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INVESTIGATION OF AFLATOXINS IN CHIPS MANUFACTURED FROM CORN

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ABSTRCT:*This study aimed to investigate the microbial content and aflatoxin in the chips manufactured from corn samples which is available in the city of Baghdad markets , 15 samples of chips manufactured from corn were collected to assess microbial content and the content of aflatoxin. The results showed that the total number of bacteria in most species, the highest number for bacteria 30 × 10^5cfu/g in the brand Buffies while the lowest number of bacteria 4 × 10^5cfu/g in Baraaem , but (Doritos , Pufak and Bad bain) were empty from bacteria.The results also showed the increase in the number of Staph. bacteria in samples, the highest number reached 18 × 10^5cfu/g in Karameesh brand and 17 × 10^5cfu/g in Zena , the lowest number of these bacteria appeared in Tarabish reached to 3 × 10^5cfu/g , while other types (Doritos ,Pufak and Bad bain) were empty from these bacteria. The results show that Puffies brand contain the highest number of the number of Coliform bacteria reached 20 × 10^5cfu/g , and the lowest number in trade brand Sanfori , Karameesh , Baraaem, while (Doritos ,Pufak and Bad bain) were empty from it. also the number of Yeasts and molds increase , Karameesh contains the highest number of them 26 × 10^5cfu/g , while the lowest number was in Baraaem 2 × 10^5cfu/g , and (Doritos ,Pufak and Bad bain) were empty from yeasts and molds. The results showed the presence of aflatoxin B1 in the types of chips studied and reached his highest concentration 1.64 PPM in brand Zena and less focus.154 PPM in brand Pop Corn while other types (Doritos ,Pufak and Bad bain)were empty from it.The aflatoxin B2 has been found in only five types (Bob corn, Baraaem, Crown, Happy, Puffies) reached the highest concentration of 0.43 PPM in Bob corn brand and less concentration of 0.13 PPM in Baraaem brand while other types were empty from it.*

KEYWORDS: Corn chips, Microbial contamination, Aflatoxin.

INTRODUCTION

Aflatoxins are produced on various grains and nuts, e.g., corn, sorghum, cottonseed, peanuts, pistachio nuts, copra, cereals, fruits, oilseeds humidity are optimal for moulds growth and toxins production (1, 2). Its presence is enhanced by factors as stress or damage to the crop due to drought before harvest, insect activity, soil type and inadequate storage conditions (3) Aflatoxins, when ingested, inhaled or adsorbed, dried fruits, cocoa, spices and beer in the field and during storage. AFs occur mainly in hot and humid regions where high temperature and through the skin, have carcinogenic,hepatotoxic, teratogenic and mutagenic effects in human and animals (rats, ferrets, ducks, trout, dogs, turkeys, cattle and pigs) (4), even at very small concentrations. When aflatoxins B1 is ingested by cows, it is transformed into its

hydroxylated product, AFs M1 and M2. Such aflatoxins is secreted in the milk and isrelatively stable during milk pasteurization, storage, and preparation of various dairy products (5).Aflatoxins are produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (6). These fungi are present in soil and plant material, cause the decay of stored grain and food. Aflatoxins may increase stress susceptibility and compromise growth efficiency. The clinical signs of aflatoxicosis are extremely varied. Signs of acute aflatoxicosis include depression, nervousness, abdominal pain, diarrhea and death (7).

The aim of this project was to screen the pathogenic bacteria and molds content of aflatoxins in samples of corn based products available in Iraq market that were widely consumed in huge amounts, as well as providing sensitive, accurate and reproducible analytical method for the detection of aflatoxins to assess the exposure of consumers to the toxins in order to bring them to the attention of the importance of monitoring the levels of aflatoxins in the corn based products and pathogens.

MATERIALS AND METHODS

Samples of corn chips from different companies as (Lucy, Salwan, Pop Corn, Doritos, Sanfori, ChaCha, Pufak, Baraaem, Zena, Crown, Happy, Bad bain, Buffies, Karameesh) were collected from Baghdad super market. Evaluation of the microbial quality of plant material the microbial quality of corn and corn products were tested according to the regulation of Pharmacopoeia (8) and Regulations. The tests were used for the quantitative evaluation of mesophilic bacteria and fungi that grow aerobically.

Aerobic plate count determination

All supplements were tested as follows: 10 g of each sample were aseptically removed and transferred to sterile blender jars. Subsequently, each sample was blended in 90 ml of 0.1% peptone (saline) for 45 sec. Because the plant samples are recognized to have a significant microbial contamination, serial dilutions were prepared so that the number of colony forming units (CFUs) in the Petri dishes would be less than 300. Duplicate 1 ml aliquots of each dilution sample were pipetted onto two separate sterile Petri dishes (9 cm in diameter); 20 ml of a liquid nutritive agar medium suitable for the cultivation of bacteria or Sabouraud agar for the cultivation of fungi, were added. After solidification of the soft agar, the Petri plates were incubated at 35°C for bacteria and 25°C for fungi, for three and five days respectively. The number of microorganisms in each sample was evaluated by multiplying the average number of colonies per plate by the dilution used. Colonies were counted and the counts were expressed as colony forming units per gram (CFU g-1).For the detection of certain bacteria, we used tests for specific microorganisms according to Pharmacopoeia and Regulations. Mold isolates were purified on PDA (potato dextrose agar) and further sub-cultured on malt extract agar (MEA) for microscopic examination and identification. Identification of plant pathogenic fungi isolated from the herbal drugs was carried out using standard determinants.

Identification of fungi

The determination of fungal colonies formed on the medium was based on the macroscopic and microscopic characteristics of the isolates. Macroscopic features include the appearance and speed of development of colonies on PDA medium, pigmentation and other substrates.

Microscopic features include the presence or absence of microconidia, form and manner of formation of microconidia and conidia cells, the appearance of macroconidia, the presence or absence of chlamydosporia, sclerotia, the biometric value characteristics of the reproductive organs of fungi in the culture and the host, and the facultative fungi. For obligate parasites we prepared native preparations and examined the fresh material under a microscope. The standard determinants of (9).

Chemical sandreagents

Aflatoxins stock standard consisted of 1000 ppb of B1 and 300 ppb of B2 was purchased from Supelco. Acetonitrile and methanol of HPLC grade were obtained from Merck. Deionized water was obtained from Elga water system. aflatoxin B1 (AFB1) 9.6 ppm of aflatoxin. All the standard solutions were stored in dark at 4°C when not in use. corn based product samples were ground and then split into four 25 g portions for each sample. Three of the portions were spiked with aflatoxins to give final levels of 10 ppb AFB1 ppm whereas the remaining portion was treated as blank.Purified extracts were analyzed by reversed-phase isocratic high performance liquid chromatography (HPLC) from Shimazu LC 10A using a Platinum C18 column (250 × 4.6 mm id, 5 μm) maintained at 40°C. A fluorescence detector from Shidmazu RF-10AXL was set at 375 nm (excitation) and 440 nm (emission). The mobile phase applied was deionized water/acetonitrile/methanol (60:20:20) with flowrate of 1.0 mL/min and injection volume of 20 μL.

Extraction and clean up

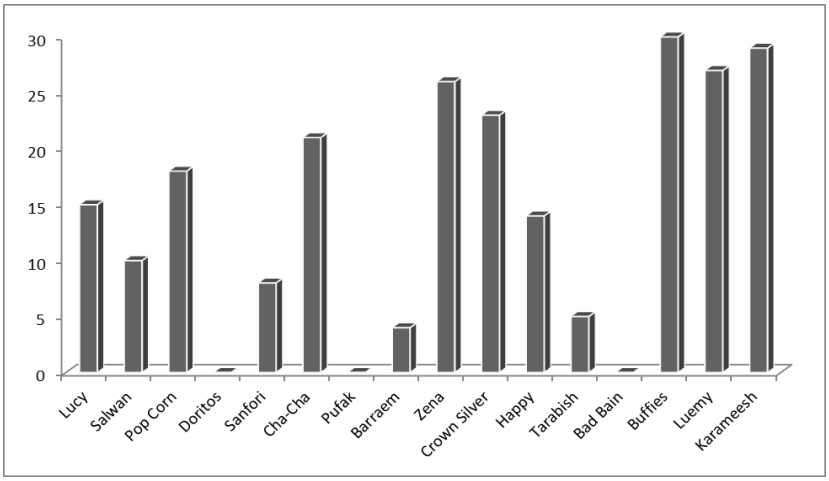
A 25 g of ground sample was mixed with 100 mL of mixture of acetonitrile and deionized water at ratio of 84:16 and shaked for 1 hour. The extract was filtered through Advantec filter paper No.131. A 9 mL of filtrate was then transferred into a test tube and pressed through Mycosep® #226 AflaZon clean-up cartridge which was obtained from Romer Labs. Inc. (10). A 2 mL of of purified extract was removed and evaporated to dryness. The dried extract was then reconstituted into 200 μL of methanol, vortexes and filter through 0.45 μm nylon membrane filter prior to HPLC analysis.

RESULTS AND DISCUSSION

Depending on the level and possibilities of microbial contamination, we found that samples microbial contamination, which is mainly caused by a large number of viable aerobic bacteria and increased number of fungi, especially molds, clearly exceeded the tolerable limit according to criteria of Pharmacopoeia and Regulations. Mixed infections were frequently noted. the highest microbial contamination was recovered from samples which is probably due to primary contamination in the field as a consequence of the manner of harvesting. Corn silk is thrown to the ground during the harvesting of corn. Among other isolated bacteria, *Staphylococcus aureus* was the most frequent in the samples.

Results shown in the Fig. (1) the total number of bacteria in the types of studied as it reached the highest number for bacteria 30 $\times 10^5$ cfu/g in the brand Buffies Followed by Karameesh brand reached to 29 $\times 10^5$ cfu/g, while the lowest number of bacteria 4 $\times 10^5$cfu/g in Baraaem ,but Doritos , Pufak and Bad bain were empty from bacteria. Fig. (2) shows the number of *Staph*. in samples, the highest number for bacteria reached 18 $\times 10^5$ cfu/g in

Karameesh brand and 17 × 10^5 cfu/g in Zena ,and 14 × 10^5cfu/g in Cha-Cha, the lowest number of these bacteria appeared in Tarabish reached to 3 × 10^5 cfu/g , while other types (Doritos , Pufak and Bad bain) were empty from these bacteria. The number of Coliform bacteria in chips types shows in Fig. (3) , the results show that Puffies brand contain the highest number of it 20 × 10^5 cfu/g , and the lowest number 2 × 10^5 cfu/g appeared in brand Sanfori , Karameesh , Baraaem, while (Doritos ,Pufak and Bad bain) were empty from it.Fig. (4) shows the number of Yeasts and molds in chips types , Karameesh contains the highest number of them 26 × 10^5 cfu/g followed by Luemy 22 × 10^5 cfu/g m then Zena 20 × 10^5 cfu/g , while the lowest number was in Baraaem 2 × 10^5cfu/g , while (Doritos ,Pufak and Bad bain) were empty from yeasts and molds.



. (1): Total number of bacteria in the types of chips

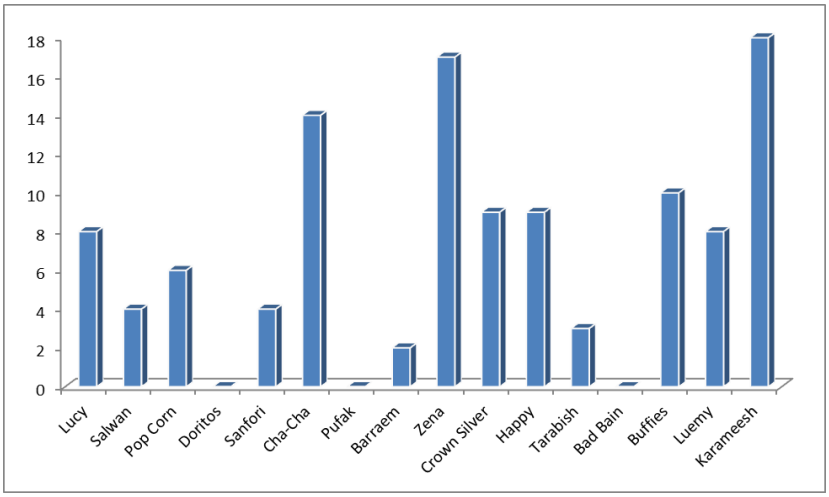


Fig. (2): Total number of *Staph*. bacteria in the types of chips

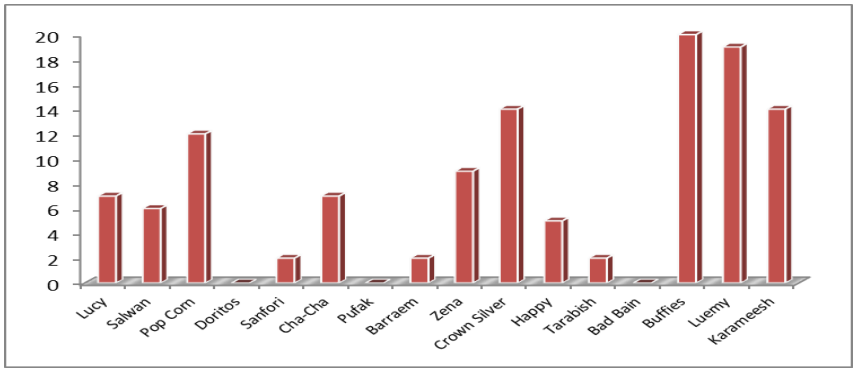


Fig. (3): Total number of *Coliform* in the types of chips

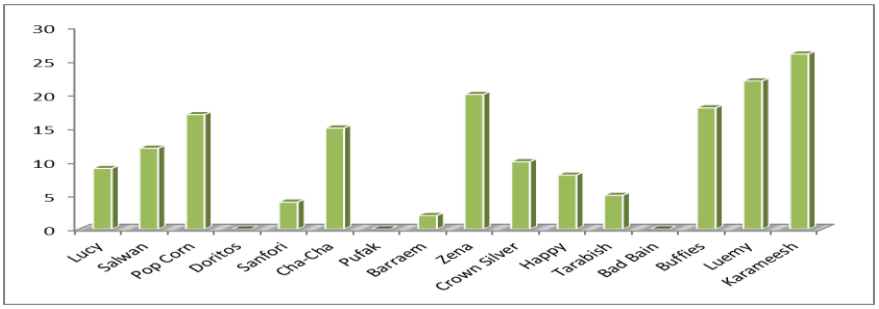


Fig. (4): Total number of Yeasts and molds in the types of chips

In the present study, AFLB1 indicating in all samples of corn chips except in Dritos , Pufax and Bad bain chips. The highest value of AFLB1 was found in Zenna, (1.64 ppm.) followed by Sanfori (0.913ppm.), the lowest value found in Pop corn(0.154ppm.) as shown in Fig (5).

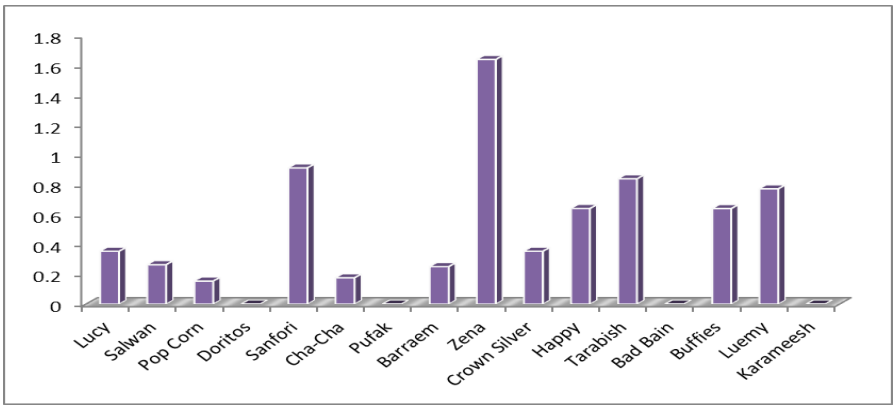


Fig. (5) : Aflatoxin B1 concentration in the types of chips

FLB2 was found in only 5 samples of chips (Fig. 6) , the highest value was found in Pop Corn (0.43 ppm.) then Crown (0.35 ppm.), Happy (0.15 ppm.), Baraaem (0.13 ppm.). while the other samples were empty from FLB2.

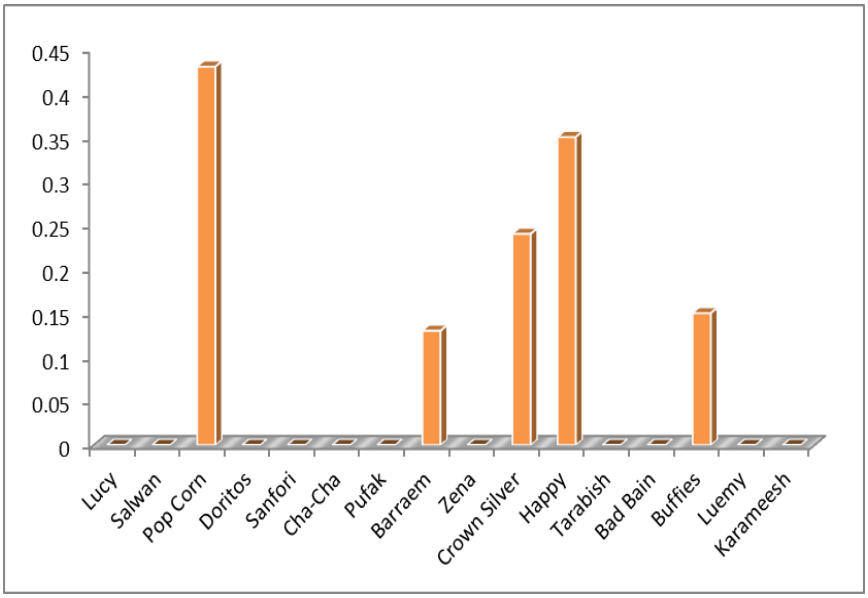


Fig. (6) : Aflatoxin B2 concentration in the types of chips

Usually no microbial problems occur with corn and products because they are kept dry. However, for the manufacturers of modern convenience. refrigerated. and specialty foods in which milled corn products are ingredients, high levels of microorganisms in grits and flour could create a problem. Some of these foods are moist and provide excellent media for the growth of microorganisms if the foods are mishandled. Contamination with psychrotrophic organisms in refrigerated foods increases the problem because these organisms are able to grow despite proper cold storage. To ensure a reasonable shelf-life and safety in modern foods, all ingredients must have a low overall microbial population and must be free of health hazards; e.g., mycotoxins, staphylococci, , and other intestinal pathogens. the presence of large numbers of these types of organisms raises spoilage or loss of quality, or create a health hazard.

The AFL are extremely potent mutagens and suspected human carcinogens. They can adversely affect human and animal health and agricultural productivity. In aquatic animals, AFL can cause abnormalities such as poor growth, physiological disorders and histological changes that decrease production. Problems can be caused by many factors such as low quality of food ingredient and in appropriate methods of feed storage, Production of AFs is mainly reported to occur by growth some strains belonging to the three major species; *A. flavus*, *A. parasiticus* and *A. nomius* (11). and less frequenty by some other *Aspergillus* species including *A. bombycis, A. pseudotamarii* and *A. ochraceus* as well as two *Emericella* species (12). Aflatoxigenic *A. flavus* strains have been reported from various crops, agricultural commodities, and soils (15, 16). According to Hamilton (1984), “in the present state of knowledge, one cannot be relied upon safe dosages of mycotoxins in foods”, hence

any dosage presents a serious hazard. The contamination of the human organism with bacteria brings about an immediate intoxication and its diagnosis is relatively simple, with an appropriate treatment being immediately applied. On the other hand, the consumption of food products contaminated with mildew results in no immediate symptoms but only in the future in the form of tumors as well as mutagenic and teratogenic effects.

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