

Title Aflatoxin production by *Aspergillus flavus* in Brazil nuts
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Abstract

Experiments were conducted to evaluate the effects of relative humidity (r.h.; 75%, 80%, 85%, 97%) and temperature (10, 13, 15, 25, 30 °C) on aflatoxin production in previously dried (3.5% moisture content; m.c.) Brazil nuts. Initially *Aspergillus* spp. were isolated from the surfaces of whole in-shell (WIS) Brazil nuts imported from Peru using *A. flavus* and *A. parasiticus* agar (AFPA). Isolates were subsequently screened for aflatoxin production using yeast extract sucrose medium. Total aflatoxin ($B_1+B_2+G_1+G_2+M_1$) was analyzed using an immunoassay technique while the presence of aflatoxin was confirmed using thin-layer chromatography. The surface of shelled half-nuts (simulating damaged or trimmed nuts), shelled whole (SW) nuts, and WIS nuts following a chlorine wash and water rinse, served as sites for inoculation ($10 \mu\text{l}$; $10^5/\text{ml}$) using an aflatoxigenic isolate. Maximum concentrations of total aflatoxin and B_1 were detected in nuts stored at 97% r.h. and at temperatures of 25–30 °C. Shelled half-nuts contained the highest total (6817 ng/g) and B_1 (4483 ng/g) aflatoxin. WIS nuts contained the least total and B_1 toxin with maximum concentrations of 93 and 49 ng/g, respectively. Aflatoxin was not detected (detection limit of 1.75 ng/g) in nuts maintained at either 10 °C (97% r.h.) or at 30 °C (75% r.h.) for up to 60 d. Maximal moisture contents (%) and water activity values (a_w) for nuts stored at these conditions were 4.50 and 0.78, and 9.14 and 0.92, respectively. Results of this study indicate that the limiting moisture content and a_w values required to control aflatoxin production ($<4\text{ng/g}$) in SW and WIS stored at 30 °C for up to 60 d are 4.5, 0.68, 5.0, and 0.75, respectively. Overall, increasing the relative humidity and temperature during storage resulted in an increase in aflatoxin and these were shown to be the most significant variables influencing toxin production in Brazil nuts.