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Reserches regarding aflatoxin production in feta chesse by direct contamination with *Aspergillus flavus*

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Abstract

The research conducted on the probe of Feta cheese were inoculated on one freshly cut surface with spore suspensions of Aspergillus flavus (toxigenic strain) and incubated at room temperature. Aspergillus strain genera were tested for aflatoxin production after growth on Feta cheese . Cheeses were sampled after at 0, 10,20,30 and 40 day and samples were examined by High Performance Liquid Chromatography (HPLC) procedures for their content of aflatoxins B_1 and G_1 . After 40 days of incubation over 48% of the molds were Aspergillus species made up 2.72 to 3.95 % and 0 to 6.05 % of the mold on cheese and in the plant atmosphere, respectively. Because the physicochemical characteristics of Feta cheese (high salt medium concentration and low pH) favor aflatoxicogenic mold growth .Aflatoxin production by Aspergillus was enhanced at a concentration of salt in Feta cheese 3,5% and at a higher concentration salt medium inhibited growth and toxin formation by both species.

Keywords: Aflatoxin **,** Aspergillus flavus , Feta cheese, mold, toxigenic strain

1. Introduction

Aspergillus flavus is the most common species producing aflatoxins occurring in most kinds of foods in tropical countries. This species is very common on maize, peanuts and cottonseed, and produces only B aflatoxins. It has been estimated that only about 30-40% of known isolates produce aflatoxin, but in this estimate other closely related species has not been taken into account. Furthermore several other extrolites are produced by *A. flavus*, including kojic acid, cyclopiazonic acid, aspergillic acid and β- nitropropionic acid, so accurate identification of both the fungus and its profile of extrolites is important, if a prevention program should be successful[5]

Aflatoxins is a potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *Aspergillus parasiticus* on variety of food products. Among 18 different types of aflatoxins identified, major members are aflatoxin B_1 and G_1. Aflatoxin B_1 (AFB1) is normally predominant in amount in cultures as well as in food products. Pure AFB1 is pale-white to yellow crystalline, odorless solid.

Aflatoxicogenic potential on cheese and other fermented dairy products is a common and recurring problem. Fungi growth on cheese and, to less extent, other fermented dairy products stored at low temperatures is a common and recurringproblem .The natural occurrence of aflatoxins in cheese has been investigated[1].

Torrey and Marth[8] reported isolation of potentially toxic molds from

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home refrigerators and cheese and other foods stored in home refrigerators. Thus, mold growth on cheese and fermented dairy products may pose potential hazards to food safety and human health. This report reviews research concerning molds and mycotoxins in cheese and fermented dairy products and assesses the significance to public health of these findings. The work can be divided into four areas:

1) incidence, types and mycotoxin-producing potential of molds in fermented dairy products;
2) experimental mycotoxin production on cheese under conditions that cheese is stored and aged;
3) natural occurrence of mycotoxins in commercial samples of cheese;

potential toxicity of *Penicillium roqueforti* and its significance in blue veined cheeses[1].

2. Materials and method

Cultures. Aspergillus flavus DSM 818 known toxigenie strains, were obtained from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, spore suspensions. Molds were grown on slants of potato dextrose agar .

Medium and incubation. The potato dextrose agar medium served to grow the molds. The spore suspension were added to 100 ml of medium (in a 500-ml Erlenmeyer flask), and cultures were incubated at 20 ^{o} C for 40 days.

Samples. A total of 35 Feta cheese samples was obtained in a milk processing industrial unit from Maramures, using standardized cow's milk. The technological process of this cheese is the same with the one used in order to obtain classic cheese, described by Costin G.M [2] what differences them is the type of starter colonies that were used.

Sampling of cheese. Growth of mold on the surface of Feta cheese became apparent after five days, and six days later the first set of cheeses was sampled. A second set was examined 20 days later, third set was examined 30 days later and the last set 40 days later .Then each Feta cheese sample was cut horizontally into a series of 0,5 cm layers.

Extraction of Toxins. A 20 g sample of cheese was blended with 100 ml of water for 2 min Waring blender jar. The pH of the solution was adjusted to 6 by addition of one N acetic acid. Then 200 ml of methanol:acetone (50:50, vol/vol) were added, and the mixture was blended for additional 3 rain. The mixture was filtered, and the filtrate was left at -20C or at a temperature below -20C for 5 to 6 h. The casein-precipitate formed by the action of cold was centrifuged at 2000 rpm for 20 rain. Then 200 ml of the supernatant liquid were transferred to a 1-1iter round-bottom flask and evaporated to about 75 ml in vacum. The remaining solution was transferred quantitatively to a separatory funnel and washed with three successive 100-ml volumes of n-hexane. The defatted solution was extracted twice with 100 ml-volumes of chloroform: once with 100 ml of chloroform:ethyl acetate (50:50 vol/vol) and then with 100 ml of ethyl acetate, respectively. These extracts were combined and passed through a filter containing anhydrous sodium sulphate into a round-bottom flask and evaporated to about 5 rot. The combination was transferred to a small glass tube and evaporated to about 1 ml in a heating block under a stream of nitrogen. The volume was adjusted to 5 ml with chloroform:hexane (50:50, vol/vol) [6]

Analysis of aflatoxin. Aflatoxin analysis was described by Tetsuhisa G.[7] , using Agilent 1200 HPLC system , ZORBAX Extend C18, 100 mm × 2.1 mm Column , Mobile phase: A = 10 mM witch ammonium acetate in water B= Methanol 40% A/60% B, Flow rate: 0.2 mL/min and 5 μL Injection volume

3. Results and discussion

Research results can be seen in table in ***Table no. 1*** regarding the production of aflatoxins by *Aspergillus flavus* indicate

that the synthesis of aflatoxins B_1 and G_1 started at day 10 and their concentrations reached maximum level after 40 days followed by a decrease aflatoxin production witch increasing salt concentration in Feta cheese

As you can see sodium chloride in ***Table no. 1*** enhanced aflatoxin production by *Aspergillus flavus.* ,toxin production by culture was markedly reduced by 3.5% sodium chloride

Growth was reduced starting with 3.5% sodium chloride , and toxin formation was drastically reduced when the medium contained 5% sodium chloride, and it was evident that *Aspergillus flavus* were able to grow and produce aflatoxins which penetrated into the inoculated Feta cheese at a concentration of sodium chloride less than 3% ,

The content of both aflatoxins in the upper layer of cheese increased (approximately fivefold for B_1 and twofold for G_1) during the interval between the first and seventh week of incubation when .The sodium chloride content of Feta cheeses is between 2 and 3,5 % and our data indicate that these levels of sodium chloride do not inhibit production of toxin by aflatoxinogenic molds.

Table no.1 Aflatoxin content (μg/100 ml) of the Feta cheese of *Aspergillus flavus* at different days of incubation and at different Feta cheese salt concentrations

Salt (%)	B_1	G_1	B_1	G_1	B_1	G_1
	after 0 days		after 10 days		after 20 days	
2	165.23 ± 0,08	189.24 ± 0,25	201,45 ± 0,75	220,45 ± 0,09	229,14 ± 0,03	247,31 ± ,01
2,5	138,55 ± 0,10	163,45 ± 0,23	159,08 ± 0,02	163,78 ± 0,01	153,73 ± 0,01	159,15 ± 0,75
3	118,12 ± 0,23	135,12 ± 0,01	128,42 ± 0,09	130,05 ± 0,08	118,19 ± 0,24	125,75 ± 0,01
3,5	101,46 ± 0,52	110,11 ± 0,24	103,09 ± 0,02	115,36 ± 0,71	108,13 ± 0,02	112,95 ± 0,02
4	79,35 ± 0,07	89,20 ± 0,01	83,54 ± 0,07	91,75 ± 0,08	89,07 ± 0,01	97,10 ± 0,01
4,5	63,30 ± 0,01	72,12 ± 0,68	65,15 ± 0,89	74,15 ± 0,78	69,19 ± 0,59	80,15 ± 0,37
5	39,36 ± 0,23	54,13 ± 0,01	41,14 ± 0,01	59,85 ± 0,09	48,17 ± 0,04	64,75 ± 0,19

Salt (%)	B_1	G_1	B_1	G_1
	after 30 days		after 40 days	
2	243,23 ± 0,01	269,14 ± 0,01	262,45 ± 0,92	289,45 ± 0,44
2,5	168,46 ± 0,52	181,21 ± 0,06	192,89 ± 0,07	223,20 ± 0,01
3	132,42 ± 0,73	141,14 ± 0,08	138,21 ± 0,01	177,03 ± 0,05
3,5	109,20 ± 0,02	122,74 ± 0,01	112,11 ± 0,05	145,46 ± 0,07
4	89,78 ± 0,58	102,98 ± 0,55	89,30 ± 0,46	123,14 ± 0,01
4,5	68,65 ± 0,01	86,69 ± 0,01	68,04 ± 0,05	95,03 ± 0,03
5	52,85 ± 0,55	69,52 ± 0,78	53,46 ± 0,01	72,45 ± 0,53

Mean ± standard error of means

4. Conclusions

This research has shown that Feta cheese is seldom held at room temperature (20 ^{0} C) for an extended period and, hence, rapid growth of toxigenic aspergilli, if present, would not be encountered and on the other hand provided critically needed information regarding the ability of sodium cloride to prevent or reduce the level of aflatoxin B_1 and G_1 residues in Feta chesse .

The study indicates that *Aspergillus flavus* was able to produce aflatoxins in Feta cheese after seeding the cheese surface with toxigenic aspergilli. Thus, the potential hazard for the natural occurrence of aflatoxins in Feta cheese is real when there are excellent conditions for growth of toxigenic aspergilla including a lower concentration of salt in cheese and this is the purpose of this research

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