Influence of storage conditions and packaging material on the multiplication of aspergillus flavus and its production of aflatoxin B1 in sifted maize meal

Chemie, Mikrobiologie, Technologie der Lebensmittel
Nout, M.J.R.; Saint-Hilaire, P.

Chem. Mikrobiol. Technol. Lebensm. *8*, 83 - 86 (1983)

Influence of Storage Conditions and Packaging Material on the Multiplication of *Aspergillus flavus* and its Production of Aflatoxin B 1 in Sifted Maize Meal

M.J.R. Nout and P. Saint-Hilaire

Department of Food Science and Technology, University of Nairobi, Kenya

Eingegangen am 15.6.1983

Summary: Sifted maize meal was inoculated with three concentrations of aflatoxin producing *Aspergillus flavus*, packaged in either commercial kraft paper or porous nylon cloth, and stored for six months under conditions representative of the Kenyan climate. Results obtained show that, (a) kraft paper was not an effective barrier against moisture migration, but served to protect against insect infestation; (b) packaging in porous nylon cloth resulted in considerable insect infestation within two months of storage; (c) the growth of *A. flavus* was not supported by the packaged product; (d) during the storage period only trace amounts of aflatoxin B1 were produced. It appears that commercial sifted maize meal is not a product at risk of being contaminated with aflatoxins during storage, and that high levels in this product would originate from maize containing aflatoxin prior to milling.

Einfluß der Lagerungsbedingungen und des Verpackungsmaterials auf die Vermehrung von *Aspergillus flavus* und dessen Produktion von Aflatoxin B1 in gesichtetem Maismehl

Zusammenfassung: Gesichtetes Maismehl wurde mit drei Konzentrationen von Aflatoxin produzierendem *Aspergillus flavus* beimpft, entweder in im Handel gebräuchliche Säcke aus Kraftpapier oder porösem Nylongewebe verpackt und sechs Monate unter Bedingungen gelagert, die dem kenianischen Klima entsprechen. Die gefundenen Resultate zeigen, daß (a) Kraftpapier keine wirkungsvolle Barriere gegen Feuchtigkeitsaufnahme bildete, hingegen half, Insektenbefall zuverhindern, (b) Verpacken in Nylongewebe einen beträchtlichen Insektenbefall innerhalb von zwei Monaten zur Folge hatte, (c) das Wachstum von *A. flavus* nicht vom verpackten Produkt unterstützt wurde, (d) während der Lagerperiode nur Spuren von Aflatoxin B1 produziert wurden. Wir schließen daraus, daß für im Handel erhältliches, gesichtetes Maismehl keine Gefahr besteht, mit Aflatoxin kontaminiert zu werden. Hohe Aflatoxingehalte in diesem Produkt müssen deshalb ihre Ursprünge im Mais haben, der Aflatoxin schon vor dem Vermahlen enthielt.

Introduction

Sifted maize meal is an important staple food in Kenya. Cereals, particularly maize, are the major source of dietary aflatoxins in this country [1]. Maize meal and maize flour were incriminated in recent cases of aflatoxicosis in the Nairobi [2] and Machakos [3] areas. Several investigators studied the conditions favouring aflatoxin production by Aspergillus species under aseptic conditions on natural foods [4]and on defined growth media [5,6]. There is evidence [7,8,9] that mycotoxin production is reduced in a natural environment where other microorganisms are present. The objective of this study is to determine the fate of *Aspergillus flavus* and its production of aflatoxin B1 in the natural environment provided by freshly milled sifted maize meal, under different conditions of storage representative of the Kenyan climate.

Materials and Methods

Microorganism

The strain of *A. flavus* used had been isolated earlier from sifted maize meal. Its ability to produce aflatoxins was demonstrated by a strong fluorescence at 366 nm of a culture grown on Aflatoxin Production

Agar [10]. Aflatoxins produced in sterile cooked maize were identified as B1 and B2 by thin layer chromatography [11].

Samples

Replicate 200 g samples of commercial sifted maize meal were either packaged in the kraft paper bags (single sheet of 95 g/m^2) used in Kenya for this product, or in coarse nylon filter cloth providing unhindered moisture migration. The samples had been inoculated with spores of *Aspergillus flavus* as follows: (a) low level contamination (untreated product) containing 100-120 *A. flavus* propagules/g; (ii) medium level contamination (*A. flavus* added) containing 300-450 *A. flavus* propagules/g; (iii) high level contamination containing 1,8 - 2,3 · 10^4 *A. flavus* propagules/g.

Storage conditions

Samples were stored umber ambient (uncontrolled) condition (A), and in large desciccators under controlled conditions (B,C,D) as follows: (A) ambient 20-23 °C, air relative humidity (RH) 45-65 %. During the storage trials, a rainy period (months 2,3,4) was followed by a dry season. The mentioned RH values represent daily averages representative of the dry, resp. rainy seasons; (B) 22 °C, RH air 70 %; (C) 35 °C, RH air 70 %; (D) 35 °C, RH air 85 %.

Water activity

Water activity (a_W) of the stored samples was determined with a Lufft A_W-value analyzer 5803, Stuttgart, Germany.

Aflatoxin B1

The samples were analysed for aflatoxin B1 using the two dimensional thin-layer chromatographic method as modified by the Swiss Food Control Laboratories [11].

Aspergillus flavus:

Viable *A. flavus* were counted using Aspergillus Differential Medium to which 30 ppm filter sterilised tetracyclin had been added after sterilisation [12]. The pour plate method was used.

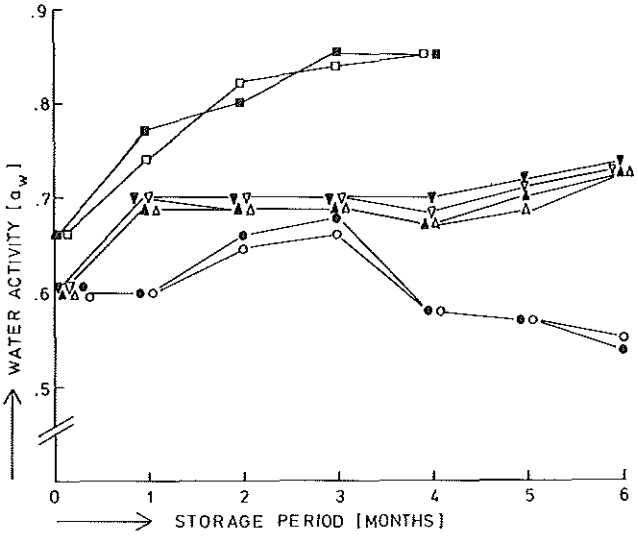
Results

Packaging

Figure 1 shows the equilibration of the water activity (a_W) of paper and filter cloth packaged samples. Irrespective of the level of *A. flavus* contamination, equilibration in the paper packaged samples occurred at the same rate as in the filter cloth packaged ones, measured at monthly intervals. This shows that the paper used does not provide a significant barrier against moisture migration. This is particularly demonstrated under storage condition (A),where a_W fluctuations caused by changes in the air RH are identical for both types of packaging. During the six months storage, no cocoons, larvae or webbing were found in the paper packaged samples, whereas the samples packaged in filter cloth contained maggots and webbing already after two month of storage.

Aspergillus flavus development

No significant multiplication of *A. flavus* was recorded (Figure 2) when stored at 20-23 °C (A) and (B). At 35 °C, a gradual reduction of viable propagules took place, in particular at 85 % RH (D). Although fungi other than *A. flavus* were not quantified, it was observed from the counting plates that *A. flavus* was gradually outgrown by other fungi, especially at



Effect of packaging material and storage conditions on water activity of sifted maize meal
(A) 20 - 23 °C, RH air 45-65 % (ambient): ● kraftpaper, ○ filtercloth;
(B) 22 °C, RH air 70 % :▼ kraftpaper, ▽ filtercloth;
(C) 35 °C, RH air 70 %: ▲ kraftpaper, △ filtercloth;
(D) 35 °C, RH air 85 %: ■ kraftpaper, □ filtercloth.

22 °C, 70 % RH (B) and 35 °C, 85 % RH (D). Under the remaining storage conditions the competitive flora did not multiply significantly.

Aflatoxin B1 production

At t_0, aflatoxin B1 could not be detected in any of the samples. Although aflatoxin B1 was present in most samples after storage, concentrations higher than 10 ppb were not recorded. The sample with high level (iii) contamination had higher aflatoxin B1 production, 1 to 7,5 ppb. The last value was obtained for condition (D) already after 1 month of storage. For the other levels of contamination, only traces of aflatoxin B1 could be detected, but medium (ii) level of contamination subjected to condition (D) had 7,5 ppb of aflatoxin B1 after 1 month of storage.

Discussion

As can be expected, moisture penetration through a single layer of kraft paper is quite rapid. However, in commercial practice a better protection from moisture might be expected than suggested by our findings, since 2 kg bags are usually distributed in double layered kraft paper balers containing 10 x 2 kg packages each. Although the moisture barrier provided by the kraft paper was found to be of limited value, it proved nevertheless effective as a protection against insect infestation.

Several strains of *A. flavus* were reportedly able to grow at 37 °C [5] and 41 °C [6]. These findings were obtained with pure cultures under conditions of a_W exceeding 0,90, grown as aerobic surface cultures on agar media. Our findings at 35 °C, RH 85 % (D) indicate that such ability to grow can be seriously affected in a densely packed product in the presence of a competitive fungal population. Under the experimental conditions used here, sifted maize meal did not support a significant multiplication of *A. flavus.*

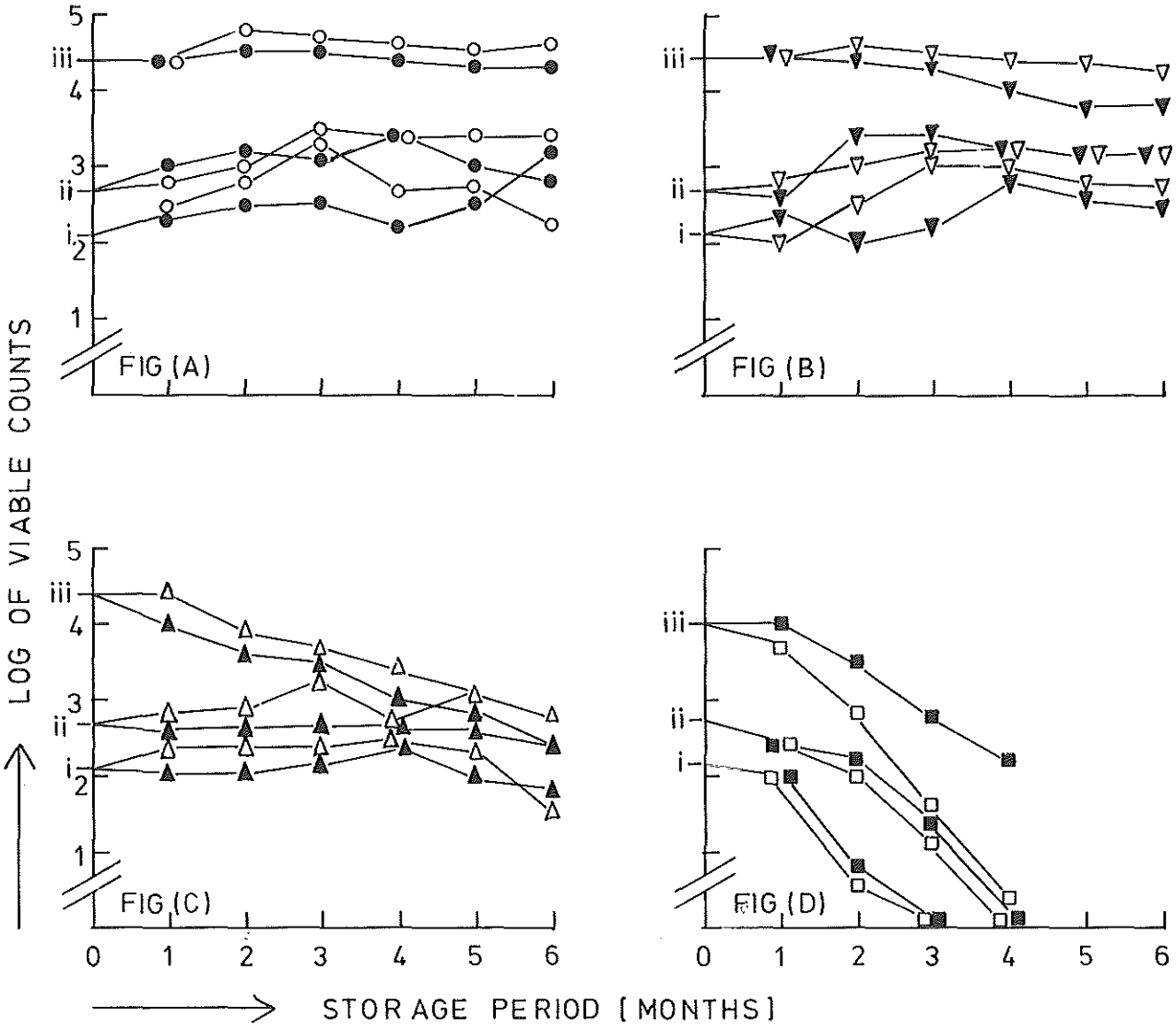


Fig. 2 Effect of packaging material and storage conditions on growth of Aspergillus flavus in sifted maize meal (contamination levels a, b and c). For legend see Fig. 1

Considering that profuse mould growth occurred on the outside of the paper bags of many samples, it is suggested that poor availability of atmospheric oxygen, caused by the dense packing of the meal and by the hindrance of the paper bag, contributed to this. Although some aflatoxin B1 could be detected after prolonged storage, the concentrations remained well below 20 ppb, which is generally considered as the highest tolerable level in human food. Aflatoxin B1 production was reported at temperatures of 13-37 °C by pure cultures of *A. flavus* strains [5], but this was mainly achieved at water activities exceeding 0,90, in stages of active growth. Aflatoxin production has been associated with active growth and subsequent sporulation [13]. This might be supported, though indirectly, by our finding that no significant toxin quantities were produced by resting or dying cultures. It is not clear to what extent the competitive flora in our samples has contributed to the low aflatoxin levels.

It appears that sifted maize meal is at considerably less risk of becoming contaminated with aflatoxins than unmilled maize kernels. Therefore, if sifted maize meal is found to contain high levels of aflatoxins [2], these are probably originating from maize kernels which had previously been contaminated during the pre-harvest stage [14] or during storage. With respect to the latter, it should be noted that in Kenya, maize is frequently stored on the cob. It will be of interest to compare the vulnerability of shelled maize and maize on the cob during farm storage. It is concluded that the emphasis of food safety control should lay on adequate storage of maize prior to processing, and on effective monitoring of mycotoxin levels in maize offered for milling.

Acknowledgements

We thank Unga Ltd., Nairobi for sifted maize meal and packaging material, the National Institute of Public Health, the Netherlands, for aflatoxin standards, and R.C.M. Maina, M.A. Murundo, S.M. Mwaura and J.N. M'Thika for technical assistance.

References

[1] Peers, F.G. and Linsell, C.A., Br. J. Cancer *27*, 473 (1973)
[2] Muraguri, N., Omukoolo, L.C., Kenji, G.M. and Condier, G.A., East Afr. Med. J. *58*, 484 (1981)
[3] Ngindu, A., Kenya, P.R., Ocheng, D.M., Omondi, T.N., Ngare, W., Gatei, D., Johnson, B.K., Ngira, J.A., Nandwa, H., Jansen, A.J., Kaviti, J.N. and Siongok, T.A., The Lancet *1*, (8285) 1346 (1982)
[4] Elaraki, A.T., Comptes Rendus des Scéances de l'Académie d'Agriculture de France *64*, 69 (1978)
[5] Northolt, M.D., Van Egmond, H.P. and Paulsch, W.E., J. Food Prot. *40*, 778 (1977)
[6] Schindler, A.F., J. Food Prot. *40*, 39 (1977)
[7] Tsuruta, O., Sugimoto, T. and Minamisawa, M., Rept. Nat. Food Res. Inst. Tokyo, Japan, No. 32, 21 (1977)
[8] Masimango, N., Remacle, J. and Ramaut, J., Ann. Nutr. Alim. *33*, 149 (1979)
[9] Wicklow, D.T., Hesseltine, C.W., Shotwell, O.L. and Adams, G.L., Abstr. Ann. Meeting Am. Soc. Microbiol. *79*, 202 (1979)
[10] Hara, S., Fennel, D.I. and Hesseltine, C.W., Appl. Microbiol. *27*, 1118 (1974)
[11] Arbeitsgruppe "Toxine" der Eidg. Lebensmittelbuchkommission, Mitt. Gebiete Lebensm. Hyg. *73*, 362 (1980)
[12] Bothast, J.R. and Fennel, D.I., Mycologia *66*, 365 (1974)
[13] Boltyanskaya, E.V., Appl. Biochem. Microbiol. *15*, 517 (1979)
[14] Gray, F.A., Faw, W.F. and Boutwell, J.L., Plant Disease *66*, 221 (1982)

Address of authors:
Dr. M.J.R. Nout
Section of Food Microbiology and Hygiene
Agricultural University
De Dreijen 12
NL-6703 BC Wageningen
The Netherlands