

## Validation of a High Performance Liquid Chromatography Method for Aflatoxins Determination in Corn Arepas

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Aflatoxins are toxic metabolites produced by *Aspergillus* species in raw materials and foodstuffs from agricultural sources. Due to the fact that aflatoxins are potentially carcinogenic, it is important to have reliable analytic methods that allow determining their levels in food matrices. The main aim of this investigation was to validate the method for aflatoxins determination by high performance liquid chromatography in corn arepas, which are widely consumed in the Colombian coffee region. The evaluated parameters were robustness, selectivity, linearity, sensitivity, repeatability, intermediate precision and recovery. The methodology was successfully validated with an optimal selectivity, linearity > 0.9998, limits of detection and quantification lower than 0.10 µg kg<sup>-1</sup>, relative standard deviation < 4.5% and recovery > 76.6%. Also, 144 corn arepas samples were analyzed and aflatoxins were found in 9.72% of them, with levels between 0.95 and 11.56 µg kg<sup>-1</sup>.

**Keywords:** validation, liquid chromatography, food analysis, aflatoxins, corn arepas

### Introduction

Aflatoxins (AF) are toxic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* in foods and feeds from agricultural sources.<sup>1</sup> Up to date, approximately 20 aflatoxins have been identified, but the most frequent in foods like corn, peanut, nuts, rice, cereals and cotton cake are AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>.<sup>2</sup> These toxins have drawn a lot of attention due to their carcinogenic potential,<sup>3</sup> mainly AFB<sub>1</sub> classified in the IA group by the International Agency for Research on Cancer (IARC) as human carcinogen due to its hepatotoxicity.<sup>4</sup> For this reason, regulations regarding aflatoxins maximum levels have been established in 100 countries, which demands the availability of reliable laboratory methods that allow to determine the contamination level by aflatoxins in food matrices of high consumption to ensure their quality and to control possible public health risks.

Given the broad array of contamination susceptible matrices, the possible concurrence of various mycotoxins and the wide concentrations range, it is necessary to validate the methods to determine the aflatoxins levels in foods. A large number of analytic techniques have been developed for mycotoxins analyses in foods,<sup>5</sup> among them thin layer

chromatography (TLC),<sup>6</sup> enzyme-linked immunosorbent assay (ELISA),<sup>7</sup> gas chromatography (GC)<sup>8</sup> and liquid chromatography (LC) with various detectors.<sup>9</sup> Nevertheless, so far, high performance liquid chromatography (HPLC) coupled to a fluorescence detector, has been the most used technique due to its high sensitivity and low costs.<sup>10</sup>

The corn arepa is the most consumed maize product in Colombia, reaching 83.1% in the central-western region of the country.<sup>11</sup> This food is one of the foodstuffs in Colombia with a high susceptibility to be contaminated with aflatoxins, because the mycotoxigenic mold may grow on maize from cultivation, storage or even when the corn arepas are being processed.<sup>12,13</sup> Therefore, surveys concerning corn and their products intended for human consumption are necessary in determining the risk of aflatoxins for consumers. The purpose of this study was to validate the HPLC with fluorescence detector method for the determination of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> in white corn arepas and to establish the aflatoxins levels in arepas commercialized in Manizales, Colombia.

### Experimental

#### Standard reactants and solutions

All used reactants were HPLC grade Honey-well and Carlo Erba brands and the water was type I Milli-Q

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Millipore 0.22  $\mu\text{m}$ . Micotox® 2002 cartridges were used for the aflatoxins purification. The standard aflatoxins with a certified concentration of 250  $\text{ng mL}^{-1}$  of AFB<sub>1</sub> and AFG<sub>1</sub>, and 75  $\text{ng mL}^{-1}$  of AFB<sub>2</sub> and AFG<sub>2</sub> in acetonitrile were acquired in Micotox Ltda® (Colombia). A 100  $\text{ng mL}^{-1}$  working solution was made, from which a series of acetonitrile solutions were prepared at the following concentrations: 0.1, 1.0, 5.0, 10, 25, and 50  $\text{ng mL}^{-1}$  for AFG<sub>1</sub> and AFB<sub>1</sub>, and 0.03, 0.30, 1.52, 3.03, 7.58, and 15.15  $\text{ng mL}^{-1}$  for AFG<sub>2</sub> and AFB<sub>2</sub>.

### Samples

A total of 144 white corn arepas samples (500 g each) from 12 trademarks commercialized in stores and local supermarkets in Manizales, Colombia, were collected, which included six handcrafted trademarks and six industrial fabrication trademarks. From each trademark, six analytic units were analyzed in two different periods during 2013 to determine their AF contamination.

### Extraction and purification

AF extraction was performed following the Colombian Technical Standards (Norma Técnica Colombiana NTC 1232),<sup>14</sup> modified by Céspedes and Díaz<sup>15</sup> and Díaz *et. al.*,<sup>16</sup> as follows: a 500 g of ground analytical sample was triturated in a food processor, from which a subsample of 25 g (in the case of hygroscopic substrates such as arepas) was extracted with 100 mL of acetonitrile:milli-Q water (84:16, v/v) during 3 min using a blender at high speed. The blended mixture was filtered through Whatman No. 4 paper, and 5 mL of the filtrate was purified with a multifunctional cleanup column (Micotox® 2002). Finally, 200  $\mu\text{L}$  of purified extract were transferred to an amber vial. This aliquot was treated with 700  $\mu\text{L}$  of milli-Q water:trifluoroacetic acid:acetic acid (7:2:1, v/v/v), and the vial was heated in a steam water bath at 65 °C for 10 min and then, when the mixture returned to room temperature, 50  $\mu\text{L}$  were injected into the loop of the HPLC pump for duplicate HPLC analysis.<sup>14-16</sup>

### HPLC analysis

The chromatographic separation was carried out in Shimadzu LC-20 AT HPLC system connected to a RF-10AXL (Shimadzu, Kyoto, Japan) fluorescence detector.<sup>17</sup> The four aflatoxins were separated in a RP18 Capital® (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) HPLC column. The best chromatographic conditions were the following: 1  $\text{mL min}^{-1}$  for the mobile phase's flow, mobile

phase with a mixture of methanol:acetonitrile:milli-Q water (17.5:7.5:65 v/v/v) in isocratic elution, injection volume of 50  $\mu\text{L}$  and column oven temperature of 50 °C. The fluorescence detection was made with excitation wavelength of 360 nm and emission of 440 nm. The four aflatoxins were separated in 20 min under the above mentioned conditions.<sup>14-16</sup>

### Validation and quality assurance of the analytical method

The method employed was validated according to the International Union of Pure and Applied Chemistry (IUPAC)<sup>18</sup> and EURACHEM<sup>19</sup> validation guides. The parameters included were robustness, specificity, linearity, sensitivity, repeatability, intermediate precision and recovery.

For the robustness tests, three variables that could affect the method performance were selected and three experiments of each one were carried out. The variables were: mobile phase composition, column oven temperature and detector's wavelength. A standard solution of 10  $\text{ng mL}^{-1}$  of AFG<sub>1</sub> and AFB<sub>1</sub>, and 3.3  $\text{ng mL}^{-1}$  of AFG<sub>2</sub> and AFB<sub>2</sub> was used and each series of experimental conditions was analyzed once. The selectivity was verified by means of an HPLC analysis of a blank sample and spiked sample with a 10  $\text{ng mL}^{-1}$  solution of AFG<sub>1</sub> and AFB<sub>1</sub>, and 3.3  $\text{ng mL}^{-1}$  of AFG<sub>2</sub> and AFB<sub>2</sub>. The linearity was determined by a triplicate analysis of standard aflatoxins solutions at six different concentrations and the blank during three consecutive days. The concentrations were; 0.1, 1.0, 5.0, 10, 25, 50  $\text{ng mL}^{-1}$  for AFG<sub>1</sub> and AFB<sub>1</sub> and 0.03, 0.30, 1.52, 3.03, 7.58, 15.15  $\text{ng mL}^{-1}$  for AFG<sub>2</sub> and AFB<sub>2</sub>. The sensitivity was established by detection limit (LOD) and quantification limit (LOQ), which were calculated according to the following equation:  $\text{LOD} = 3.3s_a/b$  and  $\text{LOQ} = 10s_a/b$ , where  $s_a$  is the standard deviation of the intercept and  $b$  is the slope of regression line, obtained from the calibration curve.<sup>20</sup> The repeatability was done by the preparation of three different concentration's solution made from the standard solution in the working interval of the method, each one was prepared by the same analyst and read six times in a short time period (1 day). The intermediate precision was done with the preparation of three different concentration's solution in the working range from the standard solution, each one prepared by a different analyst and read six times in extended period of time (day 1, 10, 20 and 30).

The recovery was determined by the analysis of a sample of arepa spiked with a solution of AFG<sub>1</sub> and AFG<sub>2</sub> at two concentration orders of 4.23 and 43.95  $\text{ng mL}^{-1}$ , respectively. The sample was spiked before the extraction

and purification phase and the recovery percentage was calculated as follows:

$$\text{Recovery (\%)} = \frac{\chi' - \chi}{\chi_{\text{spiked}}} \times 100 \quad (1)$$

where  $\chi'$  is the concentration of the spiked sample,  $\chi$  is the concentration of non-spiked sample and  $\chi_{\text{spiked}}$  is the concentration added.<sup>19</sup>

## Results and Discussion

### Chromatographic conditions determination

With the aim of obtaining a high efficiency in the separation of the four aflatoxins, some chromatographic parameters were investigated, including the mobile phase composition, the detector's wavelength and the column oven temperature. It was found that the variable that most could affect results was the mobile phase composition, because a minimal variation in acetonitrile or methanol concentration can significantly modify the retention times of each aflatoxin. It was concluded that the adequate mobile phase composition was acetonitrile:methanol:milli-Q water, (17.5:17.5:65 v/v/v); an emission and excitation wavelengths ( $\lambda$ ) of 360 nm and 440 nm, respectively in the fluorescence detector and column oven temperature of

50 °C. Under the evaluated conditions, the four analyzed aflatoxins were well separated in less than 20 min, which is within the average of the majority of published methods<sup>21</sup> and agrees with the reports of Wen *et al.*<sup>10</sup> The elution order of the peaks were AFG<sub>1</sub>, AFB<sub>1</sub>, AFG<sub>2</sub> and AFB<sub>2</sub> and the average retention times were 5.414, 7.113, 10.697 and 15.666 min, respectively.

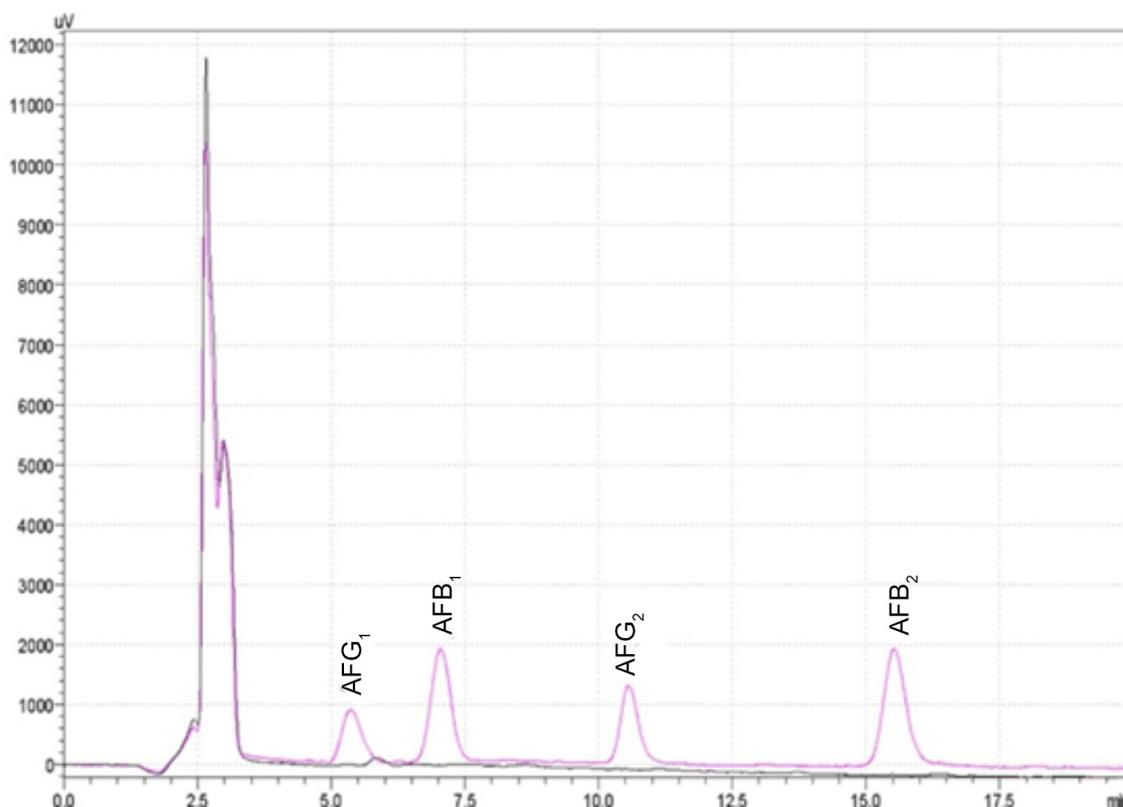
### Selectivity

This parameter studies the capacity of the method to distinguish the analyte from any other interference present in the analyzed samples.<sup>22</sup> In Figure 1, it is observed that the two compared chromatograms of a blank sample and another spiked with a standard solution, show no interfering peaks in the retention times of the four aflatoxins, which demonstrates the selectivity of the method towards other compounds naturally present in corn arepas.

These results agree with those obtained by Muscarella *et al.*,<sup>23</sup> who also did not find matrix effect interferences in samples of almonds, pistachios, corns and oats during the validation of an HPLC method for aflatoxins determination.<sup>23</sup>

### Linearity

Linearity is the capacity of the method to obtain results directly proportional to the concentration or amount of



**Figure 1.** Chromatograms of blank sample (black line) and spiked sample (pink line) with 10 ng mL<sup>-1</sup> of AFG<sub>1</sub> and AFB<sub>1</sub>, and 3.3 ng mL<sup>-1</sup> of AFG<sub>2</sub> and AFB<sub>2</sub>.

analyte in a defined range.<sup>22</sup> Each aflatoxin was evaluated using standard solutions in concentrations of 0.1 to 50 ng mL<sup>-1</sup> of AFG<sub>1</sub> and AFB<sub>1</sub>, and its third part of AFG<sub>2</sub> and AFB<sub>2</sub>. The results of the three calibration curves showed R<sup>2</sup> values of 0.9998 for each aflatoxin demonstrating this method's linearity (Table 1). These results are in agreement with the report of Huang *et al.*,<sup>24</sup> who also found R<sup>2</sup> values higher than 0.999 for all aflatoxins.

### Sensitivity

Sensitivity is the capacity of the method to discriminate small differences in the concentration of the analyte.<sup>22</sup> The LOD and LOQ for each aflatoxin are shown in Table 1.

The LOD of AFB<sub>1</sub> was 0.1 ng mL<sup>-1</sup>, which allows to quantify the contents of this aflatoxin in a arepa sample and to establish if it is according to international normativity where the maximum allowed content is 2 µg kg<sup>-1</sup>.<sup>25</sup> These results demonstrate that the validated method is very sensible for detecting and quantifying aflatoxins and concur with those obtained by other authors who have even detected a concentration of AFB<sub>1</sub> as low as 0.008 µg kg<sup>-1</sup>.<sup>24,26,27</sup>

Due to lack of homoscedasticity in the residual measurements of y axis and the 95% confidence interval of intercept, all the values of linear regression in Table 1 are not including the zero, which was resolved making an "x" axis concentration weighted linear regression to improve their linear characteristics.<sup>18</sup>

As shown in Table 2, with this kind of linear regression, the LOD and LOQ were improved, and the other linear characteristics are better.

### Precision

The precision results for the method under repeatability and intermediate precision are shown in Table 3 for each aflatoxin and tested concentrations.

These data were previously processed with a Shapiro-Wilk test in order to verify the normal distribution. Afterwards, an ANOVA one-way test was performed with the aim to proof the homogeneity of the concentration medium values in four different days. The RSD% results were among 0.2-4.4 for repeatability and 0.6-0.44% for intermediate precision. According to the regulation of the European Commission (EC) No. 401/2006,<sup>28</sup> the recommended RSD% values for each concentration must be equal or lower that those derived from the Horwitz equation, which in turn is based on the analyte concentration, regardless of the matrix and analysis method used. In the case of the method validated in this study, where concentrations lower than 1 µg mL<sup>-1</sup> were analyzed, a RSD ≤ 16% is recommended.<sup>29</sup> All the precision results were lower than 4.4%, indicating a good precision. This values were below the reference values established by the Commission Decision 2002/657/CE<sup>30</sup> and agree with the results achieved by Wen *et al.*,<sup>10</sup> who obtained RSD% values in the range of 0.7-2.7 and 0.9-3.5% for repeatability and intermediate precision, respectively. On the other hand, Chan<sup>31</sup> obtained RSD% far higher, between 9.06 and 10.13% in repeatability conditions, which suggests that the method validated here has a high precision.

### Recovery

A recovery assay was done in corn arepas spiked with a solution of AFG<sub>1</sub> and AFG<sub>2</sub> at concentrations of 72.8 and 18.1 ng mL<sup>-1</sup>, respectively. Table 4 shows the percentage of recovery in corn arepas spiked with AFG<sub>1</sub> and AFG<sub>2</sub>.

According to the regulation (EC) 401/2006 of the European Commission, the recommended value of recovery for concentrations of aflatoxins > 10 µg kg<sup>-1</sup> must be of 80 to 110%, and for higher concentrations must be of 80 to 110%.<sup>28</sup> The recovery obtained in this study for AFG<sub>1</sub> and

**Table 1.** Linearity, LOD and LOQ of AFG<sub>1</sub>, AFB<sub>1</sub>, AFG<sub>2</sub> and AFB<sub>2</sub>

Parameter	AFG <sub>1</sub>	AFB <sub>1</sub>	AFG <sub>2</sub>	AFB <sub>2</sub>
Linear equation	y = 7850.4x - 1268.3	y = 20695x - 3127.2	y = 3646.3x - 953.6	y = 6558.5x - 1366.7
R <sup>2</sup>	0.9999	0.9998	0.9998	0.9998
LOD / (µg kg <sup>-1</sup> )	0.40	0.45	0.47	0.53
LOQ / (µg kg <sup>-1</sup> )	1.34	1.49	1.55	1.76

**Table 2.** Linearity, LOD and LOQ of AFG<sub>1</sub>, AFB<sub>1</sub>, AFG<sub>2</sub> and AFB<sub>2</sub> with weighted linear equation

Parameter	AFG <sub>1</sub>	AFB <sub>1</sub>	AFG <sub>2</sub>	AFB <sub>2</sub>
Linear equation	y = 7725.6x + 626.5	y = 20299.7x + 2873.9	y = 3568.7x + 224.6	y = 6374.1x + 1433.2
R <sup>2</sup>	1.000	1.000	1.000	1.000
LOD / (µg kg <sup>-1</sup> )	0.08	0.10	0.10	0.10
LOQ / (µg kg <sup>-1</sup> )	0.26	0.33	0.33	0.33

**Table 3.** Repeatability and intermediate precision of the HPLC method for the determination of AFG<sub>1</sub>, AFB<sub>1</sub>, AFG<sub>2</sub> and AFB<sub>2</sub>

Aflatoxin	Concentration / (ng mL <sup>-1</sup> )	Repeatability			Intermediate precision		
		X (n = 6)	s	RSD / %	X (n = 6)	s	RSD / %
AFG <sub>1</sub>	2	2.12	0.06	2.80	2.31	0.05	2.18
	46	44.64	0.18	0.40	48.39	0.33	0.69
	92	91.41	0.22	0.24	97.00	0.78	0.79
AFB <sub>1</sub>	2	2.11	0.04	2.06	2.41	0.05	2.00
	46	44.44	0.43	0.96	49.51	0.35	0.71
	92	91.01	0.79	0.87	99.03	0.86	0.87
AFG <sub>2</sub>	0.6	0.76	0.03	4.42	0.84	0.04	4.31
	13.9	14.83	0.05	0.37	16.03	0.10	0.61
	27.9	30.10	0.09	0.31	32.00	0.29	0.91
AFB <sub>2</sub>	0.6	0.77	0.03	3.73	0.89	0.04	4.36
	13.9	14.83	0.05	0.37	16.03	0.10	0.61
	27.9	29.92	0.29	0.96	32.52	0.30	0.93

X: mean concentration; n: number of replicates; s: standard deviation; RSD%: relative standard deviation.

**Table 4.** AFG<sub>1</sub> and AFG<sub>2</sub> percentage of recovery

Aflatoxin	$\chi'$	$\chi$	$\chi_{spiked}$	$\chi' - \chi$	R / %
AFG <sub>1</sub>	71.1	15.3	72.8	55.78	76.6
AFG <sub>2</sub>	208.4	18.1	243.2	190.26	78.2

$\chi'$ : concentration of the spiked sample;  $\chi$ : concentration of non-spiked sample;  $\chi_{spiked}$  is the concentration added. Concentrations in ng mL<sup>-1</sup>.

AFG<sub>2</sub> was 76.6% and 78.2%, respectively, which is close to the 80% recommended. This value could be improved during sampling arepas corn aflatoxin using immunoaffinity columns. These results are similar to those reported by Huang,<sup>24</sup> who obtained recoveries of 74.7% for AFG<sub>1</sub> and 80.8% for AFG<sub>2</sub> from peanut.

#### Aflatoxins occurrence in the HPLC analyzed samples

Once the HPLC method was validated, the analysis of 144 samples of 12 different brands commercialized in Manizales, Colombia was made. Additionally, from the 144 samples analyzed with HPLC, 58 (40.27%) were randomly chosen to undergo a screening process previous to the chromatographic analysis with the ELISA technique and a concordance of 98.2% between the ELISA and HPLC results was achieved.

HPLC levels of aflatoxins in corn arepas samples analyzed with values above the LOD are shown in Table 5.

Aflatoxins were found, mainly AFG<sub>1</sub>, in 14 of the 144 analyzed samples (9.72%), at levels between 0.95 and 11.56  $\mu\text{g kg}^{-1}$ , from which seven (4.86%) showed values above the limits allowed by the national normative for total aflatoxins (4  $\mu\text{g kg}^{-1}$ ).<sup>32</sup> AFB<sub>1</sub> and AFB<sub>2</sub> were not detected

**Table 5.** Aflatoxins level ( $\mu\text{g kg}^{-1}$ ) in the corn arepas analyzed samples

Sample ID	Sampling	Type of sample	AFG <sub>1</sub>	AFB <sub>1</sub>	AFG <sub>2</sub>	AFB <sub>2</sub>
212	1	HB	1.15	–	–	–
244	1	HB	1.82	–	–	–
251	1	HB	1.72	–	–	–
252	1	HB	2.50	–	–	–
253	1	HB	2.67	–	7.22	–
254	1	HB	1.90	–	–	–
256	1	HB	0.95	–	3.82	–
131	2	IB	6.05	–	–	–
132	2	IB	11.56	–	–	–
133	2	IB	9.15	–	–	–
134	2	IB	10.99	–	–	–
155	2	IB	5.42	–	–	–
156	2	IB	6.61	–	–	–
162	2	IB	8.80	–	–	–

HB: handcrafted brand; IB: industrial brand; –: obtained value was smaller than LOD.

in any of the samples analyzed. This results are below that those reported by Arcila and Martínez,<sup>12</sup> who found that 27.8% of corn arepas samples analyzed by the method of TLC had trace amounts of total aflatoxins (< 3  $\mu\text{g kg}^{-1}$ ) and only 1.8% had 10  $\mu\text{g kg}^{-1}$  of AFB<sub>1</sub>. In the same way, this prevalence is much better than that reported by a study made in Manizales in 1996,<sup>13</sup> where the presence of total aflatoxins was detected in 100% of the arepas samples analyzed by ELISA method, the majority above the levels allowed by the international normative.

On the other hand, the results of our study indicate that the occurrence of aflatoxins in corn arepas marketed in the city of Manizales (9.72%) is relatively low compared with those reported in other countries like Ecuador (26%),<sup>33</sup> Venezuela (16.6%),<sup>33</sup> Mexico (56%),<sup>34</sup> and Peru (82%).<sup>35</sup> Moreover, this incidence is much lower than the 29% overall incidence of AFB<sub>1</sub> found in feed for poultry and pigs used in Colombia<sup>15</sup> and differ from those reported by Diaz *et al.*,<sup>16</sup> who investigated aflatoxin contamination in selected Colombian food and obtained an incidence of 8.9% of AFB<sub>1</sub>, 1.2% of AFB<sub>2</sub> and only 0.4% of AFG<sub>1</sub> and AG<sub>2</sub>.

Although AFB<sub>1</sub> has been reported as the most dangerous of the four aflatoxins studied, finding AFG<sub>1</sub> in the analyzed samples is significant because it has recently been found that oral administration of AFG<sub>1</sub> could induce chronic lung inflammation, which may provide a pro-tumor microenvironment to contribute to lung tumorigenesis.<sup>36</sup> Also, studies have shown that AFG<sub>1</sub> was the most frequently detected contaminating mycotoxin in the foodstuffs of the high incidence areas of esophageal cancer in north China.<sup>37</sup>

After applying a variance analysis (ANOVA) to the data, it was found that a statistical significant difference exists in the aflatoxins levels among the arepas brands analyzed ( $p = 0.0002$ ). A higher frequency of aflatoxins was found in the handcrafted brands, which is explained because in the small industry there is no defined operations nor standardized processes since the reception of the raw material up to the washing of the corn and exists the need to implement control measures to avoid the fungus growth.<sup>14</sup> Additionally, in the industrial process, additives or coadjuvants are added to the corn arepa to reduce the water activity, which acts directly as conservatives inhibiting the fungus growth.<sup>38</sup>

The finding of aflatoxins in the corn arepas samples shows the potential contamination with aflatoxigenic molds that begin in the corn grain and then could reduce during the storage, distribution, and processing of arepas.<sup>12</sup> Given the carcinogenic, mutagenic and genotoxic potential of aflatoxins, there are no insignificant quantities, because it is impossible to establish a toxicological reference value where the formation of tumors is not induced.<sup>11</sup> According to numerous studies, the aflatoxins consumption, even at very low levels (i.e., 1 ng kg<sup>-1</sup> p.c. day<sup>-1</sup>), contributes to liver cancer development.<sup>25,39-43</sup> In Colombia, it has been estimated that the exposure to consumption of corn and corn products is of <0.699 and <0.198 µg kg<sup>-1</sup> p.c. day<sup>-1</sup>, respectively, which indicates that the population might be at risk.<sup>11</sup>

## Conclusions

The validated method for AFG<sub>1</sub>, AFB<sub>1</sub>, AFG<sub>2</sub> and AFB<sub>2</sub> determination in white corn arepas by HPLC presented

satisfactory values of robustness, selectivity, linearity, LOD and LOQ. In the same way, the acceptance criteria for validating the methodology were achieved, with a RSD% lower to 4.5% in the precision assays and a recovery above 76.6%. Thus, this method can potentially be applied for the determination of aflatoxins in other food matrices with little or no modification.

Aflatoxins were found in 9.72% of the analyzed samples at levels between 0.95 and 11.56 µg kg<sup>-1</sup>, from which seven (4.86%) had values above the limits allowed by the local normative for total aflatoxins.

It was found that the samples contained primarily AFG<sub>1</sub>, which is one of the most frequently detected mycotoxins in grains and foodstuffs and also has been linked to lung and esophageal cancer in north China.

The results obtained comprise the first report in the world about aflatoxins determination in corn arepas using the HPLC with a fluorescence detector method.

## References

1. Soriano-del-Castillo, J. M.; *Micotoxinas en Alimentos*; 1ª ed., Ediciones Díaz de Santos: Madrid, 2007.
2. Urrego Novoa, J. R.; Díaz, G. J.; *Rev. Fac. Med. Univ. Nac. Colomb.* **2006**, *54*, 108.
3. <http://www.ansci.cornell.edu/plants/toxicagents/aflatoxin/aflatoxin.html> accessed on February 28, 2014.
4. <http://monographs.iarc.fr/ENG/Monographs/vol100F/> accessed on February 28, 2014.
5. Köppen, R.; Koch, M.; Siegel, D.; Merkel, S.; Maul, R.; Nehls, I.; *Appl. Microbiol. Biotechnol.* **2010**, *86*, 1595.
6. Stroka, J.; van Otterdijk, R.; Anklam, E.; *J. Chromatogr. A* **2000**, *904*, 251.
7. Ghali, R.; Hmaissia-khlifa, K.; Ghorbel, H.; Maaroufi, K.; Hedili, A.; *Food Control* **2008**, *19*, 921.
8. Trucksess, M. W.; Brumley, W. C.; Nesheim, S.; *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 973.
9. Liu, S.; Qiu, F.; Kong, W.; Wei, J.; Xiao, X.; Yang, M.; *Food Control* **2013**, *29*, 156.
10. Wen, J.; Kong, W.; Wang, J.; Yang, M.; *J. Sep. Sci.* **2013**, *36*, 3709.
11. Instituto Nacional de Salud; *Evaluación de Riesgo de la Aflatoxina B1 (AFB1) en Arepa de Maíz en Colombia*, 2013.
12. Arcila García, M. P.; *Biosalud* **2002**, *1*, 45.
13. Arango Mejía, M. C.; *Biosalud: Revista de Ciencias Básicas* **2002**, *1*, 45.
14. Instituto Colombiano de Normas Técnicas y Certificación; *NTC 1232 Granos y Cereales. Método de Análisis de Aflatoxinas de Ocurrencia Natural (B1, B2, G1 y G2)*. ICONTEC: Bogotá, 1996.
15. Céspedes, A. E.; Díaz, G. J.; *J. AOAC Int.* **1997**, *80*, 1215.

16. Díaz, G. J.; Perilla, N. S.; Rojas, Y.; *Mycotoxin Res.* **2001**, *17*, 15.
17. Manso, S.; Pezo, D.; Gómez-Lus, R.; Nerin, C.; *Food Control* **2014**, *45*, 101.
18. Thompson, M.; Stephen, L. R.; Wood, R.; *Pure Appl. Chem.* **2002**, *74*, 835.
19. [https://www.eurachem.org/images/stories/Guides/pdf/MV\\_guide\\_2nd\\_ed\\_EN.pdf](https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf) accessed on February 2015.
20. Miller, J. C.; Miller, J. N.; *Statistics for Analytical Chemistry*, 3<sup>rd</sup> ed., Ellis Horwood PTR Prentice-Hall: New York, 1993.
21. Alvito, P. C.; Sizoo, E. A.; Almeida, C. M. M.; Egmond, H.; *Food Anal. Method.* **2010**, *3*, 22.
22. <http://www.chem.agilent.com/Library/primers/Public/5990-5140EN.pdf> accessed in February 2015.
23. Muscarella, M.; Iammarino, M.; Nardiello, D.; Lo Magro, S.; Palermo, C.; Palermo, D.; Centonze, D.; *Food Addit. Contam.* **2009**, *26*, 1402.
24. Huang, B.; Han, Z.; Cai, Z.; Wu, Y.; Ren, Y.; *Anal. Chim. Acta* **2010**, *662*, 62.
25. <http://www.fao.org/docrep/007/y5499s/y5499s00.htm> accessed in February 2015.
26. Fu, Z.; Huang, X.; Min, S.; *J. Chromatogr., A* **2008**, *1209*, 271.
27. Sook, H.; Kim, H. J.; Ok, H. E.; Hwang, J. B.; Chung, D.; *Food Chem.* **2007**, *102*, 385.
28. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:070:0012:0034:EN:PDF> accessed in February 2015.
29. <http://www.bii.mx/documentos/horwitzCm10.pdf> accessed in February 2015.
30. <http://cemu10.fmv.ulg.ac.be/OSTC/2002657EC.pdf> accessed in March 2014.
31. Chan, D.; MacDonald, S. J.; Boughtflower, V.; Brereton, P. J.; *J. Chromatogr., A* **2004**, *1059*, 13.
32. Ministerio de Salud y Protección Social; Resolución No. 4506, *Por la Cual se Establecen los Niveles Máximos de Contaminantes en los Alimentos Destinados al Consumo Humano y se Dictan Otras Disposiciones*, Bogotá, 2013.
33. Fernández, G.; Negrón, G.; Isea, G.; Sánchez, E.; *Rev. Fac. Agron. (LUZ)* **2000**, *X*, 63.
34. <http://www.ejournal.unam.mx/bot/074-02/BOT74207.pdf> accessed in January 2015.
35. Caballero, J.; Arbaiza, T.; Lucas, O.; *Rev. Inv. Vet. Perú* **2001**, *12*, 1.
36. Liu, C.; Shen, H.; Yi, L.; Shao, P.; Soulika, A.; Meng, X.; Xing, L.; Yan, X.; Zhang, X.; *Toxicology Letters, in press*, DOI:10.1016/j.toxlet.2014.11.002.
37. Zhang, X. H.; Xie, T. X.; Li, S. S.; *Biomed. Environ. Sci.* **1998**, *1*, 140.
38. <ftp://ftp.fao.org/docrep/fao/005/y1390e/y1390e00.pdf> accessed in February 2015.
39. Comisión Europea, *Diario Oficial de la Unión Europea*, Reglamento No. 165/2010, 2010.
40. Guengerich, F. P.; Johnson, W. W.; Ueng, Y. F.; Yamazaki, H.; Shimada, T.; *Environ. Health Perspect.* **1996**, *104*, 557.
41. Peers, F. G.; Linsell, C. A.; *Br. J. Cancer* **1973**, *27*, 473.
42. Jackson, P. E.; Groopman, J. D.; *Best Pract. Res., Clin. Gastroenterol.* **1999**, *13*, 545.
43. [http://ec.europa.eu/food/fs/ifsi/eupositions/cac/archives/cac\\_item10a\\_es.html](http://ec.europa.eu/food/fs/ifsi/eupositions/cac/archives/cac_item10a_es.html) accessed in February 2015.

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