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Mould contamination and aflatoxin residues in frozen chicken meat-cuts and gIBLETS

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Abstract

Mould contamination of frozen chicken meat and gIBLETS has a particular public health significance in the field of food safety due to its related food spoilage and production of mycotoxins. The objectives of this study were firstly, to investigate the incidence of mould contamination in frozen chicken meat-cuts and gIBLETS. Secondly, to estimate aflatoxin residues in these products. The public health importance of the prevalent mould genera and aflatoxin residues was discussed. Frozen gizzards had the highest total mould count followed by frozen liver, breast and thigh respectively. The prevalent mould genera were *Aspergillus*, *Penicillium*, *Cladosporium* and *alternaria*. *Aspergillus niger*, *flavus*, *parasiticus* and *versicolor* were the identified *Aspergilli*. Examined frozen chicken samples had variable residual concentrations of total aflatoxins. Thus, strict hygienic precautions during processing of frozen chicken products should be adopted to reduce mould contamination and mycotoxin production.

Key words: Mould, frozen chicken meat, aflatoxins

Introduction

Chicken meat and gIBLETS are very important sources for animal-derived proteins, essential amino acids, and a vast array of vitamins and minerals.

Freezing is a very useful method of meat preservation for prolonged times, however, in some cases due to bad storage conditions or fluctuation of freezing temperatures, mould contamination may occur. Mould contamination of frozen chicken meat and gIBLETS may lead to their spoilage and production of mycotoxins with potential health hazards to human due to their carcinogenic effects,

liver diseases and organ damage³.

The incidence of meat contamination with different mould genera was investigated in different localities of the world such as Australia, Japan, Italy and Spain^{6,8}. Despite of the large-scale consumption of different chicken meat and gIBLETS by Egyptian population, few published reports are available about the incidence of mould contamination and aflatoxin residues in such products. Thus, this study was undertaken to estimate the incidence of mould contamination in frozen chicken meat-cuts and gIBLETS. Furthermore, identification of prevalent mould genera was carried out. Aflatoxin residues were measured in

the examined samples. Public health significance of aflatoxins and the existing mould genera were discussed.

Materials and Methods

Food Samples collection:

Eighty random samples of frozen chicken breast, thigh, gizzards and livers (twenty of each) were collected from different localities in Zagazig city, Egypt. Samples were identified, packed and transferred to the laboratory in icebox and subjected to the mycological examination.

Preparation of samples:

Twenty five grams from each sample were aseptically excised and homogenized in 225 ml of sterile buffered peptone water 0.1% at 2500 rpm for 2 min using a sterile homogenizer (type M-P3-302, mechanic, precyzina, Poland). Such homogenate represents the dilution of 10⁻¹, and then decimal dilutions were done.

Determination of the total mould count:

The total mould count (TMC) was determined by culturing duplicate plates on each of malt extract agar media and Czapeck-Dox agar with 5% NaCl (Oxoid, Basingstoke, UK) followed by incubation in dark at 25 °C for 5-7 days. During the incubation time, the plates were examined daily for the star-shape mould growth, which is picked up under aseptic conditions with its surrounding cultivated medium and transferred into malt extract slope agar (Oxoid) then kept for further examination. Estimation of TMC was obtained by counting of the cultured agar plates of acidified malt extract agar and osmophilic moulds on Czapeck-Dox agar¹). Mould counts were converted into base logarithms of colony forming units per g of meat product samples (log cfu/g).

Identification of isolated moulds:

The identification of isolated mould genera were carried out based on their micromorphological properties¹⁰). In brief, the isolates were sub-cultured on malt extract agar and Czapeck-

Dox agar, incubated at 25 °C for 5-7 days. The identification of the colonies was carried out by careful observation and measurements of the macroscopical and microscopical characteristics of the mould colonies, which were recorded in data sheet.

Macroscopical examination:

The cultures were examined daily for the rate and pattern of growth during the incubation period. Observations were made for the consistency of the surface growth; the pattern of folding (rugae); the distinctness of the colony margin and for the presence of pigment either on the surface or the reverse of the colony or diffusing into the surrounding medium. Both the surface and backside of the colony were examined.

Microscopical examination:

From the periphery of 5-7 days old mould colony, a triangular piece was transferred to a clean glass slide. With two mycological needles, the piece of the colony was distributed with one or two drops of 70 % alcohol. One drop of lactophenol stain was added after evaporation of the alcohol. Then covered by a clean cover slide followed by gentle pressure to remove the excess of fluid and air bubbles as well as to depress the hyphae and other structures for facilitating microscopic examination. The prepared slides were examined under low power and oil immersion lens to characterize the measurements and morphological structures of the mould growth, concerning the conidial stage, head, vesicle, sterigmata, conidiophore and conidia.

Quantitative estimation of total aflatoxins:

The quantitative estimation of total aflatoxins (B1+B2+G1+G2) by flourometer (VICAM. Series 4) was done following the method published before⁴) with slight modifications. In brief, 25 g of ground samples with 5 g NaCl were extracted in 100 mL methanol: water (80:20) three times. The extracts were diluted 4 times with DDW and filtrated using glass microfibre filter; 4 mL filtered diluted extract passed through AflaTest®-P affinity column at a rate of about 1-2 drops/second. Elution of affinity column by passing 1.0

mL HPLC grade methanol through column at a rate of 1.2 drops/second and collecting all of the sample elute (1 mL) in a glass cuvette. Then add 1.0 mL of AflaTest® Developer to elute in the cuvette. Mixing well and place cuvette in a calibrated fluorometer. Reading of aflatoxin concentration after 60 seconds. The detection limit from 0.1 ppb to 300 ppb.

Statistical analysis:

Statistical significance was evaluated using the Tukey–Kramer HSD test (JMP statistical package; SAS Institute Inc., Cary, NC). In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results and Discussion

Inadequate freezing of chicken meat-cuts and giblets may result in mould growth, especially, if the initial microbial load is high. Thus, investigating the mould contamination and the resultant aflatoxin production is a matter of importance for public health.

In this study, the incidence of mould contamination of frozen chicken meat-cuts and giblets distributed in Zagazig city, Egypt was done. The obtained results revealed that frozen gizzards had significantly higher TMC followed by frozen livers, breast and thigh. The recorded mean \pm SD (Log cfu/g) values in such samples were 3.60 ± 0.14 , 3.47 ± 0.32 , 3.17 ± 0.11 , 2.69 ± 0.12 respectively (Figure 1).

In line with these results, Iacumin *et al.*⁶⁾ recorded a mould contamination in 41% of the examined sausage samples collected from different cities in Italy. However, the level of mould contamination recorded in this study was much lower than the level recorded by Ismail and Zaky⁷⁾ who recorded 100% mould contamination in luncheon samples collected from Assiut city markets, Egypt.

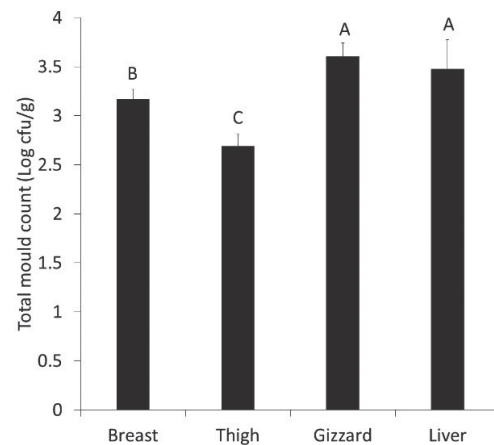


Figure 1. Mould contamination of different frozen chicken meat samples

Total mould count in the examined frozen chicken meat-cuts and giblets. Values represent means \pm SD (Log cfu/g) of twenty samples for each product and each sample was measured in duplicates. Columns with different letters differ significantly at $P < 0.05$.

Mould contamination of frozen chicken meat samples in this study indicates inadequate sanitary measures performed during processing of such products leading to increase of the initial microbial load. Additionally, inadequate storage conditions like fluctuation in freezing temperature may lead to high mould contamination of such products. The conditions of the environment in the manufacturing rooms, stores, refrigerators and shops are very suitable for the development of moulds inside the products, but more frequently on the surface of various sorts of meat and meat products⁹⁾. Serious contamination takes place from soil and water, raw meat becomes contaminated from meat contact surfaces, equipment, utensils, handling by workers and during transportation¹²⁾.

Four mould genera were identified in this study, namely, *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria*. Frozen chicken gizzards and livers had the highest incidence percentages of *Aspergillus* (90% & 80%), *Penicillium* (75% & 60%) and *Alternaria* (25% & 10%), while breast and thigh samples had the highest incidence of

Cladosporium (25% each) (Figure 2). This finding goes in agreement with Pitt and Hocking¹⁰, who reported that, the common isolated mould genera from retail meats are *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Mucor* and *Rhizopus*. Additionally, Iacumin *et al.*⁶, mentioned that *Penicillium* and *Aspergillus* were the mainly isolated moulds from sausage marketed in Italy. Furthermore, Saccomori *et al.*¹¹ observed high growth of *Penicillium polonicum* and *Penicillium glabrum* in frozen chicken nuggets.

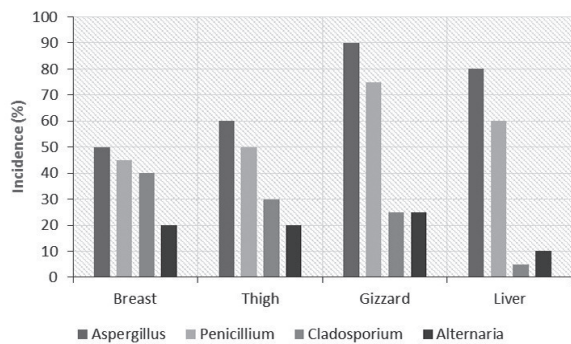


Figure 2. Incidence (%) of different mould genera isolated from frozen chicken meat samples

Penicillium and *Aspergillus* can grow over a wide range of pH from 2 to 11; over a water activity value ranges from 0.620 to 0.995; over a temperature ranges from -10 to around 60°C and over a wide range of nutrient limitations¹⁰, these reasons may explain the frequent isolation of these two moulds from frozen chicken meat-cuts in Egypt and other countries.

We further identified the different isolated *Aspergilli*. *A. niger*, *A. flavus*, *A. parasiticus*, and *A. versicolor* were the dominant *Aspergillus* species as clear in figure 3. This finding goes partially in correspondence with Ismail and Zaky⁷) and Mizakova *et al.*⁹) who could isolate these *Aspergilli* from luncheon in Egypt and fermented meat products in Slovakia.

Generally, mould growth may introduce a meat product to consumers containing aflatoxins, ochratoxin and other mould metabolites like antibiotics and allergens, which represent a

potential health hazard to consumers too. Thus, total aflatoxin residues were further estimated in this study as clear in figure 4.

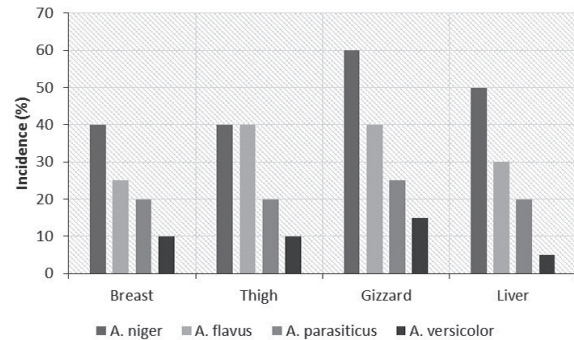


Figure 3. Incidence (%) of different Aspergilli isolated and identified from examined frozen chicken meat samples

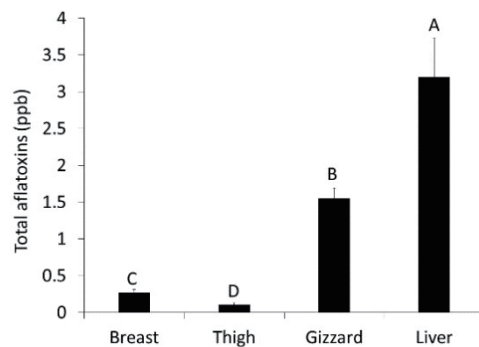


Figure 4. Total aflatoxin residues in frozen chicken meat-cuts and giblets

Total aflatoxin residues in the examined frozen chicken meat-cuts and giblets. Values represent means \pm SD (ppb) of twenty samples for each product and each sample was measured in duplicates. Columns with different letters differ significantly at $P < 0.05$.

Parallel to mould contamination level, frozen livers had the highest aflatoxin residues followed by frozen gizzards, breast and thigh-cuts. The estimated concentrations were nearly matching with Abd-Elghany and Sallam²). Despite of being below the maximum permissible limits (20ppb)⁵, aflatoxins are known as dietary human carcinogens of fungal origin and have a well-documented genotoxic effects³). Thus, contaminated samples should not be consumed and strict precautions should be given during freezing of chicken meat

and giblets to avoid initial high microbial load. In addition, chicken feed should be investigated for their mycotoxin contents.

Conclusions

Mould contamination of meat products marketed in Egypt was clearly observed in this study indicating unsatisfactory hygienic measures adopted during processing and freezing of chicken meat-cuts and giblets. The prevalent mould species were *Aspergillus* and *Penicillium*. The growing moulds may represent potential health hazards for consumers. Thus, strict hygienic measures should be followed during the processing and preservation of chicken meat.

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