Sterigmatocystin: Incidence, fate and production by *Aspergillus versicolor* in Ras cheese

E. A. M. Abd Alla*, M. M. Metwally, A. M. Mehriz and Y. H. Abu Sree*

Summary

The occurrence, distribution, and stability of sterigmatocystin (STG) in Ras cheese were investigated. An incidence value for STG in market samples of Ras cheese was 35% with a mean value of 22.2 µg/kg. In experimental Ras cheese from milk contaminated with STG, 80% of the toxin was retained in the curd while 20% was found in the whey. The temperature for cheese ripening affected the toxin content. At 6 °C the toxin concentration was hardly affected, but at 20 °C the concentration was reduced by 16% after 90 days. In Ras cheese contaminated with spores of *Aspergillus versicolor,* toxin production started after 45 days of the ripening, reached a maximum after 90 days, and declined thereafter. Cow's milk favoured toxin formation in comparison with buffaloe's milk. Aged cheese (more than 6 months) inhibited toxin production.

Introduction

Sterigmatocystin (STG) is a toxic metabolite produced by about fifteen species of fungi. The principal producer is *Aspergillus versicolor* [3].

STG has proved to be mutagenic in *Salmonella typhimurium* [2, 9, 16] and in cultured chinese hamster cells [10]. STG was also found to be carcinogenic for rats [12, 13] and for mice [7, 19]. *A. versicolor* has been isolated from grains and grain products, fruits and marmalade, grapefruit juice, dried meat products and cheese [8, 11].

Therefore, STG occurrence in food is considered a potential hazard to man, although its incidence as a natural food contaminant is so far limited. One reason for this limitation could be that STG is a precursor for aflatoxin B_1 synthesis by the mold [19]. Thus, detection of STG in the products would depend on the stage of the aflatoxin B_1 synthesis.

Stability of STG in the presence of lactic acid bacteria was studied by EL. SAYED ABD ALLA [5], who found that the disappearance of STG from contaminated milk depended on the type of microorganism, storage period, and the type of milk. Actually, STG has been detected in hard cheese in concentrations ranging from 500–600 µg/kg in 1 cm thick surface layer [11].

This study was carried out to determine natural occurrence of STG in local hard cheese (Ras cheese), the fate of STG during cheese making, and formation of STG by *A. versicolor* on Ras cheese during ripening.

Materials and methods

Materials

Cheese samples

One hundred samples of hard cheese (Ras cheese) were collected from local markets of Cairo, Giza and Kalubia Governorates. Samples were kept under refrigeration until analysis.

Department of Dairying, Faculty of Agriculture, Cairo University, and * Mycotoxin Laboratory, Food and Dairy Science Department, NRC, Dokki, Egypt

Cheese milk

Fresh buffaloe's and cow's milk obtained from the herds of the faculty of Agriculture, Cairo University, were processed into Ras cheese.

Chemicals and solvents

All chemicals and solvents used were of high purity and were purchased from E. Merck, Darmstadt, Germany.

Thin layer chromatography

TLC Silica gel 60 plates (Ant. 5553) were purchased from E. Merck, Darmstadt, Germany.

Sterigmatocystin standard

STG was obtained from Sigma, Chemical Comp., USA.

Methods

Organism

Aspergillus versicolor (FRR 599) was used throughout the study. The fungus was cultivated on potato dextrose agar (PDA) slants for 10 days at 25 °C until they were well sporulated. The spores were harvested with sterile 0.05% tween 80 to give a final spore concentration of approximately 5×10^5 spores/ml.

Artificial contamination of milk

Fresh buffaloe's and cow's milk were contaminated with 125 and 250 µg STG/kg of each milk, to investigate the distribution of STG between curd and whey during cheese manufacture and its stability during cheese ripening.

Cheese making

Ras cheese was manufactured as described by ABDEL-TAWAB [1]. Cheese blocks were round and weight about 2 kg. The blocks were waxed and stored in ripening room at either 6 °C or 20 °C at 90% relative humidity (RH) till the required age.

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Table 1. STG content of hard cheese rind (1 cm thick layer) collected from local markets

Area	Total sample	Total positive	[%]	Range of concentration [μg/kg]		Mean [μg/kg]	S.D. (±)
Cairo	50	20	40	16.0	37.7*	25.7	7.6
Giza	25	7	28	18.8	23.7	21.3	2.0
Kalubia	25	8	32	10.0	37.0	23.5	9.6
Total	100	35	35	14.9	32.8	23.5	6.4

* Only one sample contained 62.8 μg/kg

Cheese inoculation

The outer surface of cheese blocks of proper age was inoculated with a spore suspension of *A. versicolor.* Inoculated cheeses were kept in the ripening room for the required period before STG determination.

Sterigmatocystin analysis

STG was extracted by the method of FRANCIS et al. [6]. 36 g of the sample were extracted with 200 ml of a mixture of acetonitrile–water (170 + 30 v/v) containing 4% KCl. 50 ml of filtrate were vigorously shaken for 30 s with 50 ml of 50% $CaCl_2$ solution (w/v). The top layer was extracted with 50 ml distilled water and then with hexane. The bottom layer was then extracted twice with methylene chloride 50 and 25 ml. The methylene chloride extracts (bottom layer) were combined and purified by passing through cupric carbonate diatomaceous earth column. The recovery of STG was 85–91%. The purified extract was evaporated under nitrogen to dryness. TLC technique for STG determination was carried out as described in AOAC (1990).

Results and discussion

Natural occurrence of sterigmatocystin in Ras cheese

To survey the presence of STG in Egyptian hard cheese (Ras cheese), one hundred cheese samples were collected from three administrative districts: Cairo, Giza and Kalubia. The samples were analyzed for the STG contents in the outer 1-cm thick layer and the inner layer. Results in Table 1 pointed out that STG presence was confined to the outer layer of 1 cm thickness. The inner layers were free from the toxin. Thirty five of the samples contained STG with a mean value of 22.23 μg/kg and the range were between 10.0 and 37.7 μg/kg. Only one sample contained 62.8 μg/kg. More cheeses were contaminated in Cairo than in the other 2 districts. These results are in line with that reported by NORTHOLT et al. [11] who found that nine out of 39 samples of cheeses were contaminated, the toxin also existed in the outer layer, and the toxin concentration ranged from 5 to 600 μg/kg. The presence of the toxin in the outer layer was expected since the mold grows on the surface of the cheese. On the other side the presence of the toxin proves that Ras cheese was contaminated with the toxigenic fungi and thus, the surface 2-cm layer of any molded cheese should be discarded for safety.

Fate of STG during Ras cheese processing

Cow's milk artificially contaminated with STG at levels 125 and 250 μg/kg was made into Ras cheese. STG content of milk, curd and whey was determined. The results obtained are presented in Table 2. It is clear from the results that only about 90% of the total STG added to cheese milk could be extracted during determination of STG in milk contaminated with either low or high level (125 and 250 μg/kg), respectively.

Table 2. Distribution of STG in curd and whey during cheese making (a)

Treatment	Low level		High level	
	Concentration of STG [μg/kg]	Recovery [%]	Concentration of STG [μg/kg]	Recovery [%]
Added STG	125	–	250	–
Extracted STG				
Milk	111.3[b]	100[b]	225[b]	100[b]
Curd	89.3[b]	80.2[b]	179[b]	79.7[b]
Whey	22.0[b]	19.8[b]	45.6[b]	20.3[b]

(a) Average of four replicates
(b) Calculated on the basis of actual amount of STG extracted from milk

Table 2 shows the distribution of STG between curd and whey during cheese processing. 80% of the toxin originally present in milk was found in the curd and only 20% was found in the whey. This was expected since STG is insoluble in aqueous solvents, therefore it resides in the curds.

Stability of STG during cheese ripening

The concentration of STG (μg/kg) in cheese during ripening at 6 ± 2 °C and 20 ± 2 °C are shown in Fig. 1 and

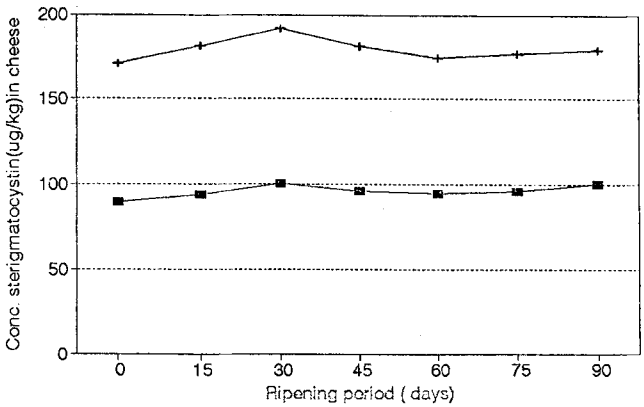

Figure 1. Concentration of sterigmatocystin in Ras cheese made from milk containing 125 (■) and 250 (+) μg/kg during ripening at 6 ± 2 °C

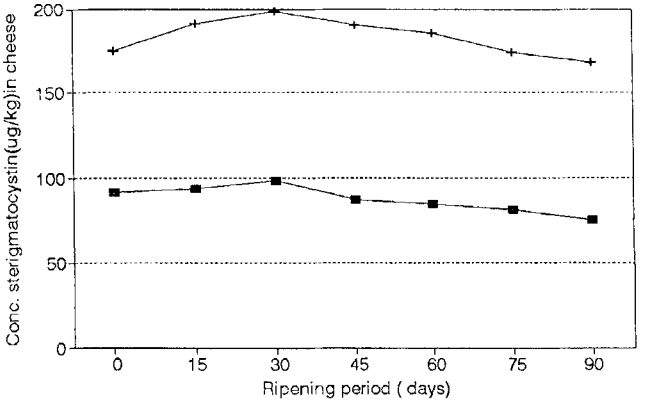

Figure 2. Concentration of sterigmatocystin in Ras cheese made from milk containing 125 (■) and 250 (+) µg/kg during ripening at 20 ± 2 °C

2 respectively. It is clear from the first diagram that there was no reduction in toxin content of cheese made from milk contaminated with 125 and 250 µg/kg of STG during ripening at 6 ± 2 °C. Figure 2 shows that a gradual decrease in STG content of Ras cheese made from cow's milk contaminated with 125 µg/kg was observed after 30 days of ripening at 20 ± 2 °C. When milk containing 250 µg/kg was used, the STG content of the resultant cheese slightly decreased only after 60 days of the ripening period at the same temperature. At the end of ripening period (90 days) the toxin in cheese was reduced by 16 and 4% when low and high concentrations of STG were used in cheese making, respectively. It could be assumed that some of the ripening bacteria were able to work on the toxin and change some into another metabolite.

It is clear from the above results that the toxin is highly stable and the health hazard of contaminated milk is still the same when converted into Ras cheese. This high stability of STG was also reported by VAN EGMOND et al. [17] who reported that the toxin was stable for 3 months at a temperature up to 16 °C.

Formation of STG by *Aspergillus versicolor* during ripening of Ras cheese

Fresh Ras cheeses made from buffaloe's and cow's milk were inoculated with aqueous suspension of *A. versicolor* spores. The inoculated cheese blocks were ripened either at 6 ± 2 °C or 20 ± 2 °C. Circular sectors of each cheese block were analyzed for the toxin after 0, 15, 30, 45, 60, 90, 120 and 150 days of ripening. The toxin was determined in horizontal 1 cm thick layers of each of the cheese sectors. Results of STG contents of cheese are presented in Tables 3 and 4.

In all the cheese and at both ripening temperatures toxin production started between 30 and 45 days of ripening. Concentrations reached maximal values at 90 days and then declined. It was observed that cow's milk was favoured for toxin production more than buffaloe's milk especially at 90 days of the ripening period. With regard to the total ripening period the differences of toxin production in both milk varieties are smaller. It is clear from these results that ripening temperatures actually affected both the toxin production and the rate of degradation. While the low ripening temperature enhanced toxin production, the high ripening temperature retarded the decrease of toxin.

Table 3. Formation of sterigmatocystin by *A. versicolor* mold during Ras cheese ripening at 6 ± 2 °C

Type of cheese	Ripening period [days]							
	0	15	30	45	60	90	120	150
Toxin concentration** [µg/kg]								
Control*	0	0	0	0	0	0	0	0
Cow	0	0	0	89	250	330	150	40
Buffaloe	0	0	0	178	230	280	140	50

* There was a cow's and buffaloe's cheese control
** Average of duplicate determination

Table 4. Formation of sterigmatocystin by *A. versicolor* mold during Ras cheese ripening at 20 ± 2 °C

Type of cheese	Ripening period [days]							
	0	15	30	45	60	90	120	150
Toxin concentration** [µg/kg]								
Control*	0	0	0	0	0	0	0	0
Cow	0	0	0	36	180	300	180	106
Buffaloe	0	0	0	180	200	256	220	160

* There was a cow's and buffaloe's cheese control
** Average of duplicate determination

These results are in agreement with that of NORTHOLT et al. [11] who found that nine mouldy cheeses with *A. versicolor* out of 39 cheeses with ages varying from 2 to more than 7 months contained the toxin in a concentration range from 500 to 600 µg/kg.

Cheese age was also found to have an effect on mold growth and toxin production. Aged cheese was neither a good medium for the mold growth nor for toxin production. Buffaloe's as well as cow's cheese with 3 months of age did not sustain good *A. versicolor* growth and the toxin was not detected before 60 days after inoculation. STG concentration in the above mentioned aged cheese was 10 and 12 µg/kg for cow's and buffaloe's cheese, respectively, when kept for 60 days at 20 ± 2 °C after inoculation. At refrigerator temperature the toxin levels were 20 and 25 µg/kg for cow's and buffaloe's cheese, respectively. This may indicate, that low temperature favours toxin production.

Finally, this study clearly proved that all precautions should be taken to prevent cheese contamination with fungi to avoid mycotoxins formation and its hazardous effect to human.

References

[1] ABDEL-TAWAB, C. F., and G. A. HAMDY, Principals of Dairy Industries (in Arabic) Cairo, Egypt, 1963.
[2] AMES, B. N., W. E. DURSTON, E. YAMASAKI and F. O. LEE, Proc. Natl. Acad. Sci. USA 70 (1973) 2281–2285.
[3] COLE, R. J., in: Mycotoxins and other fungal related food problems. Ed. by J. V. RODRICKS, pp. 68–89. Advanced Chem. Ser. 149. Am. Chem. Soc., Washington, D. C., 1976.
[4] DE-BOER, E., and M. STOLK HOROTHUIS, J. Food Protection 40 (1977) 533.

[5] EL-SAYED A. M. ABD ALLA, Egyptian J. Dairy Sci. 22 (1995) 319–327.
[6] FRANCIS, O. J., G. M. WARE, A. S. CARMAN and S. KUAN, J. Assoc. Off. Anal. Chem. 68 (1985) 643.
[7] FUJII, K., H. KURATA, S. ODASHIMA and Y. HATSUDA, Cancer Res. 36 (1976) 1615–1617.
[8] HOLZAPFEL, C. W., I. F. H. PURCHASE and P. S. STEYN, S. African. Med. J. 103 (1966) 1100–1151.
[9] KUCZUK, H. K., P. M. BENSON, H. HEATH and A. W. HAYES, Mutat. Res. 53 (1978) 945–949.
[10] NODA, K., M. UMEDA and Y. UENO, Carcinogenesis 2 (1981) 945–949.
[11] NORTHOLT, M. D., H. P. VAN EGMOND, P. S. SOENTORO and W. E. DEYLL, J. Assoc. Off. Anal. Chem. 63 (1980) 115–119.
[12] OHTSUHO, K., M. SAITO and H. KIMURA, Food Cosmet. Toxicol. 16 (1978) 143–149.
[13] PURCHASE, I. F. H., and M. E. PRETORIUS, J. Assoc. Off. Anal. Chem. 56 (1973) 225.
[14] PURCHASE, I. F. H., and J. J. VAN DER WATT, Food Cosmet. Toxicol. 8 (1970) 289–295.
[15] SCOTT, P. M., W. VAN WALBEEK, B. KENNEDY and D. ANYETI, J. Agric. Food Chem. 20 (1972) 1103.
[16] UENO, Y., K. KUBOTA and Y. NAKAMURA, Cancer Res. 38 (1978) 536–542.
[17] VAN EGMOND, H. P., M. D. NORTHOLT and W. F. PAULOCH, in: Proceedings 4th Meeting on Mycotoxins in Animal Diseases. pp. 87–89. ADAS Weybridge, Great Britain 1981, cf. Food Chemistry 11 (1983) 289.
[18] ZWICHER, G. M., and W. W. CARLTEN, Food Cosmet. Toxicol. 12 (1974) 491–497.
[19] YAMAZAKI, M. in: Toxicology and Pathology of Mycotoxins. Ed. by K. VRAGUCHI, and M. YAMAZAKI, p. 65, John Wiley and Sons, New York 1978.

Correspondence to:
EL-SAYED A. M. ABD ALLA, National Research Centre, Dokki-Cairo, Egypt

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Simultanbestimmungsmethoden für die Elemente Kupfer, Zink, Eisen und Mangan sowie Natrium, Kalium, Calcium und Magnesium mittels Flammen-Atomabsorptionsspektrometrie (F-AAS)

U. Gottelt, G. Henrion*, R. Kalähne* und M. Stoyke

Zusammenfassung

Die Elemente Na, K, Ca, Mg, Cu, Zn, Fe und Mn sind insbesondere für die Lebens- und Futtermitteluntersuchung aus ernährungsphysiologischen Gründen von Bedeutung. Es werden zwei Simultanbestimmungsmethoden für diese Elemente mittels Flammen-Atomabsorptionsspektrometrie (F-AAS) vorgestellt. Die Empfindlichkeit der F-AAS ist für diese Untersuchungen ausreichend. Die Elemente Na, K, Ca und Mg können nach Zusatz von SCHINKELlösung (Cäsiumchlorid-Lanthanchlorid-Pufferlösung) und erfolgter Kalibration bzw. Standardaddition simultan bestimmt werden. Die Nachweisgrenzen von Na, K, Ca und Mg werden durch die simultane Bestimmung der Elemente, die angewandten Wellenlängen und die Art der untersuchten Proben (30 mg Na, 24 mg K, 1,9 mg Ca und 0,46 mg Mg je kg Frischsubstanz) beschränkt. Für Cu, Zn, Fe und Mn konnte ebenfalls eine Simultanbestimmungsmethode mit den Nachweisgrenzen von 0,61 mg Cu, 0,14 mg Zn, 3,8 mg Fe und 0,44 mg Mn je kg Probe erarbeitet werden.

Die Methoden wurden mit den Standardreferenzmaterialien CRM No 278 (Mussel Tissue), CRM No 185 (Bovine Liver) und CRM No 189 (Wholemeal Flour) überprüft und an ausgewählten Proben getestet.

Summary

Simultaneous determination of the elements copper, zinc, iron and manganese as well as sodium, potassium, calcium and magnesium by flame atomic absorption spectrometry (F-AAS).

The elements Na, K, Ca, Mg, Cu, Zn, Fe and Mn are very important for the examination of human food and foodstuff in the light of nutritional physiology. Two simultaneous determination methods for these elements with flame atomic absorption spectrometry (F-AAS) are presented. The sensitivity of the F-AAS is sufficient for these examinations. Using the calibration and the method of standard addition resp. and "SCHINKELlösung" (caesiumchlorid lanthanumchlorid buffer solution), the simultaneous determination of Na, K, Ca and Mg is possible. The detection limits of Na, K, Ca and Mg depend on the simultaneous determination of the elements, the used wavelengths and the kind of the examined samples (30 mg Na, 24 mg K, 1,9 mg Ca and 0,46 mg Mg per kg fresh matter). The detection limits for Cu, Zn, Fe and Mn with the simultaneous determination are 0,61 mg Cu, 0,14 mg Zn, 3,8 mg Fe and 0,44 mg Mn per kg sample.

The new methods are tested with the standard reference materials CRM No 278 (Mussel Tissue), CMR No 185 (Bovine Liver) and CRM No 189 (Wholemeal Flour) and with selected samples.

Einleitung

Die Flammen-AAS ist eine der am häufigsten eingesetzten Routineverfahren zur Bestimmung von mittleren und höheren Gehalten an Metallen in organischen Materialien. Sie hat den Vorteil, billig, universell und sehr flexibel zu sein [1–3]. Ihr Haupteinsatzgebiet liegt im Konzentrationsbereich mg/l.

In der Lebens- und Futtermittelanalytik stellt sich häufig die Frage nach den Gehalten an Mengen- und Spurenelementen. So sind z. B. die Elemente Ca, Mg, Na, K, Mn, Fe, Cu und Zn von Interesse für eine gesunde Ernährung

Institut für Veterinär-Pharmakologie und Toxikologie GmbH, Bernau, und * Humboldt-Universität zu Berlin, Institut für Angewandte Analytik und Umweltchemie, Berlin, Deutschland