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STABILITY OF AFLATOXINS M_1 AND M_2 DURING MANUFACTURE AND STORAGE OF ICE CREAM

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ABSTRACT

Ice cream was made from artificially contaminated milk with aflatoxin M_1 and M_2 to investigate the effect of manufacturing steps and storage period on AFM_1 and AFM_2 content and its stability. Results obtained during the course of this investigation, show the percent of AFM_1 degradation was 4% and AFM_2 was 5% after cream adding, and more reduction of 9.7% for AFM_1 and 12.3% for AFM_2 after adding mixture before ice cream manufacture from artificial contaminated milk with aflatoxin. Results show that the highest losses of AFM_1 and AFM_2 were about 30.7% and 24% respectively after adding gelatin during ice cream manufacture which might be due to the strong association of AFM with gelatin as casein during cheese making. During processing of ice cream, the pasteurization played an important role. Results cleared that the highest losses of 38.7% of AFM_1 and 45% of AFM_2 were after pasteurization and homogenization before ice cream manufacture. Results cleared also no loss of AFM_1 and AFM_2 during aging of mixture and hardening of ice cream. Regarding the effect of storage period on AFM content of ice cream at the present work, the distraction and the ratio of distraction were observed during 10 weeks of storage in freezer. Data showed that the percent of reduction of AFM_1 was 9.5% at the first week of storage, but there is no effect of AFM_2 during the first week of storage of ice cream. More degradation of AFM_1 of about 9.5% from the first week to 28.5% at the end of 4^{th} week. Furthermore, AFM_1 was stable from 4^{th} week to 6^{th} week and then, became constant 33.3% of degradation until the end of storage period. On the other hand, slow reduction of AFM_2 of about 8% from the second week to 25% at the 7^{th} week, after then AFM_2 was stable to 40% of reduction until the end of storage period. It is evident that AFM_1 and AFM_2 were reduced about to 10% and 15% from total toxins, respectively during frozen storage of ice cream. The percent of AFM_1 reduction about 66.6% and 65% of AFM_2 at the end of ice cream manufacture from the initial contamination, therefore ice cream will be safe for human health because 76.6%-80% of milk toxin were detoxified during processing and storage period from 6-7 weeks.

Keyword: Aflatoxin; Milk;ice cream,TLC Method

INTRODUCTION

Ice cream is a frozen product which may contain a variety of ingredients in addition to milk cream and sugar and it's a good source of energy and many nutrients such as fat, proteins ,vitamins and mineral salts as calcium and phosphor for consumption, (Clarence., et al 1982). However, ice cream could also be a source of toxic substances such as aflatoxin (AFM). Aflatoxin is a group of several toxic secondary fungal metabolites produced by some species of *Aspergills as A. flavus, A. parasiticus, and A. anomies* (van Egmond,1989, Blanco et.al, 1993 and Lopez et .al ,2003). Aflatoxins B_1 and M_1 are known as hepatotoxins and hepatocarcinogens and the deleterious effects in humans, especially children, of consuming AFM_1-

contaminated milk are of considerable concern (Qian, *el al.*, 1984, Chu, 1991 and motawee, *et al.*, 2004). Aflatoxin M_1 is the 4-hydroxy derivative of aflatoxin B_1 and Aflatoxin M_1 is the 4-hydroxy derivative of aflatoxin B_1 (Van Egmond. H.P & Dragacci .S ,1989) . Aflatoxin M1 appears in milk and milk products as the result of the intake of aflatoxin B1-contaminated feed by dairy cows(De longh,H.et.al 1964, Van Egmond & Dragacci, 2001, Veldman,et.al,1992 and Nassib,T.A et.al,2005a). Occurrence of aflatoxin in milk and milk products can be due to three possible causes, AFM_1 present in raw milk as a consequence of carryover of AFB_1 from contaminated cow feeds to milk, synthesis of AF(B_1,B_2,and G_2) by *Aspergillus Flavus and Aspergillus parasiticus* growing on cheese(Applebaum,et.al 1982 and Zerfiridis, 1985) and occurrence of these toxin in milk and dried milk to enrich the milk used to make cheese and other dairy products. Consumption of aflatoxin-contaminated feed by lactating cows results in excretion of an aflatoxin B_1 metabolite, aflatoxin M_1, in the milk. From controlled feeding studies, an estimate is that aflatoxin B_1 at 30 ng/g of feed will result in measurable (1ng/ml) aflatoxin M_1 in milk (Stoloff. L and Wood .G,1981,). Several countries control contamination of milk and dairy products by AFM_1, and they have established regulations for AFM_1 in these products. The current permissible levels for AFM_1 in milk range from 0.05 to 0.5 µg/kg, except for infant milk for which lower levels exist (FAO, 1997 and Maria & Herminia 2000). Whereas the food and Drug Administration has established an action level of 0.5 g/kg AFM_1 in whole, low fat, and skim milk (US Food and Drug Administration, 1996).

MATERIALS AND METHODS.

Aflatoxin M_1 and M_2 Standards were obtained from SIGMA (Deisenhofen-Germany) Fresh milk was obtained from a farm of 6% fat and refrigerated at 5°C until it was processed Milk was spiked with 3 µg/kg during manufacture of ice cream. Cream was prepared by fore warming the whole milk to 40°C and then, separating it with a hand operated separator (Subitas, Istambul,Turkey). Both fresh milk and cream were pasteurised at 64 °C for 30 min.The manufacture of ice cream was carried out according to El-saddik., et al 1968.

Extraction of aflatoxin M_1 and M_2

1-For Fluid Milk

According to AOAC Method (1995). 50 ml of fluid milk were shacked (at room temp.), by 10 ml of salt solution , and 120 ml of chloroform ($CHCl_3$) in 250 ml separator funnel 60 s. The layer was separated after about 2 min. and the lower of ($CHCl_3$) layer was drained into 150 ml Erlenmeyer. After that centrifugation of mixture was carried out to break emulsion. Then ca 10 g Na_2SO_4 to $CHCl_3$, were added and stirred occasionally 3 min., and filtrated through paper whatman No:2 into 100ml graduate (volume was recorded). The final filtrate was saved for column chromatography.

2-For Yoghurt and Ice Cream

50 gm ice cream was shaking vigorously with NaCl solution 10 ml (saturated) 35 °C and warmed 120 ml $CH3Cl_3$ at 38 °C and mixed with

sample and salt solution in separator funnel, and the mixture was centrifuged at 4000 rpm for 10 min. The chloroform layer was separated.Chloroform layer was filtered throw filter paper (Watman No. 1) into a graduated cylinder. The filtrate volume was recorded. (Stublefield 1979, with some modification by Nassib,et.al 2005b)

Column Clean up chromatography.

Silica gel 60 containing 1% water was washed with methanol and $CHCl_3$ before activation. Silica gel (2g)was slurried in chloroform in the column (1.0 cm x 30cm) or 2g activated silica gel flitted a column on $CHCl_3$. Anhydrous sodium sulfate granular (1.5- 2 g) was added to the $CHCl_3$ above the settled silica gel. Ice cream filtrate was drained through the column into a 150 ml beaker, and finally the inside of the column was rinsed with chloroform and drained through the column. the column was then washed with acetic acid/toluene(1: 9,v/v) (25ml) and with acetonitrile: ether: hexane(2:3:5,v/v/v), and the washes were discarded. AFM was eluted with 30 ml of acetone: chloroform (1:4,v/v) into a 125ml Soxhlet flask and the eluent was evaporated. The dry residue was transferred quantitatively with chloroform and the extract evaporated to dryness for TLC chromatography, (AOAC 1995).Chromatograms of ice cream were developed first in ether :methanol : water (95+4+1 v/v/v). Developed plates were air dried turned through 90 °C and redeveloped in chloroform: acetone: isopropanol (87+10+3,v/v/v). Milk and cream used to prepare frozen dessert mixes and the mixes at various stages of manufacture and storage were analysed for AFM . frozen products were thawed and mixed before extraction.

RESULT AND DISCUSSION

The obtained results dealing with ice cream revealed that manufacture steps and storage period for ten weeks of ice cream played an important role in degradation of milk toxins; (Aflatoxin) $AFM_1$and AFM_2 content.

Table(1):Effect of manufacture stages on AFM1 and AFM_2 content of ice cream

Stages of Manufacture	AFM_1 (µg/kg)	AFM_2 (µg/kg)	% Of Degradation (µg/kg)	
			AFM_1	AFM_2
Fresh milk	3.0	3.0	0.0	0.0
Cream	2.88	2.85	4.0	5.0
Mixture	2.60	2.50	9.7	12.3
Adding gelatine	1.80	1.90	30.7	24.0
After Pasteurisation of Mix.	1.22	1.20	32.2	36.8
Homogenisation of Mix	1.15	1.10	5.7	8.3
After aging of Mix.	1.15	1.10	0.00	0.00
After freezing of ice cream	1.05	1.00	8.7	9.1
After hardening of ice cream	1.05	1.00	0.00	0.00

Results in table (1) viewed that AFM_1 was 3.0 ppb and AFM_2 was 3.0 ppb in fresh milk at the beginning of ice cream manufacture as control. The percent of AFM_1 degradation was 4% and AFM_2 was 5% after cream adding,

and more reduction of 9.7% for AFM_1 and 12.3% for AFM_2 after adding mixture before ice cream manufacture from artificially contaminated milk with aflatoxin. similar results are in agreement with Wiseman and Marth (1983 a), who reported that an apparent increase of AFM_1 reduction after sherbet mixture was prepared from naturally contaminated milk with AFM during ice cream making. Results indicated that the highest losses of AFM_1 and AFM_1 about 30.7% and 24%, respectively after, adding gelatin during ice cream manufacture which might be due to the strong association of AFM with gelatin as casein during cheese making (Blanc, et.al 1983, Brackett & Marth,1982 and Youssef & Marth 1989). During processing of ice cream, the pasteurization played an important role. Results cleared that the highest losses of 38.7% of AFM_1 and 45% of AFM_2 after pasteurization and homogenization before ice cream manufacture. Results obtained during the course of this investigation could be confirmed by Purchase,et.al 1972, Kiermeier and Mashaley,1977, Abd Alla 1983, and Motawee et.al 2003, who used the same degree of temperature and the same time of pasteurization, but El-deep, et.al 1992 and Mashaly, et.al 1986 found the lowest degradation of AFM_1about 9.5% only. Opposite results were observed by Wiseman and Marth, (1983 b) who observed that the AFM_1 content in milk was stable during pasteurization. Also Van Egmond, 1983 noted that AFM of milk is not reduced by heat treatment such as pasteurization and sterilization. These different results depend on different factors such as degree of temperature and length of time of treatment which affect the degree of toxin binding by milk casein. For example, high temperature increased the interaction of the toxin with casein and gelatin. The high concentration of toxin might led to saturation of casein binding sites leaving some free toxin easy for extraction and the method of extraction might also cause different results. Results in table (1) also cleared no loss of AFM_1 and AFM_2 during aging of mixture and hardening steps of ice cream manufacture. Regarding the effect of storage period on AFM content of ice cream at the present work, the distraction and the ratio of distraction were observed during 10 weeks of storage in freezer.

Table(2): Effect of storage Period on Aflatoxin M_1 and M_2 content for ice cream

Storage Period of ice cream	AFM_1 (µg/kg)	AFM_2 (µg/kg)	% Of Degradation (µg/kg)	
			AFM_1	AFM_2
Zero time after manufacture	1.05	1.00	0.0	0.0
1 WEEK	0.95	1.00	9.5	0.0
2 WEEKS	0.87	0.92	17.1	8.0
3 WEEKS	0.82	0.90	22.0	10.0
4 WEEKS	0.75	0.85	28.5	15.0
5 WEEKS	0.75	080	28.5	20.0
6 WEEKS	0.75	0.80	28.5	20.0
7 WEEKS	0.70	0.75	33.3	25.0
8 WEEKS	0.70	0.60	33.3	40.0
9 WEEKS	0.70	0.60	33.3	40.0
10 WEEKS	0.70	0.60	33.3	40.0

Data in table (2) showed that the percent of reduction of AFM_1 was 9.5% at the first week of storage but there is no effect of AFM_2 during the first week of storage of ice cream. More degradation of AFM_1 of about 9.5% from the first week to 28.5% at the end of 4th week, furthermore, AFM_1 was stable from 4th week to 6th week and then, became constant (33.3%)of degradation until the end of storage period. On the other hand, slowly reduction of AFM_2 about 8% from the second week to 25% of reduction to 7th week, thenafter, AFM_2 was stable of (40%) until the end of storage period of ice cream after manufacture. It is evident that AFM_1 and AFM_2 were reduced to about 10% and 15% from total toxins, respectively, during frozen storage of ice cream. Results were in agreement with Kiermeier & Mashaley ,1977, Abd Alla 1983, Abdl salam, et.al,1983, Wiseman & Marth, (1983a) Stoloff,et.al 1975 and Motawee,et.al 2003. The percent of AFM_1 reduction was about 66.6% and 65% of AFM_2 at the end of ice cream manufacture from artificially contaminated milk. Therefore, ice cream will be safe for human consumption because 76.6%-80% of milk toxin were detoxified during processing through ten weeks of storage.

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ثبات سموم الألبان (الأفلاتوكسينات م١، م٢) فى الأيس كريم أثناء التصنيع والتخزين

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قد تم تصنيع أيس كريم من لبن ملوث صناعيا بالسموم الفطرية(الأفلاتوكسين) من النوع م١ ،م٢ لمعرفة مدى وجودها وثباتها أثناء مراحل تصنيع الأيس كريم وكذلك أثناء تخزينة بعد التصنيع مباشرة لمدة عشرة أسابيع تحت الصفر. وجد أن نسبة التكسير للأفلاتوكسين م١ ٤% ، م ٢ ٥% بعد إضافة القشدة وزادت نسبة التكسير الى ٧و٩% ، ٣ و١٢ لكل من م١،م٢ على التوالى بعد إضافة الخليط. ثم زادت الى ٧و٣٠%،٢٤% لكل من م١، م٢ على التوالى بعد إضافة الجيلاتين. أما عن البسترة فقد لعبت دورا كبيرا أثناء التصنيع، ودلت النتائج على أن أعلى نسبة فقد فى تلك السموم كانت ٧و٣٨ % من م١ و٤٥% من م٢ بعد البسترة والتجنيس للخليط مباشرة. وأوضحت النتائج أيضا أنة لايوجد أى فقد من السموم الفطرية م١ وم٢ أثناء مرحلة التعتيق والتصليب. أما عن تأثير فترة التخزين لمدة ١٠ أسابيع على محتوى الأيس كريم من م١ و م٢ فإن الدراسة أوضحت أن النسبة المئوية للفقد من السموم الفطرية م١ كانت ٥و٩% خلال الأسبوع الأول من التخزين بينما لا يوجد معدل فقد من السموم الفطرية من النوع م٢ فى الأسبوع الأول من التخزين وزاد معدل الفقد من م١ من ٥و٩% الى ٥و٢٨% حتى نهاية الأسبوع الرابع ثم ثبت معدل الفقد حتى الأسبوع السادس حيث وصل الى ٣و٣٣% حتى نهاية فترة التخزين بينما كان معدل التكسير من م٢ بطيئا من ٨% فى الأسبوع الثانى حتى ٢٥% فى الأسبوع السابع تدريجيا بعد ذلك ثبت بعد أن وصل إلى ٤٠% حتى نهاية فترة التخزين. وهذا يؤكد أن نسبة الفقد الكلية م١ و م٢ كانت٦٦,٦ % و ٦٥% علي التوالي بعد التصنيع مباشرة وكانت نسبة الفقد ١٠ % و ١٥% من إجمالى السموم المضافة لكلا النوعين علي التوالي أثناء التخزين تحت تجميد. ونستنتج من هذة الدراسة أن الأيس كريم بصفة عامة أمن لصحة المستهلك لأن ٧٦%-٨٠%من السموم الفطرية المتوقع أن تكون موجودة به يزول مفعولها السمى أثناء التصنيع والتخزين من ٦ إلى ٧ أسابيع.