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Research Article

# Intake time safercorn tortilla by Aspergillus sp. growth in culture

Abaroa-Aguirre MF<sup>1</sup>, Sánchez-Godoy EG<sup>2</sup>, Escamilla-Violante R<sup>3</sup>, and Ruvalcaba Ledezma JC.<sup>3</sup>\*

<sup>1</sup>Nutrition Counselor (UNIVA) Atemajac Valley University, Zapopan Jalisco, México
<sup>2</sup>Professor degree in Nutrition (UNIVA) Atemajac Valley University, Zapopan Jalisco, Mexico
<sup>3</sup>Full-time Research Professor in the Academic Area of Medicine (ICSA-UAEH) Institute of Health Sciences, Autonomous Hidalgo State University, Pachuca City, Hidalgo, Mexico
\*Corresponding Author E-mail: dcspjcarlos@gmail.com

# ABSTRACT

A descriptive longitudinal study tracked 48, 72 and 96 hours, in order to determine the safest corn tortilla consumption time was made, in terms of growth of Aspergillus sp UFC results obtained from the purchase of corn tortillas tortilla from a colony with higher socioeconomic status suggest that growth of yeast and fungal microorganisms occurs, UFC / Time quantified and detected as the optimum time of consumption the first 48 hours of purchase these, the fastest growing 72-92 hours incubation, the growth apparently UFC synergy fungi which may be involved as a result of the same reproduction of the fungus occurs. So the presence of fungi in this genus is related to the generation of aflatoxins under conditions where crops potato dextrose was made in agar at 25 degrees Celsius where the substrate and temperature play a decisive role for growth fungus.

Keywords: Aspergillus sp, yeast, corn tortilla, potato dextroseagar.

## **INTRODUCTION**

In 1960, the death of thousands of poults was registered in England; such event happened a week after the first symptoms appeared. Necropsies revealed hemorrhages and necrotic areas in the poults liver; later the correlation between the death of the turkeys and their consumption of peanut from Brazil was established. It is worth saying that such peanuts were invaded by *Aspergillus flavus*<sup>4</sup>. Aflatoxins are secondary metabolites produced by the fungi *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*<sup>25,31</sup>. It is known that they generate mycotoxins capable of causing carcinogenic, mutagenic or teratogenic damage<sup>23,25,31</sup>.

Those have been detected in corn<sup>25</sup>, peanut products and grains in general<sup>31</sup>. Aflatoxins; B1 (AFB1), B2 (AFB2), M (AFM), G1 (AFG1) and G2 (AFG2). The most important is the AFB1 regarding its toxigenic degree<sup>23,25,31</sup>. It was possible to detect the AFM as one of the secondary metabolites of AFB1 in blood and urine<sup>15</sup> and in cord blood<sup>16,25</sup>. It is certain that these aflatoxins have causal association with liver cancer<sup>7,10</sup>.

Aflatoxin contamination of corn occurs during cultivation and storage. Aflatoxin B (blue fluorescence) and G (green fluorescence) occur when Aspergillus flavus and / or Aspergillus parasiticus grow in various foods that serve as ideal for this substrate. Usually these foods are basic grains such as corn, wheat, rice, beans, sorghum, barley, oilseeds (walnuts), and dried fruits. The aflatoxin contamination of corn represents a potential risk for the Mexican population, mainly because it is a staple food and ingested as omelette, with a consumption of  $325 \text{ g} / \text{day}^{3,18}$ .

Swallowing (AFB1) isassociated with liver toxicity in humans and farm animals. Epidemiological studies in Africa and Asia indicate that there is a positive correlation between liver cancer and consumption of food contaminated with aflatoxins<sup>3,18</sup>.

There is evidence of the effect of aflatoxins in animals, both mutagenic and carcinogenic<sup>10</sup>. We know for example that in acute outbreaks these can cause embryonic death, toxicity for the fetus, decrease eggs production and the sizes of these. In 1999, the FAO and WHO noted that food for human consumption must remain free of mycotoxins du to the fact that these can decrease productivity of ruminants and occasionally cause death<sup>9</sup>.

Several experiments have been conducted in order to diagnose the effects of aflatoxins to animals. For example, in rabbits it was found that after being administered with 100 micrograms of aflatoxins per every kg of pregnancy weight, 47% showed to have a high mortality rate. Some of the rabbits who remained alive experienced liver damage. However, those who were administered 50 micrograms per kg during 10 days had a birth weight rate of zero<sup>27</sup>. Testicular steroid genesis has also been observed in rats<sup>32</sup>. It has been observed that aflatoxins can also drastically affect trout<sup>14</sup> as well as humans; in 1974 there was a food poisoning outbreak where 397 cases were recorded and 106 died<sup>19</sup>.

Risk factors for hepatocellular carcinoma<sup>14,19,23</sup> were also detected as well as aflatoxigenic milk<sup>7,8</sup> affecting umbilical  $cords^{18,25}$  which are the result of ingesting aflatoxigenic food during pregnancy obtaining low weight at birth as an adverse effect<sup>1,14</sup> Corn and other foods such as fruit juice represent a substrate for the growth of *Aspergillus*. Corn is one of the most common foods of the Mexican population; it represents one of the fundamental means for the development of aflatoxins, including cereals<sup>20,25,26,31</sup>.

A mechanism to decrease the concentration of aflatoxins is provided by the lime, which during nixtamalization changes the pH<sup>2,28</sup>. Aflatoxins have a half-life in the human body among 2 to 3 months. The effects of aflatoxins in humans have not been studied yet in a systematic way<sup>6,32</sup>. Consumption of these toxins has been associated to some of the chronic malnutrition effects in children undergoing growth; among which we find liver and kidney damage, carcinogenesis and immunosuppression<sup>5,11,13,17</sup> wasting and stunting have also been implicated in the pathogenic process of aflatoxins<sup>12,29</sup>.

Aflatoxins have been detected in body fluids including urine and have been associated with malnutrition<sup>22,30</sup>. In the study entitled "Aflatoxins in body fluids and food of Nigerian Children with protein-energy malnutrition<sup>21</sup>" several types of aflatoxins were detected in urine samples from patients and controls in varying concentrations, which later were analyzed and showed to have high levels of those (90.9%), followed by kwashiorkor (84.6%), marasmus (81.8%) and marasmus kwashiorkor (60%), in descending order. However, the observed differences in detection rates in the urine were not significant (p> 0.05)<sup>21</sup>.

That's why this research aims to determine the time in which a corn tortilla should be consumed before contaminated.

Aflatoxins are a group of toxic substances closely related to the fungus *Aspergillus flavus* and *Aspergillus parasiticus*, which grow in peanuts, corn, soybeans, barley, wheat and other grains, particularly under hot and humid conditions. Some contamination may occur before harvest, but the major source of exposure to humans resulting from inadequate post harvest storage that facilitates the growth of the fungus.

Some important features of these toxins are its ability for bioconcentration, bioaccumulation and stability. The toxin most toxic and carcinogenic risk, is aflatoxin B1. It has been shown to cause cancer in rats, mice, hamsters, rainbow trout, ducks, woodchucks, shrews, guinea pigs, sheep and monkeys. Aflatoxin B1 causes mainly liver cancer, and is the most potent carcinogen known<sup>7,10</sup>

**Aim:** The purpose of this work is to determine the time of intakes a festcorn tortilla for growth of *Aspergillus spp UFC* in Agrapotato dextrose.

### MATERIAL AND METHODS

A longitudinal descriptive study 8 samples per week was based tracking planting 32 samples for 4 weeks, with follow-up at 48, 72 and 96 hours of planting corn tortilla potato dextrose agar incubated at 25 degrees Celsius.

# **RESULTS**

Of 4 experiments designed to track each corresponding to eight boxes tortilla 4 samples each in duplícate cultura was detected yeast-growth in 262326 100% of the culture dishes for 48, 72 and 96 hours and generally of 4 experiments in one 25% fungal growth was observed, in 75% if growth was observed. (Table 1).

#### Table1. Growth of yeast and fungi in 4 experiments to monitor the 48, 72 and 96 hours of incubation on potato dextrose agar at 25 degrees Celsius

Tracing	Experiment 1	Experiment 2	Experiment 3	Experiment4
Yeast	+	+	+	+
Aspergillus sp	-	-	+	+

Source: Direct, samples	tested in the laboratory	of nutrition UNIVA
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Average detected in yeast for 48 hours was 754 CFU, 72 hours and 96 hours 832 833 there is no significant difference in these (Table 2).

Table 2. Total	samples / week 8	(4) 32 samples to	o monitor the 48.	72 and 96 hours of y	veasts
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	48 hours	72 hours	96 hours
Yeast total	24,128	26624	26,656
Average	754	832	833

Source: Direct, samples tested in the laboratory of nutritionUNIVA

The growth of fungi Aspergillus sp corresponding to the track 48,72 and 96 hours with optimum growth appeared after 72 between 72 and 96 hours, although in tortill as you left the environment "without medium" from 3 days they are bought tortillas and generally showed whitish, slightly transparent, sticky aspect of the tortilla filaments, which was recorded in general slower growth in the culture médium was at 48 hours, although the first and second experiment showed no UFC Aspergillus, growth generally occurs after 72 hours of monitoring, 96 hours no significant difference was detected, ratherit appears as a time of stability to crops where fungus growth (Table 3) demonstrated.

Table3. TotalUFCdetected inpotato dextroseagarat 25 degreesCelsius				
Aspergillus sp	48 hours	72 hours	96 hours	

Table3. TotalUFCdetected inpotato of	dextroseagarat 25 degreesCelsius
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Aspergillus sp	48 hours	72 hours	96 hours
UFC total	0	34	36

Source: Direct, samplestested in the laboratory of nutrition UNIVA

## DISCUSSION

When wespeak of Mexican corn recalls the many dishes of our regions, that make us salivate and evoke pleasant memories and important moments of our lives always accompanied with holiday dishes prepared on the basis corn. Tortilla and related products are the products most consumed by the population, noting that among the lower socio economic level there is a greater dependence, corn provides about 70% of the calories and 50% of the daily protein intake.

The tortillas are considered excellent energy or heat source because of its high starch content. They are also a good source of calcium because lime is added during cooking for obtaining nixtamal. In addition, the alkaline cooking increases the bioavailability of one of the most important B vitamins, niacin, without affecting other vitamins found in suitable in the original, such as thiamine, pyridoxine and riboflavin grain levels.

*Int. J. Pure App. Biosci.* **3** (3): 22-27 (2015)

The experiments denote the probability of the existence of *Aspergillus sp* growth, most growth between 72 and 96 hours of growth, although growth could be considered poor, it is important to remember that this could be explained because the tortilla samples correspond to a colony high socioeconomic level, context could be a determining present low growth of the fungus, but this shows that the fungus is present in maize, although this proved that the nixtamalization process with lime that is added during cooking of this decreases the fungus and aflatoxic up to 90-97%, it should be noted that in vulnerable neighborhoods where the context has unpaved streets facilitates contact of the earth or dust and this could increase that presence of the fungus in the tortillas<sup>2,28</sup>.

Recently, there has been a daily practice in the marketing of tortillas in grocery stores and something surprising is retained for sale in coolers and has been observed in samples tortilla afternoon and night immediately after buying them already with the Player of the fungus mycelium, that is, observable by separating filaments to heat tortillas, this could represent a daily practice of risk.

A family to their daily tortillas, but his remaining consumed the previous day and even 2 to 3 days, he kept that to daily to consume later, daily placed in a basket or basket hanging from the ceiling of the kitchen freshly made tortillas. By showing these denote tortillas fungal growth. This means that the economic and cultural aspects of vulnerability are involved in processes of exposure to the fungus. Both died of cancer, liver and bones, so it is important to investigate to respect, to guide respect to the intake of corn tortillas and other foods aflatoxigenic people.

The results obtained in this study show areas of opportunity in the field of public health, not only for research but to take actions to improve the quality of life and impact on life itself by simply recommending eating tortilla that is daily made, avoiding cereals and oilseeds that have an unpleasant smell and taste as well as analyze their characteristics.

In experiments where the samples were left to the environmental conditions in which families do not have refrigerators, it was observed in the same way that after three days and the mycelium of the fungus and 7-8 days rather mold growth is present. A limitation applicable in this paper have not determined biochemically identifying the fungus, but preparations are made with blue and safranin was unmistakable structure of *Aspergillus sp.* Characteristic of *Aspergillus flavus*. However, the results obtained allow the recommendation not to consume tortillas they present with 3 days of being purchased independently of the social context in which these come with this will decrease the risk of exposure to metabolites "Aflatoxin" generated by this fungus.

Experiments where the environment tortillas were left without being refrigerated for three days already show similarly filaments or mycelium of fungi, this could serve as a criterion of exposure to humans who eat these tortillas fungus *Aspergillus sp* plus if we remember that economically vulnerable families do not have refrigerators could represent risk the tortillas intake 3 or more days of being purchased.

The omelette consumed was described to have poor quality, with whitish filaments or yarns when separating them, represents risk for low weight at birth if ingested during the first two months of pregnancy; synergism is even possible for the mothers who smoke. Poor quality omelette is apparently involved in the mortality effect of the product, as one of the possible causes of this event. The foods that mostly impact the outcomes of pregnancy in terms of aflatoxigenic effects are corn tortillas, peanuts, pistachios and junk foods<sup>24</sup>.

## CONCLUSIONS

By employee laboratory methodology was possible to determine the presence of Aspergillus between 72 and 96 hours of having bought the tortillas under the conditions of the culture medium and 25 degrees Celsius.

Theomeletteisone of the staples, culturally daily in Mexico and that is why the need for further research to reduce the concentration of *Aspergillus spp* arises, so it is essential to end the practice of selling these in coolers, improve conditions of their transport businesses where the tortilla is made and keep track of improving lifestyles as one of the determinants of health that we must influence from health programs considering the social context, this will improve quality of life and reduction of health risks, lower cost health and family level.

Do not eat corn omelette after three days you have bought them, especially if they are not under refrigeration, remember that to avoid exposure decreases the risk of adverse effects on public health.

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#### REFERENCES

- 1. Abdulrazzaq, Y.M. *et al.*, Fetal exposure to aflatoxins in the United Arab Emirates, *Annal of Tropical Pediatrics.*, Departament of Paediatrics, Faculty of Medicine& Health Sciences, UAE University, Al Ain United Arab Emirates (2002)
- 2. Anguiano Ruvalcaba, G.L. Verver Vargas Cortina, A.Y Guzman De Peña, D., Inactivación de aflatoxins B1 y aflatoxicol tradicional del maíz y su regeneración por acidificación de la masa. *Salud Pública de México.*, **47**(5): (2005)
- Azziz-Baumgartner, E. Lindblade, K. Gieseker, K. Schurz Rogers, H. Kieszak, S. Njapau, H. Romero Schleicher, Leslie, F. McCoy, Misore, A. DeCock, K. Rubin, C. Slutsker, L., Case– Control Study of an Acute Aflatoxicosis Outbreak, Kenya, 2004. *Environ Health Perspectives.*, 113(12): 1779-1783 (2005)
- 4. Bourgeois, C.M. Mesche, J.F.Y. Zucca, J., Micotoxins. *Microbiología alimentaría* edit acribia, S.A Zaragoza España (1994)
- Chao, T.C. Maxwell, S.M. Wong, S.Y., An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: a clinic pathological study. *Journal Pathology.*, 164(3): 225-3 (1991)
- 6. Drusch, S. Ragab, W., Mycotoxins in fruit juices, and dried fruits., *Journal of Food Protection.*, University of Kiel, Heirich-Hecht-Platz 10, 24118 Kiel, Germany. (2003)
- 7. Fernandez-Escartin, E., *Microbiología e Inocuidad de los Alimentos*, Universidad Autónoma de Querétaro. (2000)
- 8. Garrido, N.S. Iha, M.H. Santos Ortolani, M.R. Duarte Fávaro, R.M., Ocurrence of aflatoxins M1 and M2 in milk comercialized in Riberao preto.SP, Brazil.Food Additives and Contaminants. (2004)
- 9. Galan-Alejo, L.C. Y Rodríguez-Jerez, J.J., La contaminación por micotoxins. *Universidad Autónoma de Barcelona.*, 1-3 (2003)
- 10. Gonzalez-Salas, R. Desafios en la lucha contra las micotoxins. Universidad de Granma Cuba. (2003)
- 11. Gong, Y.Y.*et al.*, Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *British Medical Journal.*, **325**(7354): 20 (2002)
- 12. Gong, Y.Y. Egal, S. Hounsa, A. Turner, P.C. Hall, A.J. Cardwell, K.F. and Wild, C.P.,. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa; the critical role of weaning. *International Journal of Epidemiology.*, **32:** 556-562 (2003)
- 13. Hendrickse, R.G., Clinical implications of food contaminated by aflatoxins. *Annal Academic Medecine of Singapore.*, **20(1):** 84-90 (1991)
- 14. Hobbs, B.C.Y. Gilbert, R.J., Higiene y toxicología de los alimentos. Edit. Acribia, S.A. 36. (1986)
- 15. Joensyn, F.E. Maxwell, S.M. Hendrickse, R.G., Human fetal exposure to ochratoxin A and aflatoxins. *Annal of Tropical Paediatrics.*, **15(1):** 3-9 (1995)
- 16. Joensyn, F.E., Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls. *Mycopathologia.*, **152(1)**: 35-40 (2001)
- Jonathan Williams, H. et al., Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition.*, 80(5): 1106-17 (2004)

**Ruvalcaba LJC** *et al* Int. J. Pure App. Biosci. **3** (3): 22-27 (2015)

- Johnni, H.D. Lauren, W.L. Yanique, A.R. Stephanie Kieszak, Robert, F.B. Dana Flanders, W. Carlos Campana, John Mwihi, George Ogana, Sopiato Likimani, Masja Straetemans, Michael, A.M., Comprehensive Assessment of Maize Aflatoxin Levels in Eastern Kenya, 2005–2007. *Environ Health Perspectives.*, 119: 1794-1799 (2011)
- 19. Lunn, R.M. et al., mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan. *Journal News.*, **28**(2): 53(1997)
- 20. Maia, P.P. Pereira Bastos De Siqueira, M.E., Ocurrence of aflatoxins B1, B2, G1 and G2 in some Brazian pet foods. Food additives and Contaminants, (2002)
- 21. Onyemelukwe, G.C. Ogoina, D. Ibiam, G.E. And Ogbadu, G.H., Aflatoxins in body fluids and food of Nigerian children with protein-energy malnutrition, *African Journal of Food, Agriculture, Nutrition & Development.*, 1-15 (2012)
- 22. Oshikoya, K.A And Senbanjo, I., Path physiological changes that affect drug disposition in protein energy malnourished children., *Nut Metab.*, **6**: 50 (2009)
- 23. Ramis-Verges, M., *Microbiología de los alimentos* "Características de los patógenos microbianos" Hongos Toxigenic: *Aspergillus* Editorial Acribia, S.A Zaragoza España. (1998)
- 24. Ruvalcaba Ledezma Jesús Carlos, Interían Gómez Leticia, Flores Salinas Eduardo Efrain and Raygoza Anaya Miguel. Aflatoxigenic Feeding and its Possible Implications after Pregnancy. *Biomedical & Pharmacology Journal.*, **7**(1): 183-193 (2014)
- 25. Santos-Chona, O.M., Importancia de la aflatoxinas en seres humanos. *Universidad de Bucaramanga*. (2001)
- 26. Suarez, G., Regresan mazapanes de EU contaminación. Mural. (2004)
- 27. Teglia, M.C. *et al.*, Influencia de la aflatoxina B1 en crías de conejas intoxicadas. Estudio de las lesiones y determinación de la acumulación de aflatoxinas. (2004)
- 28. Torres, P. Guzmán-Ortiz, M. Ramírez-Wong, B., Revising the role of pH and thermal treatments in aflatoxin content reduction during the tortilla and deep frying processes. *Journal of Agricultural and Food Chemistry.*, (2001)
- 29. Turner, P.C. Collinson, A.C. Cheung, Y.B. Gong, Y. Hall, A.J. Prentice, A.M. And Wild, C.P., Aflatoxin exposure in uteri causes growth faltering in Gambian infants. *International Journal of Epidemiology.*, **36(5)**: 1119-1125 (2007)
- 30. Tchana, A.N. Moundipa, P.F. And Tchouanguep, F.M., Aflatoxin contamination in food and body fluids in relation to malnutrition and cancer status in Cameroon. *Int. J. Environ. Res. of Public Health.*, **7:** 178-188 (2010)
- 31. Valdivia-Flores, A.G. Quezada Taristán, T. Ortiz Martínez, R. Martínez De Anda, A., Implicaciones de la contaminación de alimentos por aflatoxinas, *Investigación y Ciencia.*, **23**: 2-10 (2000)
- 32. Verma, R.J. and Fair, A., Effect of aflatoxins on testicular steroidogenesis and amelioration by vitamin E. *Food and Chemical Toxicology*., (2002)