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EFFECTS OF PAN BREAD MAKING ON ZEARALENONE LEVELS IN ARTIFICIAL CONTAMINATED WHEAT FLOUR

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The ability of *Saccharomyces cerevisiae* (Baker's yeast) in reduces of zearalenone during fermenting dough at 25°C and 30°C for 30 and 45 min was studied. The results indicate that content of zearalenone was reduced to 9.44, 19.22, 37.6 and 63.16µg/kg in dough made from flour artificially contaminated with 25, 50, 75 and 100 µg/kg, respectively when the fermentation process at 25°C for 45 min. On the other hand, during fermentation at 30°C the decrease in the content of zearalenone is greater than ever, particularly with increasing duration or time of fermentation to 45 min. The content of zearalenone after fermentation at 30°C for 45 min was 7.52, 17.14, 34.19 and 60.38µg/kg in dough made from flour artificially contaminated with 25, 50, 75 and 100µg/kg, respectively. The reduction percentage of zearalenone after baking pan bread at 180°C for 25 min was 55.98, 62.78, 62.5 and 60.52%.These results indicate that fermentation and baking are effective in reducing zearalenone.

Key words: Zearalenone, *Saccharomyces cerevisiae*, fermentation and baking

Introduction

Zearalenone (ZEA) is a mycotoxin mainly produced by *Fusarium graminearum* and *Fusarium culmorum*, which are common soil fungi in temperate and warm countries, and are regular contaminants of cereal crops worldwide [1, 2]. ZEA is mainly with corn and wheat but it occurs also in barley and sorghum amongst other commodities frequently used in human and animal diets. The International Agency for Research on Cancer (IARC) has categorized ZEA as a class 2A carcinogen [3]. ZEA produces estrogenic effects in humans and animals leading hyperestrogenism. Hyperestrogenism may be manifested enlargement of the reproductive organs. ZEA can cause severe reproductive and infertility problems in farm animals, particularly in swine [4]. Specifically, ZEA can act as an estrogen analog and cause alterations in the uterus of the reproductive tract of swine and affect follicular and embryo development [5]. The function of ZEA as an estrogen analog is important because swine are monogastric animals with responses similar to humans in many respects [6]. Even though most African countries have a climate characterized by high humidity and high temperature which favor growth of moulds, little information is available on the occurrence of *Fusarium* toxins particularly ZEA in foods and feeds. High contaminations of the raw materials are an ongoing problem. Regulatory issues are not available in the field of food exhibition and retailing, and mycotoxin problems have already been associated with some food contamination in some areas in Africa [2]. In Egypt, several commodities were reported to contain ZEA especially corn, wheat and rice and walnut [7, 8]. Corn from Egypt was also found contaminated with high levels of ZEA that ranged from 9.8 to 38.4 mg/kg [9]. According to FAO [10], ZEA was regulated in 1996 by 6 countries, but by the year 2003 the toxin ZEA was regulated in foods and animal feeds by 16 countries. Limits for ZEA in maize and other cereals, currently vary from 50 to 1000 µg/kg. Although various prevention strategies have been implemented in many countries, they were not enough to manage the problem efficiently in the developing world. In the recent years, many dietary strategies involving microorganisms have been under investigation. Many species of bacteria and fungi have been shown to enzymatically degrade mycotoxins [11]. However, the question still remains on the toxicity of the degradation products. *Saccharomyces cerevisiae* has been utilized in food fermentation for several centuries. In the African and Asian countries, fermentation is used as a predominant mode of food processing and preservation. Many yeast species, especially *Saccharomyces cerevisiae*, play a predominant role in food fermentation along with lactic acid bacteria [12]. In the African fermented foods such as fermented maize dough (Kenkey) as well as sorghum beer (Pito), *Saccharomyces cerevisiae* constitutes a predominant component of fermentation microflora and the biodiversity of yeast microflora in these foods has been well documented [13]. Mycotoxin binding by *Saccharomyces cerevisiae* and lactic acid bacteria have been reviewed recently [14]. The aim of this work was to study the ability of *Saccharomyces cerevisiae* in reduce of ZEA during fermenting dough at 25°C and 30°C for 30 and 45 min, also study effect of baking Pan bread at 180°C on content of ZEA.

Materials and method:

Materials

Wheat flours (72% extraction) were obtained from the North Cairo Mills Company, Egypt. *Saccharomyces cerevisiae* (Baker's yeast) was obtained from Al-Hawamdia Company fresh comprised yeast according to the Egyptian standard (191/2005).

Methods

Preparation of artificial contaminated wheat flour

One milligram of ZEA was purchased from Sigma, chemical Co. (St. Louis, MO, U.S.A). ZEA was dissolved in 1 ml methanol. Then taken appropriate size of focus and complemented the larger size makes it easier to control added to wheat flour. Four wheat flour samples (1kg each) were aged in various concentrations (25, 50, 75 and 100µg/kg). Each of the contaminated wheat flour samples was used for preparation of Pan Bread. This mixture was stirred until the solvent had evaporated.

Pan bread making

Pan bread was prepared by mixing 100g of wheat flour (72% extraction) with 5 g of Baker's yeast, 1.5 g of sodium chloride and 75–80 ml of water and hand blended for about 6 min to form the dough. The dough was lift to rest for 30 min at 25°C & 30°C and 85% relative humidity and was then divided into 150g pieces. The pieces were booted in metal pans that had been left to ferment for about 45 min at the same temperatures and relative humidity according to AACC [15]. And then were baked at 180°C for 25 min the resulted pan bread was allowed to cool at room temperature for 2 h before being packed in polyethylene bags and stored at room temperature for determination of ZEA.

Extraction procedure

Twenty grams of sample was weighted into a 250mL blender jar with 2 g NaCl and extracted with 50mL of extraction solution (acetonitrile: water 90:10 v/v). The mixture was blended for 2 min and centrifuged for 3 min at 3,000 rpm.

The extract was filtered through filter paper (Whatman No.1). 10 ml filtrate was mixed with 40 ml water, followed by filtration through a glass microfibre filter.

Immunoaffinity Clean-up

Ten milliliter of filtrate was passed through Zeara-latest™ immunoaffinity columns at about 1-2 drops/s. 10ml water was used to wash the loaded immunoaffinity column at a steady flow rate. ZEA was eluted with 1 ml of methanol. The methanol elute was filtered through a 0.45µm micro filter and collected in a clean vial for the following HPLC.

HPLC/fluorescence Analysis

The HPLC system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Watres 2475 Multi- Wavelength Fluorescence Detector. The fluorescence detector was operated at wavelength of 274 nm for excision and 440 nm for emission, and a data workstation with software Breeze 2. A phenomenex C_{18} (250 X 4.6 mm i.d.), 5 µm from waters corporation (USA). The mobile phase consisted of a mixture of acetonitrile: water: methanol (48:50:3, v/v/v). A 20 µl of the reconstituted extract was injected onto the column at a flow rate of 1.0 ml/min. ZEA content in samples was calculated from chromatographic peak areas using the standard curve.

Statistical analysis

The statistical analysis was performed using the SPSS software program (SPSS Institute, 2011, version 19.0) and the differences in ZEN concentrations between time and temperatures dough fermentation were analyzed by ANOVA test. $P \leq 0.05$ was considered to be statistically significant.

Results and discussion

Influence of manufacture process of Pan Bread on content of ZEA:

Effect of dough fermentation

The effect of using *Saccharomyces cerevisiae* (Baker's yeast)on content of ZEA during fermentation of artificially contaminated wheat flour dough at 25°C and 30°C for 30 and 45 min was presented in *Table 1 and 2*. The content of ZEA was reduced to 13.71, 24.18, 43.88 and 71.43µg/kg in dough made from flour artificially contaminated with 25, 50, 75 and 100 µg/kg, respectively when the fermentation process at 25°C for 30 min. While decreased the content of ZEA more with more time fermenting dough to 45 min. On the other hand, during fermentation at 30°C the decrease in the content of ZEA is greater than ever, particularly with increasing duration or time of fermentation to 45 min. The content of ZEA after fermentation at 30°C for 45 min was 7.52, 17.14, 34.19 and 60.38µg/kg in dough made from flour artificially contaminated with 25, 50, 75 and 100µg/kg, respectively.

Low incubation temperature (25°C) during the growth of the cells seems to adversely affect the binding and the optimal and above-optimal growth temperature did not affect the binding. Temperature is known to affect the cell wall composition with lower temperature resulting in reduced cell growth, cell wall dry mass, mannan and β-glucan levels [16]. Culture conditions including pH, temperature, oxygenation rate, nature of the medium, and concentration or nature of the carbon

source strongly modulate the quantity and structural properties of β-D-glucans, mannans and chitin in cell walls. Moreover, the cell cycle stage also interacts with the cell wall composition. For example, budding induces strong changes in the distribution of the structural components of the cell wall such as chitin. Changes in the cell wall components at low growth temperatures might have resulted in reduced binding molecules on the cell surface.

Cell wall of *Saccharomyces cerevisiae* consists of network of β-1,3 glucan back bone with β-1,6 glucan side chains, which is in turn attached to highly glycosylated mannoproteins which make the external layer [17].

The proteins and glucans provide numerous easily accessible binding sites with different binding mechanisms such as hydrogen bonding, ionic or hydrophobic interactions [18]. Binding of different mycotoxins such as aflatoxin, ochratoxin and ZEA to yeast cell surface has been reported earlier and the binding has been attributed to cell wall glucans in case of ochratoxin and ZEA [19]. β-D-glucans are the yeast component responsible for the complexation of ZEA, and that the reticular organization of β-D-glucans and the distribution between β-(1, 3)-D-glucans and β-(1, 6)-D-glucans play a major role in the efficacy and add at weak hydrogen and van der Waals bonds are involved in the chemical complex formation between ZEN and β-D-glucans. Thus, the chemical interaction is more of "adsorption type" than "binding type". Molecular modeling was performed to validate the concept on other mycotoxins such as aflatoxin B_1, deoxynivalenol and patulin [20].

The reduction percentage of ZEA after fermentation dough at 30°C for 30 min was only 58.8, 55.1, 46.98 and 34.42%, while it was 69.9, 65.72, 54.41 and 39.62% after fermentation dough for 45 min from flour artificially contaminated with 25, 50, 75 and 100µg/g, respectively. The percentage of ZEA reduction increased with time of fermentation dough shown in *(Figure 1)*. There was observed relationship between the degree of reduction and the initial level of ZEA contamination during fermentation dough . And, as it was expected, a positive relationship was observed between the increase of fermentation time and that of the percentage of reduction on ZEA-contaminated wheat flour

Table 1: Concentrations of ZEA determine in artificially contaminated wheat flour after dough fermentation at 25°C (n=3)

Time of fermentation dough (min)	Spiking level of ZEA (µg/kg)			
	25	50	75	100
After mixed directly	23.26 ±0.34	46.98 ±0.4	73.71 ±0.12	96.45 ±0.17
30	13.71 ±0.46	24.18 ±0.25	43.88 ±0.52	71.43 ±0.39
45	9.44 ±0.18	19.22 ±0.44	37.6 ±0.41	63.16 ±0.51

Table 2: Concentrations of ZEA determine in artificially contaminated wheat flour after dough fermentation at 30°C (n=3)

Time of fermentation dough(min)	Spiking level of ZEA (µg/kg)			
	25	50	75	100
After mixed directly	23.26 ±0.34	46.98± 0.4	73.71 ±0.12	96.45± 0.17
30	10.3 ±0.26	22.45± 0.28	39.76 ±0.71	65.58± 0.73
45	7.52 ±0.1	17.14± 0.19	34.19 ±0.38	60.38± 0.92

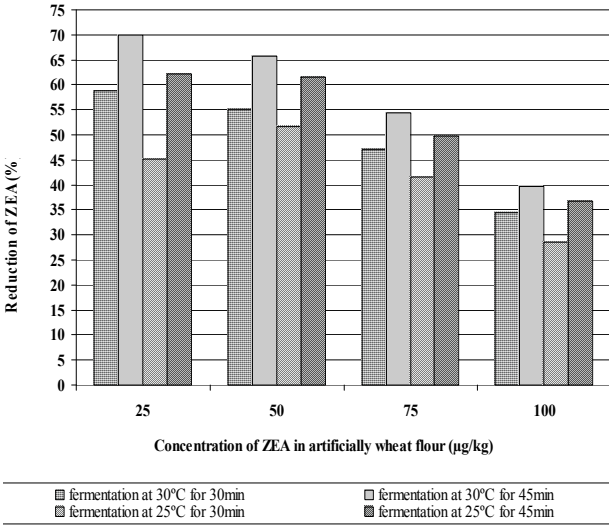

Figure 1: The percentages of ZEA reduction during fermented dough

Effect of baking

ZEA concentration as affected by baking of artificially contaminated flour dough during Pan bread making is shown in *Table 3* and *Figure 2*. The residual amount of ZEA was 3.31, 6.38, 12.82 and 23.84µg/kg after baking dough artificially contaminated with different concentration of ZEA i,e 25, 50,75 and 100µg/g, respectively. The reduction percentage of ZEA after baking pan bread at 180°C for 25 min was 55.98, 62.78, 62.5 and 60.52%.The results showed a positive correlation between the initial contamination level and the reduction of ZEA after baking. The levels of ZEA in cereal-based foods were reduced significantly by extrusion processing, and reduction of 83% of ZEA in corn-based foods was obtained with this process [21]. However, there remains a need to demonstrate that the toxicity or biological activity of ZEA has been reduced or completely eliminated in cereal-based foods using extrusion processing [22]. *Figure 3a* showed the HPLC chromatogram of ZEA standard where *Fig. 3b* showed the sample with zearalenone level of 25µg/kg after fermentation at 30 °C for 30 min. while *Fig. 3c* HPLC chromatogram of zearalenone in sample pan bread.

Table 3: Concentrations of ZEA in Pan Bread after baking at 180 °C for 25 min. (n=3)

Treatment	Spiking level of ZEA (µg/kg)			
	25	50	75	100
Fermentation dough at 30°C for 45 min	7.52 ±0.1	17.14 ±0.19	34.19 ±0.38	60.38 ±0.92
Baking at 180 °C for 25 min	3.3.1 ±0.15	6.38 ±0.29	12.82 ±0.76	23.84 ±1.6
Reduction (%)	55.98	62.78	62.5	60.52

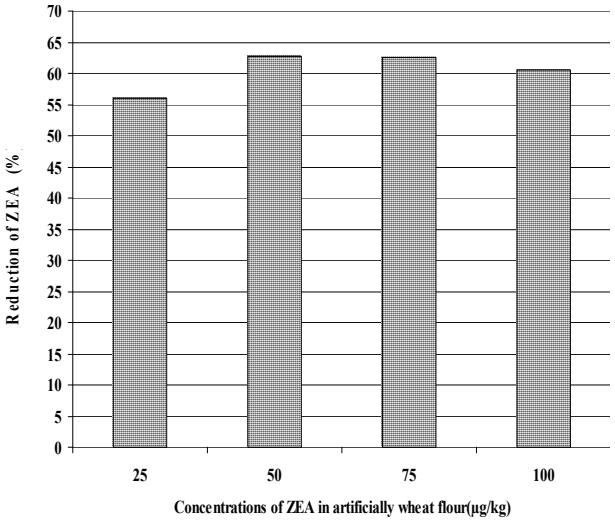

Figure 2: Effect of baking at 180 °C for 25 min on content of ZEA in dough after fermentation at 30°C for 45 min

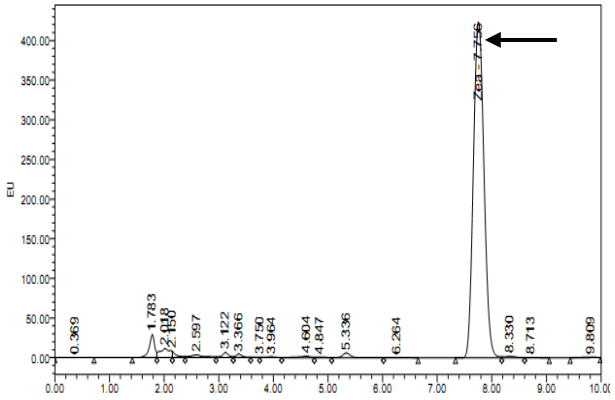

Figure 3 a: HPLC chromatogram of zearalenone standard

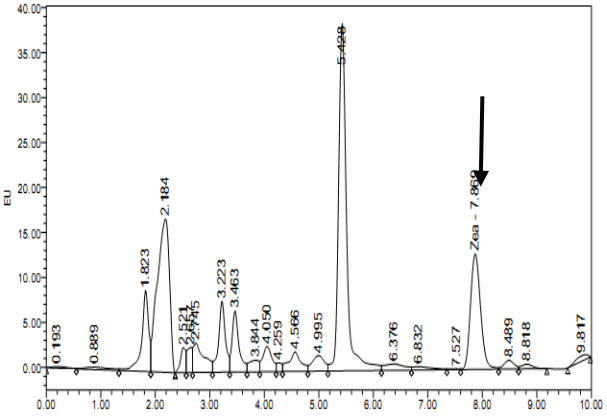

Figure 3 b: HPLC chromatogram of sample with 30°C for 45min

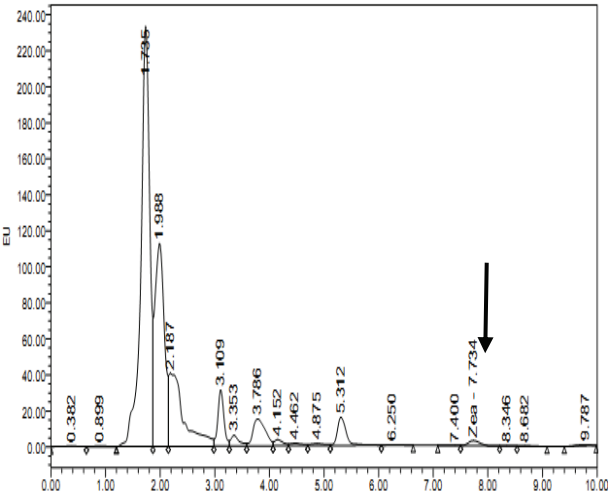

Figure 3c: HPLC chromatogram of zearalenone in sample pan bread

Conclusion

It could be concluded that the fermentation of dough at 30°C for 45 min was best into reduce the content of ZEA, Because this could be a good growth of yeast cells in the dough and is doing and its perfect. So attention must be paid to the process of fermentation and conditions of particular temperature that would influence the activity of yeast cells used. It also shows the importance of yeast cells to reduce the levels of ZEA**.** Also the results of the study show that the residual of the ZEA in the bread was within allowable limits internationally.

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