairo Univ., Egypt.

Speck, M.L. (ed) (1976): Compendium of methods for microbiological examination of food. American Public Health Association, Washington, DC.

Stadhouders, J. (1993): Bacillus cereus in milk and milk products. Bulletin of the International Dairy Federation. No. 287: 3-5.

Tudor, E.A. and Board, R.G. (1993): Food-spoilage yeasts. The yeasts. Vol. 5: Yeast technology. Eds A.H. Rose & J.S. Harrison. 2nd Ed. London: Academic Press: 435-516.

Varnam, A.H. and Sutherland, J.P. (1994): Milk and Milk Products: Technology, Chemistry and Microbiology. Chapman and Hall, London, UK.