

# Decomposition and detoxification of aflatoxin B<sub>1</sub> by lactic acid

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## Abstract

**BACKGROUND:** A degradation study of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) was carried out using a combination of physical and chemical methods. AFB<sub>1</sub> was heated at 80 °C in the presence of acetic, citric and lactic acids for various time periods. The cytotoxicity of the degraded AFB<sub>1</sub> and its products were determined by MTT assay.

**RESULTS:** The results showed that among the three organic acids lactic acid was most efficient in degrading AFB<sub>1</sub>. Although complete degradation was not observed, up to 85% degradation of AFB<sub>1</sub> was obtained when heated for 120 min. Degradation of AFB<sub>1</sub> was confirmed by the reduced toxicity on HeLa cells using MTT assay. Treatment with lactic acid resulted in the conversion of AFB<sub>1</sub> into two degradation products. These products were observed at lower retention factors of 0.63 and 0.38, which were identified as AFB<sub>2</sub> and AFB<sub>2a</sub>, respectively. The cytotoxicity of AFB<sub>2a</sub> exhibited much reduced toxicity on HeLa cells compared to that of AFB<sub>1</sub>.

**CONCLUSION:** The results have shown the efficiency of lactic acid in degrading AFB<sub>1</sub>. This study suggests that lactic acid may be considered for use in the food and feed industry since it is present naturally in food and is considered safe.

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**Keywords:** aflatoxin B<sub>1</sub>; aflatoxin B<sub>2a</sub>; degradation; lactic acid; cytotoxicity assay

## INTRODUCTION

Aflatoxins (AFs) are a group of structurally related metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*.<sup>1,2</sup> These fungi can grow and produce aflatoxins under favourable temperature and moisture conditions in various food materials such as groundnut, tree nuts, dried fruits, spices, herbs and a number of food grains.<sup>3,4</sup> In order of their toxicity, the four major AFs are AFB<sub>1</sub>, AFG<sub>1</sub>, AFB<sub>2</sub> and AFG<sub>2</sub>. They are known to be genotoxic, hepatotoxic, mutagenic, carcinogenic and immunosuppressive in animals and humans.<sup>5</sup> Among them, AFB<sub>1</sub> is the most toxic and has been classified as Group I human carcinogen by the International Agency for Research on Cancer.<sup>6</sup> AFB<sub>1</sub>-8,9-epoxide, the carcinogenic metabolite which is activated by cytochrome P450, is responsible for the mutagenic activity of AFB<sub>1</sub>.<sup>7</sup> It has been reported that the double bond in the terminal furan ring and the lactone ring in the coumarin moiety of AFB<sub>1</sub> are the main sites responsible for its toxicity.<sup>8,9</sup>

Removal or degradation of AFB<sub>1</sub> from food materials and the environment has become a necessity. AFB<sub>1</sub> is highly oxidized and hence stable to dry heat up to its melting point, i.e. 260 °C. Several degradation techniques involve a combination of heat treatment and moisture content. Factors such as temperature, moisture (acidic or alkaline) and time period determine the level of degradation.<sup>10,11</sup> Use of chemicals for degrading AFB<sub>1</sub> has been practised in the food industries. Ammonia is one of the most effective methods for AFB<sub>1</sub> degradation and has been accepted for use by the corn production industry. Ammoniation of AFB<sub>1</sub> leads to the formation of a degraded product, AFD<sub>1</sub>, which has reduced toxicity and mutagenic potential.<sup>12</sup> Chlorine gas has been successfully used for inactivating 75% of AFB<sub>1</sub> in corn meal, copra

meal and groundnuts without the formation of new compounds.<sup>13</sup> Although aqueous chlorine is used as a sanitizer in the food industries, the safety of chlorinated foods remains a concern. In the presence of acids, AFB<sub>1</sub> is converted to less toxic derivatives such as AFB<sub>2a</sub>, AFD<sub>1</sub> and AFB<sub>2</sub>.<sup>14–17</sup> It was reported that a higher level of degradation and loss of mutagenicity of AFB<sub>1</sub> in maize and sorghum was achieved with heat treatment in the presence of citric and lactic acid.<sup>18</sup> Safara *et al.*<sup>19</sup> demonstrated the efficacy of citric acid in reducing the level of AFB<sub>1</sub> in rice grains. The natural occurrence of organic acids in food makes them suitable in aiding the degradation of AFB<sub>1</sub> in food. Lactic acid bacteria and lactic acid in fermented foods have also been exploited for the detoxification of AFB<sub>1</sub>.<sup>20,21</sup>

The present study evaluated the efficacy of organic acids in degrading AFB<sub>1</sub>, and detoxification of AFB<sub>1</sub> was studied in animal cell lines.

## MATERIALS AND METHODS

### Aflatoxin B<sub>1</sub> purification and photometric quantification

An AFB<sub>1</sub>-producing strain of *Aspergillus* was isolated in the laboratory and identified as *Aspergillus flavus* Link 1809 at the

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