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# A physical method for segregation of fumonisin-contaminated maize

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**Abstract**

A batch of naturally contaminated maize was treated with water and different concentrations of NaCl in water by immersing for 5 min and removing the upper buoyant fraction. Both the buoyant and non-buoyant fraction were analysed for fumonisin B1. With water alone upto 74% of the toxin was removed in the buoyant layer. Maximum effective removal was up to 86% with saturated NaCl solution. Thirty per cent NaCl solution and above can be effectively used for physical decontamination of fumonisin-contaminated maize. 

*Keywords:* Fumonisin B1; Density segregation; Decontamination; Contaminated maize

## 1. Introduction

Fumonisin B1 is a heat stable mycotoxin produced by *Fusarium moniliforme* (Alberts et al., 1990; Gelderblom et al., 1988). Fumonisin B1 has been shown to cause leukoencephalomalacia in horses (Wilson et al., 1992), pulmonary edema in pigs (Harrison, Colvin, Greene, Newman, & Cole, 1990) and liver cancer in rats (Gelderblom, Semple, Marasas, & Farber, 1992). Naturally-occurring high levels of fumonisin B1 have been epidemiologically correlated with human oesophageal cancer in the Transkei region of South Africa (Rheeder et al., 1992; Sydenham et al., 1990) and China (Chu & Li, 1994; Yoshizawa, Yamashita, & Luo, 1994). Recently, fumonisin-contaminated maize and sorghum was found to be associated with a foodborne disease in humans (Bhat, Shetty, Amruth, & Sudershan, 1997) and a feedborne disease outbreak in poultry (Shetty, Sudershan, Rao, & Bhat, 1997).

Fumonisin B1 has been shown to occur naturally throughout the world, mainly in maize and maize-based foods and feeds (Marasas, 1996). Indian maize, sorghum and feeds were shown to be contaminated with fumonisin B1 (Shetty & Bhat, 1997). The levels of fumonisin B1 naturally occurring in foods and feeds and its demonstrated ability to cause several diseases make fumonisin B1 a real cause for concern in both health and economic respects (Marasas, 1996).

Considerable reduction in fumonisin B1 levels was observed with chemical detoxification procedures such as ammoniation (Norred, Voss, Bacon, & Riley, 1991) and alkaline hydrolysis (Scott & Lawrence, 1994). However, the treated substrate was found to be toxic when fed to rats. Similarly, reduction observed at higher temperatures (Scott & Lawrence, 1994) was also hypothesized to yield a toxic substrate. Sydenham, van der Westhuizen, Stockenstrom, Shephard, and Thiel (1994) reported a physical decontamination procedure which involves the separation of the screenings from the kernels. Up to 70% of fumonisin B1 is removed with the screenings. Huff (1980) showed that removal of low density grains with a sucrose gradient reduced the aflatoxin content by 90%. The present study was undertaken to assess the efficiency of density segregation in fumonisin B1 decontamination.

## 2. Materials and methods

### 2.1. Contaminated maize

Naturally-contaminated maize kernels containing $1.93 \pm 0.4$ ug/g of fumonisin B1 were collected from the field and stored at $-20°C$.

### 2.2. Density segregation

#### 2.2.1. Single step

Five hundred grams of contaminated maize (in triplicate) was added to 1 l of water (0% NaCl), 10% NaCl,

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20% NaCl, 30% NaCl or saturated NaCl solution, stirred and allowed to stand for 5 min. After 5 min, the top buoyant fraction and bottom non-buoyant fraction were separated, washed with excess water, blotted, dried in a blow-air oven (at 50°C) overnight and weighed.

#### 2.2.2. Serial

To find out the individual and cumulative effects, 750g of contaminated maize (in triplicate) was added to 1.5 l of water and buoyant fractions were separated. The buoyant layer was processed as shown above. The non-buoyant fraction was then added to 1.5 l of 10% NaCl solution and the non buoyant fraction again passed through 20%, 30% and saturated NaCl solutions. The buoyant fractions of water, 10%, 20%, 30% and saturated NaCl solutions were washed with excess water, blotted, dried, weighed and stored as above. The non-buoyant fraction of the saturated NaCl solution was also treated similarly.

### 2.3. Analysis of fumonisin B1

The different fractions were finely ground and analysed for fumonisin B1 using the method of Stack and Eppley (1992). A 250g portion of the ground fraction was extracted with 50 ml of methanol–water (3:1). In the case of some buoyant samples, where less material was available, the entire amount was used and methanol–water (3:1) was added accordingly. After extraction, the extract was filtered through Whatmann #4 filter paper and a 10 ml aliquot was applied to a Bond-Elut SAX cartridge (Supelclean, 500 mg adsorbant 2.5 ml capacity, Supelco, MO) which had been conditioned with 5 ml of methanol water (3:1). Subsequently, the cartridge was washed successively with 8 ml of methanol–water (3:1) and 3 ml of methanol. Finally, fumonisin B1 was eluted with 20 ml of 0.5% acetic acid in methanol. The eluate was evaporated to dryness under a flow of nitrogen at 50°C. The residue was redissolved with 250 μl of acetonitrile–water (1:1) and analysed by HPLC after derivatizing with o-phthalaldehyde reagent (100 μl OPA reagent for 25 μl of the redissolved sample).

The HPLC system consisted of Waters pump, Bondclone RP C18 column (3.9 x 150 mm, Phenomenex), Shimadzu fluorescence detector and Shimadzu Chromatopak integrator. The detector was set at an excitation wavelength of 335 nm and emission of 440 nm. Mobile phase consisted of acetonitrile–water–acetic acid (50:50:1) at the flow rate of 1 ml/min. Quantitation of the fumonisin B1 in the samples was achieved by extrapolating the area signal at the same retention time as standards with that found with the samples.

### 2.4. Statistical analysis

The data were subjected to analysis of variance utilizing the least square difference of mean (LSD) to determine the significance of differences between mean values.

## 3. Results and discussion

On treating with water and different concentrations of NaCl, lower density kernels separate into the buoyant fraction. It was observed that with water alone, a majority of the contaminated kernels went into the buoyant layer and were found to contain high amounts of fumonisin B1 (38.3 ± 3.78 μg/g, Table 1). With increasing concentrations of NaCl, more maize entered the buoyant fraction. The fumonisin B1 content in the buoyant grains increased with the increasing concentration of NaCl although the fumonisin B1 concentration per gram of the kernels became less. In the buoyant fraction of water 74% of the fumonisin B1 was removed, representing 4% of the total kernels. No difference occurred in either the amounts of the low density kernels in the buoyant fraction or the fumonisin B1 removed between the water and 10% NaCl solution.

Table 1
Concentration of fumonisin B1 and weight of contaminated grains in maize samples treated individually with water and different concentration of NaCl

| Treatment | Fraction | Weight (g) | Fumonisin B1 concentration μg/g | Total fumonisin B1 content μg/g | Fumonisin B1 recovery (%) |
|---|---|---|---|---|---|
| Water | Buoyant | 18.0 ± 2.16 | 38.3 ± 3.78 | 681 ± 19.82 | 73.8 ± 1.49*a |
| | Non-buoyant | 472 ± 5.89 | 0.51 ± 0.04 | 242 ± 21.58 | – |
| 10% NaCl | Buoyant | 29.0 ± 5.35 | 24.9 ± 4.59 | 711 ± 10.3 | 74.9 ± 1.49a |
| | Non-buoyant | 464 ± 3.06 | 0.5 ± 0.03 | 234 ± 16.06 | – |
| 20% NaCl | Buoyant | 55.3 ± 6.65 | 12.8 ± 1.43 | 698 ± 16.67 | 76.3 ± 1.5a |
| | Non-buoyant | 440 ± 8.52 | 0.49 ± 0.03 | 217 ± 16.24 | – |
| 30% NaCl | Buoyant | 96.4 ± 7.88 | 8.43 ± 1.00 | 809 ± 93.29 | 82.9 ± 4.8b |
| | Non-buoyant | 407 ± 2.9 | 0.40 ± 0.1 | 164 ± 41.13 | – |
| Saturated NaCl solution | Buoyant | 106.83 ± 3.06 | 9.81 ± 0.65 | 1041 ± 71.02 | 86.3 ± 1.6b |
| | Non-buoyant | 394 ± 4.32 | 0.42 ± 0.04 | 164 ± 14.35 | – |

*Values designated with different letters are significantly coefficient at 5% level.

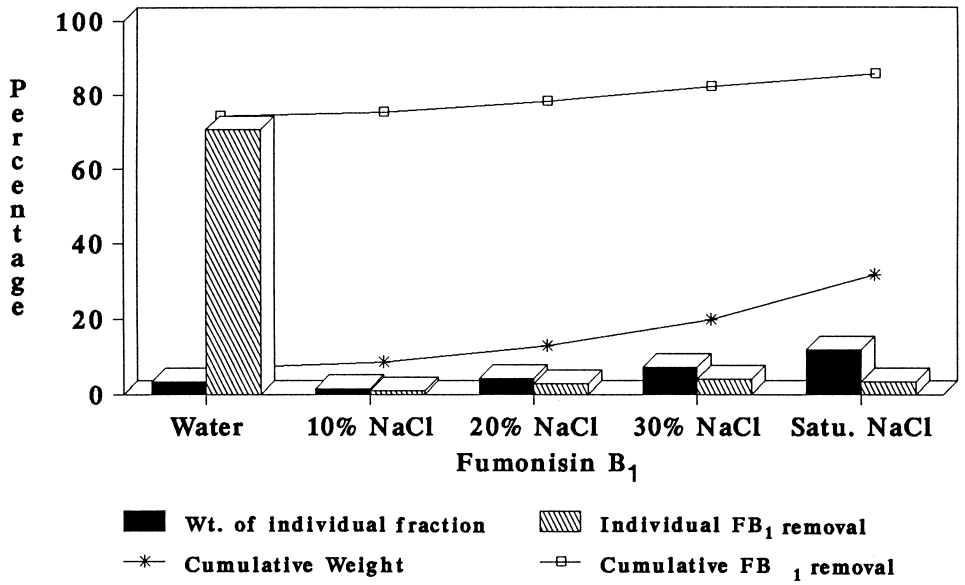


Fig. 1. Individual and cumulative removal of contaminated grains and fumonisin $B_1$ in maize treated serially with water and different concentrations of NaCl.

Although more low density kernels were removed with 20% NaCl solution, fumonisin B1 removal was not statistically significant. Maximum fumonisin B1 removal (86%) was seen with the saturated NaCl solution, but the removal is not significantly higher than that found with 30% NaCl solution.

Maize used in the present study contained $1.93 \pm 0.45$ μg/g of fumonisin B1. The distribution of the toxin in the batch was not very homogeneous and hence a considerable variation was observed. From the results, it is clear that only around 10% more of the fumonisin B1-containing kernels were removed with the sodium chloride solution. This may account for high levels of the toxin in highly contaminated batches of maize making the process highly economical. Ordinary salt (NaCl) is available freely and cheaply in rural India. Also the farmers are familiar with salt water treatment for the removal of ergoty bajra (Bhat, Roy, & Tulpule, 1975). Healthy maize in the non-buoyant fraction can be used for the preparation of local dishes such as unleavened Indian bread and porridge. The salt solution and the scum containing the contaminated grain can be used as a natural fertilizer.

Individual removal of fumonisin B1 by each concentration of NaCl in water when treated serially is shown in Fig. 1. The results are similar to the samples treated individually.

Earlier studies have shown that grains contaminated with aflatoxins have lower densities than normal kernels (Koltun, Gardner, Dollear, & Rayner, 1974; Huff, 1980; Newbanij, Saib, Chung, Seitz, & Deyol, 1986). This density difference is successfully utilized for decontamination. Results of the present study are similar to the earlier report of Huff (1980), who successfully removed up to 90% of the aflatoxin with 40% sucrose solution. But the use of a sucrose gradient may not be applicable in the field condition. Although NaCl treatment imparts a salty taste to grains, washing with water completely removes the salty taste.

In certain rural parts of India, 20% NaCl solution is traditionally used to remove the sclerotia from ergot contaminated bajra (Bhat et al., 1975). In view of this, popularization of this procedure, as an effective household decontamination procedure, may not be difficult.

Because of the inability of the chemical and thermal processing treatments, to remove fumonisins, more attention needs to be given to physical decontamination procedures. Available decontamination procedures, such as segregation of screenings (Sydenham et al., 1994) and steeping (Canela, Pujol, Sala, & Sanchis, 1996), are also found to be effective in the decontamination of fumonisin B1. The present method, alone or in combination with the other physical methods, may be a very effective and inexpensive way of fumonisin removal. However, more studies are needed in this regard with more samples with different levels of fumonisin B1.

## 4. Conclusion

Fumonisin B1-contaminated kernels have a low density and up to 86% of the toxin can be removed in the buoyant fraction when contaminated maize is treated with saturated NaCl solution. Thirty percent NaCl solution or more can be effectively used for the decontamination of fumonisin B1.

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## References

Alberts, J. F., Gelderblom, W. C. A., Thiel, P. G., Marasas, W. F. O., van Schalkywyk, D. J., & Behrend, Y. (1990). Effects of temperature and incubation period on production of fumonisin B1 by *Fusarium moniliforme*. *Appl. Environ. Microbiol., 56*, 1729–1733.

Bhat, R. V., Roy, D. N., & Tulpule, P. G. (1975). Ergot contamination of bajra. In *Proceedings of Nutrition Society of India, Hyderabad: National Institute of Nutrition* (pp. 7–11).

Bhat, R. V., Shetty, P. H., Amruth, R. P., & Sudershan, R. V. (1997). A foodborne disease outbreak due to the consumption of fumonisin contaminated sorghum and maize. *J. Toxicol. Clin. Toxicol., 35*, 249–255.

Canela, R., Pujol, R., Sala, N., & Sanchis, V. (1996). Fate of fumonisin B1 and B2 in steeped corn kernels. *Food Addit. Contam., 13*, 511–517.

Chu, F. S., & Li, G. Y. (1994). Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in region with high incidence of oesophageal cancer. *Appl. Environ. Microbiol., 60*, 847–852.

Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R., & Kriek, N. P. J. (1988). Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol., 54*, 1806–1811.

Gelderblom, W. C. A., Semple, E., Marasas, W. F. O., & Farber, E. (1992). The cancer-initiating potential of fumonisin B mycotoxins. *Carcinogenesis, 13*, 433–437.

Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E., & Cole, J. R. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. *J. Vet Diagn. Invest., 2*, 217–221.

Huff, W. E. (1980). A physical method for the seggregation of aflatoxin-contaminated corn. *Cereal Chemistry, 57*, 236–238.

Koltun, S. P., Gardner Jr, H. K., Dollear, F. G., & Rayner, E. F. (1974). Physical properties and aflatoxin content of individual cat eye fluorescent cotton seeds. *Journal of the American Oil Chemistry Society, 51*, 178–180.

Marasas, W. F. O. (1996). Fumonisins: History, worldwide occurrence and impact. In: L. S. Jackson, J. W. DeVries, & L. B. Bullerman (Eds.), *Fumonisins in Food, Advances in Experimental Medicine and Biology* (Vol. 392, pp. 1–18). New York: Plenum Press.

Newbanij, M., Saib, P. A., Chung, D. S., Seitz, L. M., & Deyol, C. W. (1986). Ergosterol versus dry matter loss as quality indicator for high-moisture rough rice during holding. *Cereal Chemistry, 63*, 315–320.

Norred, W. P., Voss, K. A., Bacon, C. W., & Riley, R. T. (1991). Effectiveness of ammonia treatment in detoxification of fumonisin-contaminated corn. *Food Chem. Toxicol., 29*, 815–819.

Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Shephard, G. S., & van Schalkwyk, D. J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human oesophageal cancer in Transkei. *Phytopathology, 82*, 353–357.

Scott, P. M., & Lawrence, G. A. (1994). Stability and problem in recovery of fumonisin added to corn based foods. *J. AOAC Int., 77*, 541–545.

Shetty, P. H., & Bhat, R. V. (1997). Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in sorghum, maize and poultry feeds. *J. Agric. Food Chem., 45*, 2170–2173.

Shetty, P. H., Sudershan, R. V., Rao, J. P. K., & Bhat, R. V. (1997). Outbreak in layer hens due to consumption of fumonisin contaminated feed. *British Poultry Science, 38*, 475–479.

Stack, M. E., & Eppley, R. M. (1992). Liquid chromatographic determination of fumonisin B1 and B2 in corn and corn products. *J. AOAC. Int., 75*, 834–837.

Sydenham, E. W., Thiel, P. G., Marasas, W. F. O., Shephard, G. S., van Schalkwyk, D. J., & Koch, K. R. (1990). Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei. *Southern Africa. J. Agric. Food Chem., 38*, 1900–1903.

Sydenham, E. W., van der Westhuizen, L., Stockenstrom, S., Shephard, G. S., & Thiel, P. G. (1994). Fumonisin contaminated maize: physical treatment for the decontamination of bulk shipments. *Food Addit. Contam., 11*, 25–32.

Wilson, T. M., Ross, P. F., Owens, D. L., Rice, L. G., Jenkins, S. J., & Nelson, H. A. (1992). Experimental production of ELEM. A study to determine the minimum toxic dose for ponies. *Mycopathologia, 117*, 115–120.

Yoshizawa, T., Yamashita, A., & Luo, Y. (1994). Fumonisin occurrence in corn from high and low risk areas for human oesophageal cancer in China. *Appl. Environ. Microbiol., 60*, 1626–1629.