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# FATE OF OCHRATOXIN A IN BISCUITS PREPARED FROM MIXTURES CONTAINING BARLEY OR BARLEY MALT FLOUR

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## ABSTRACT

Naturally contaminated barley grains with fungi producing ochratoxin A (OTA) were used in this study. Seven samples were examined to find out the effect of malting process on the level of ochratoxin A in barley grains by high performance liquid chromatography (HPLC). The samples represented the whole stages (4 stages) of malting process starting with cleaned barley grains and ending with dried malt. Then 10 mixtures of wheat flour with barley or malt or carmeled malt flour were used in different rates to make biscuits. 14 samples of raw materials used and biscuits made of it were analyzed to follow up the concentration of OTA in the final product. The results showed that malting process significantly affected the level of OTA in barley grains, where OTA concentration decreased from 0.9 to 0.0 μg/Kg in the malt. The results also revealed that in spite that the samples of raw materials had OTA in concentrations of 0.900, 0.026 and 0.846 μg/Kg for barley, malt and carmeled malt, respectively, the biscuit samples prepared from each of them had no OTA. It is worth noting to mention that, the isolated fungi capable to accumulate OTA in barley grains and malt were *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergillus niger* in this study.

**Key words:** Barley, Ochratoxin A, Malting, Carmeled malt, Biscuits, *Aspergillus ochraceus.*

## INTRODUCTION

Mycotoxin term is derived from the Greek word "*mykes*" which means fungus and Latin word "toxicum" which means poison (Egmond, 1995).

Mycotoxins are by-products of mould metabolism that produce toxic reactions known as "mycotoxicoses" upon inhalation or consumption by humans or animals. The toxic metabolites appear in mould after the maximum growth can be reached. Hence, the mycotoxicoses differs from the infectious fungal diseases called mycoses (*e.g*., blastomycosis, histoplasmosis, and coccidioidomycosis) which require fungal replication within the host to produce disease (Gloag, 1981).

Ochratoxins were first isolated and characterized by Van Der Merue *et al.* in 1965 during a routine laboratory screening tests designed in South Africa to detect fungal products in foodstuffs. Ochratoxins A, B and C are toxins predominantly produced by *Aspergillus ochraceus*, *Penicillium viridicatum* and other *Penicillium* species.

Ochratoxin A (OTA), the most common of the ochratoxins is a mycotoxin of considerable concern for human health and is classified as a possible genotoxic human carcinogen. It has been shown to be hepatoxic,

nephrotoxic, teratogenic, carcinogenic and immunosuppressive to animals and possibly to humans (Kuiper–Goodman and Scott, 1989).

OTA residues in foods of animal origin were higher in kidneys from pigs (2-100 μg/kg) and lower levels were found in liver, muscles, blood and adipose tissue. OTA residues in poultry were 19 μg/kg and 1.5 : 2.5 μg/kg in kidneys and liver, respectively, with lower residues in blood. OTA was not detected in eggs (Egmond *et al.*, 1994).

OTA has been known as intermittent contaminant of stored cereals, cereal products and coffee beans for 30 years (Trucksess *et al.*, 1999). Cereals normally account for 50-80% of average consumer intake of OTA. The Kuiper–Goodman and Scott review in 1989, estimated the safe level for daily intake at 1.2–5.7 ng/Kg of body weight. This somewhat lower than the levels estimated as safe by the World Health Organization's Joint FAO/WHO Expert Committee on Food Additives. That study suggested 16 ng/Kg of body weight per day as an acceptable intake (WHO, 1991), while the Nordic Countries Working Group (1991) proposed a figure of 5 ng/Kg of body weight per day.

Malting barley are generally carried out at moisture levels of less than 16% to safeguard the germination potential. Under these conditions, growth of *Penicillium verrucosum* is extremely unlikely. Surveys by Brewing International Research (BIR) suggest that OTA is normally undetectable in good-quality malting barley, while malts generally contain less than 0.5 μg/kg. Consequently, prevention of OTA formation by specific moulds in cereals would have a significant impact on levels of human exposure (Baxter *et al*., 2001).

The aim of this investigation is to study the effect of malting process on the level of OTA in malting barley and follow up the fate of the toxin in one of cereal products (biscuit) which contains this malt to be sure that this product is safe for human health.

## MATERIALS AND METHODS

**Materials:**

Barley and malt samples were collected from Sakha, El-Sharkia regions and Al-Ahram for Beverages Co., Cairo. Wheat flour 72% extraction rate was purchased from Bread Training Center, Cairo, Egypt. Other materials used for making biscuits, *e.g*., baking powder, sugar, etc. were obtained from Mansoura local markets. Ochratoxin A standard and Czapek broth medium were of Sigma products, so was Potato Dextrose Agar medium (PDA).

**Methods:**

**1- Isolation and identification of fungi associated with barley and malt samples:**

Samples of barley and malt (11 samples) were immersed for two minutes in 1% sodium hypochlorite solution, then washed three times with sterile distilled water. The grains were then blotted between folds of sterile filter paper and sand in petri dishes (11 cm-in diameter) onto the surface of

potato dextrose agar medium (PDA). The medium was supplemented with streptomycin sulphate (250 mg/L) for bacterial elimination. The plated samples were incubated at 27°C for 5-7 days. By using the dissecting microscope, conidia were picked up with the tip of an agar-moistened sterile loop and carefully streaked across the surface of plain agar plates and individually transferred to PDA. The fungal isolates were identified locally according to Samson *et al*. (1981), Botton *et al*. (1985), and Pitt and Hocking (1997).

**2- Determination of OTA by high performance liquid chromatography (HPLC):**

OTA was determined by using HPLC technique according to the method of A.O.A.C. (1995). Waters 600 E HPLC system was used, supplied with 474 waters fluorescence detector set at 333 and 460 nm as excitation and emission wave lengths. $C_{18}$ Nucleosil column 250 x 4 mm (Macherey Naggel Co., Germany) was used. Water + acetonitrile + acetic acid (99 + 99 + 1) were used as mobile phase at flow rate 1 ml / min. OTA analysis was carried out in the National Research Centre (NRC), Dokki, Giza, Egypt.

**3- Effect of malting process on the level of OTA in barley grains:**

Malting process begins with cleaned barley grains, which are then steeped in water for three days at 16°C to increase the moisture content to 42-46%, then the grains are allowed to germinate for two days at 13-16°C. Finally the grains dried at 45-60°C. Carmeled malt was heated at 120°C. The above samples were obtained from Al-Ahram for Beverages Co., Cairo, Egypt.

**4- Fate of OTA in raw materials and biscuit samples:**

The mixtures of wheat flour with barley or malt or carmeled malt flour were used to make biscuits in different rates. All samples were examined for OTA existence in biscuit samples after baking in addition to the raw materials samples.

## RESULTS AND DISCUSSION

**Fungi producing OTA in barley and malt:**

Eleven samples of fungal isolates from barley and malt were examined for detection of fungi producing OTA cultivated on Czapek broth medium. From Table (1), it could be concluded that species of *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium verrucosum* were capable to produce OTA, while the other fungal isolates didn't. The presented data in Table (1) revealed that OTA was detected in 27.27% (3 out of 11) of the total samples analysed. Those results agree with many investigators (Szebiotko *et al*., 1981; Pitt *et al.*, 1987; Cretnic and Pepeljnjak, 1990; Skrinjar and Dimic, 1992; Abarca *et al*., 1994; Krivobok *et al*., 1995 and Moss, 1996).

**Natural occurrence of ochratoxin A (OTA) in barley, malt and carmeled malt:**

It is obvious from Table (2) that the OTA levels in the examined samples were ranged from 0.0 to 0.900 in barley, 0.0 to 0.026 in malt and 0.864 μg/kg in carmeled malt. However, the presented data also revealed that OTA was detected in 50% (6 out of 12) of the total samples analyzed.

**Table (1): Ability of OTA production by fungi isolated from barley and malt.**

| No. | Fungi | OTA | No. | Fungi | OTA |
|---|---|---|---|---|---|
| 1- | *Alternaria alternata* | N.D | 7- | *Fusarium* sp. | N.D |
| 2- | *Alternaria ndilans* | N.D | 8- | *Mucor* sp. | N.D |
| 3- | *Aspergillus flavus* | N.D | 9- | *Penicillium verrucosum* | D |
| 4- | *Aspergillus niger* | D | 10- | *Rhizopus* sp. | N.D |
| 5- | *Aspergillus ochraceus* | D | 11- | *Slachybotrys* sp. | N.D |
| 6- | *Cladosporium* sp. | N.D. | | | |

**D = detected by TLC.**
**N.D = not detected by TLC.**
**Medium used is Czapek broth.**

On studying the results shown in Table (2), it is observed that barley samples collected from Sakha region had low concentrations of OTA (0.0 to 0.040 μg/kg) comparing with sample taken from Al-Ahram for beverages Co., which had 0.900 μg/kg. The lowest level of OTA was in El-Sharkiya region sample (0.024 μg/kg).

It is clear that the low levels of OTA detected in the present investigation coincides with those given by Gareis (1999) being 0.035 μg/kg. This referred to fresh barley grains used immediately after harvest in addition to good transport and handling conditions (Vasanti and Ramesh, 2000). On the other hand, the samples obtained from Al-Ahram for beverages Co. were stored for one year.

**Table (2): Levels of OTA in natural contaminated barley, malt and carmeled malt.**

| Samples | Barley | | | | | | | | Malt | | | Carm-eled malt Al-Ahram for bevera ge Co. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sakha region | | | | | | El-Sharkia region | Al-Ahram for beverage Co. | Abou Hammad (Sharkia) | Al-Ahram for beverage Co. | | |
| | Giza 123 | Giza 124 | Giza 125 | Giza 126 | Giza 128 | Bweet | | | | Type 1 | Type 2 | |
| OTA Conc. (μg/kg) | 0.040 | N.D. | 0.037 | N.D. | N.D. | N.D. | 0.024 | 0.900 | N.D. | 0.026 | N.D. | 0.864 |

**N.D. = Not detected by HPLC**

In this regard, Szebiotko *et al*. (1981) found that two months after harvest, the percentage of contaminated samples was 5-7% and OTA content was not higher than 140 μg/kg. While, after one year of grain storage

with high moisture content showed increase of contamination level was (1-3 mg/kg).

As for malt and carmeled malt samples, the data given in Table (2) showed that the levels of OTA decreased in malt samples. It ranged from 0.0 to 0.026 μg/kg. This result is ordinary according to many investigations showed that malting process reduced the level of OTA of barley grains from 0.04 to 0.0 and from 0.90 to 0.026 (Baxter, 1996).

The surprising observation was for the carmeled malt sample, which showed high level of OTA (0.864 μg/kg) in Table (2). But, this could be simply elucidated where the carmeled malt was stored for one year in Al-Ahram for beverages Co. and the effect of storage conditions was illustrated before.

**Effect of malting process on the level of OTA in barley grains:**

Data given in Table (3) showed that malting process (4 stages) affecting the level of OTA in barley malt (specially the steeping stage). The experiment began with cleaned barley grains in which OTA level was 0.900 μg/kg. Then the grains were steeped in water for three days at 16°C.

After steeping for one day the level of OTA decreased to be 0.785 μg/kg. Two days steeping in water resulted in a big drop in OTA level to become 0.053 μg/kg. After the final stage of steeping, OTA level became 0.014 μg/kg as shown in Table (3). This means that the steeping stage during malting causes loss in OTA level equal 98.4% in the examined samples.

About this point, Baxter (1996) found that during trials using malting barley inoculated with *Penicillium verrucosum* to give high levels of OTA, greater than 90% of OTA was demonstrated to be lost during steeping alone (the initial stage of malting).

In another investigation, no OTA was found in malt processed on a laboratory scale from naturally contaminated barley containing 420 or 830 ng/g. Only traces of OTA (8-31 ng/g) were present in malt made from sound barley inoculated at steeping with 2 strains of *Penicillium viridicatum*, and none was found when *Aspergillus ochraceus* was used by Scott (1996).

From Table (3), it is observed that no OTA was detected in the next stage of malting where the grains were germinated for two days at 13 : 16°C. So, no OTA was found in dried malt (the final stage).

**Table (3): Effect of malting process on OTA level in barley.**

| No. | Stages of malting process | Samples | OTA concentration (μg/kg) |
|---|---|---|---|
| 1 | Cleaning | Cleaned grains | 0.900 |
| 2 | Steeping in water | Steeped for one day | 0.785 |
| 3 | | Steeped for two days | 0.053 |
| 4 | | Steeped for three days | 0.014 |
| 5 | Germination | Germinated for one day | N.D |
| 6 | | Germinated for two days | N.D |
| 7 | Drying | Dried malt | N.D |

**N.D. : Not detected by HPLC.**

**Fat of OTA in raw materials and biscuits prepared from them:**

Raw materials of wheat, barley, malt and carmeled malt flour were analyzed for their OTA content. Results were expressed as μg/kg. The obtained data presented in Table (4).

Based on the data in Table (4) it appears that barley, malt and crameled malt flour had different amounts of OTA 0.900, 0.026 and 0.864 μg/kg, respectively, while wheat flour hadn't any amount of OTA.

From the same Table, it can be seen that the biscuit samples made from wheat flour and its mixtures with barley or malt or carmeled malt flour were analysed for their OTA content after backing. The results in Table (4) show that the biscuit samples were safe from OTA.

Osborne (1979) showed that OTA was not degraded during bread making (220°C for 25 min), whereas 62% of the toxin were destroyed after baking of biscuits (180°C for 5.5 min), which have a much lower water content than bread.

Table (4): Fate of OTA of raw materials and biscuit samples.

| No. | Samples | | OTA Concentration μg/kg |
|---|---|---|---|
| 1 | Raw materials | Wheat flour (72% ext.) | N.D. |
| 2 | | Barley flour | 0.900 |
| 3 | | Malt flour | 0.026 |
| 4 | | Carmeled malt flour | 0.864 |
| 1 | Biscuit samples (after baking): | 100% wheat flour. | N.D |
| 2 | | 90% wheat flour + 10% barley flour | N.D |
| 3 | | 85% wheat flour + 15% barley flour | N.D |
| 4 | | 80% wheat flour + 20% barley flour | N.D |
| 5 | | 95% wheat flour + 5% malt flour | N.D |
| 6 | | 90% wheat flour + 10% malt flour | N.D |
| 7 | | 85% wheat flour + 15% malt flour | N.D |
| 8 | | 97.5% wheat flour + 2.5% carmeled malt flour | N.D |
| 9 | | 95.0% wheat flour + 5.0% carmeled malt flour | N.D |
| 10 | | 92.5% wheat flour + 7.5% carmeled malt flour | N.D |

**N.D.: Not detected by HPLC.**

## CONCLUSION

In the light of data presented in this study, the malting process significantly reduced the natural occurred OTA content in barley grains.

This fact directs our attention to a very important point, which indicates that the malt used in several food manufactures is prepared from barley without serious OTA contamination, and that foods don't present a health hazard from OTA. This conception coincides with Guldberg (1997).

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## وجود أوكراتوكسين (أ) فى البسكويت المحضر من خلطات تحتوى على دقيق الشعير أو المولت

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**قسم الصناعات الغذائية – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .**

استخدمت فى هذه الدراسة حبوب شعير ملوثة طبيعياً بالفطريات المنتجة للأوكراتوكسين (أ) ، وقد تم إختبار عدد ٧ عينات لمعرفة تأثير عملية تحضير المولت على مستوى أوكراتوكسين (أ) فى حبوب الشعير بإستخدام جهاز (HPLC) . هذه العينات تمثل المراحل المختلفة لعملية تحضير المولت ( ٤ مراحل ) بدءا من حبوب الشعير بعد عملية التنظيف وإنتهاء بالمولت المجفف . بعد ذلك تم إستخدام ١٠ خلطات من دقيق القمح ودقيق الشعير أو المولت أو المولت المكرمل بنسب مختلفة لعمل البسكويت . وقد تم تحليل ١٤ عينة تمثل المواد الخام السابق ذكرها وعينات البسكويت المحضر منها لتتبع تركيز الأوكراتوكسين فى الناتج النهائى . وقد أظهرت النتائج المتحصل عليها أن عملية تحضير المولت قد أثرت تأثيراً معنوياً على مستوى أوكراتوكسين (أ) فى حبوب الشعير المستخدمة فى إنتاج المولت حيث إنخفض معدل أوكراتوكسين (أ) من ٠,٩ إلى صفر ميكروجرام / كجم فى الحبوب . كما أظهرت النتائج أيضاً أنه بالرغم من وجود أوكراتوكسين (أ) فى المواد الخام المستخدمة فى صناعة البسكويت بتركيزات ٠,٩ و ٠,٠٢٦ و ٠,٨٤٦ ميكروجرام / كجم فى الشعير والمولت والمولت المكرمل ، على الترتيب ، إلا أن عينات البسكويت المحضرة من كل منهم أصبحت خالية من أوكراتوكسين (أ) . ومن الملاحظات التى تستحق الذكر فى هذه الدراسة أن الفطريات التى تم عزلها ولها القدرة على إنتاج أوكراتوكسين (أ) فى حبوب الشعير والمولت هى و *Penicillium verrocosum* و *Aspergillus ochraceus* و *Aspergillus niger* .