

1 *Review*

## 2 **Aflatoxins in Mozambique: Impact and Potential for** 3 **Intervention**

4 **Edgar Cambaza**<sup>1,2\*</sup>, **Shigenobu Koseki**<sup>1</sup> and **Shuso Kawamura**<sup>1</sup>

5 <sup>1</sup>Laboratory of Food Process Engineering, Graduate School of Agriculture, Hokkaido University, Japan

6 <sup>2</sup>Department of Biological Sciences, Faculty of Science, Eduardo Mondlane University, Av. Julius Nyerere, nr.  
7 3453 Maputo, Mozambique

8 \* Correspondence: edy@bpe.agr.hokudai.ac.jp; Tel.: +81-80-2876-1106

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10 **Abstract:** Aflatoxins are a known cause of primary liver cancer in Mozambique since  
11 pre-independence epidemiological studies. However, their impact goes beyond the public health,  
12 affecting the country's economy and raising legal concerns. As a developing country endemic for  
13 *Aspergillus*, the nation has been struggling to keep pace with external trade quality demands,  
14 delicate policy making, still dealing with the farmers' limitations to control the contamination. The  
15 contamination shows variations over time, space and the different commodities. Considering the  
16 recommendations of the Codex Alimentarius, the major crops will be highly implicated unless  
17 there is major intervention from the authorities to control the toxins.

18 **Keywords:** aflatoxins, Mozambique, impact, health, economy, regulation

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### 20 **1. Introduction**

21 Aflatoxins are not only a health and agricultural issue. They started as the cause for the loss of  
22 several turkeys in England [1,2], later the root of outbreaks of human aflatoxicosis in places such as  
23 India [3] and Kenya [4], and liver cancer in Mozambique [5]. However, the problem is far more  
24 complex, affecting trade and policymaking [6,7]. For example, limits for aflatoxin consumption have  
25 been actively discussed by the International Agency for Research on Cancer and Codex  
26 Alimentarius, among other organizations.

27 Mozambique has no specific legislation for aflatoxins [8] but the matter has already been  
28 discussed in academic and professional circles. These discussions were influenced by factors such as  
29 the pioneer realization of aflatoxin impact in the onset of primary liver cancer [5,9], rejection of  
30 peanuts by international buyers [7] and the casualties in Kenya [10].

31 This review aimed to provide a glance of how the aflatoxins are affecting the health, economy  
32 and the legal environment in Mozambique and provide some comments about it.

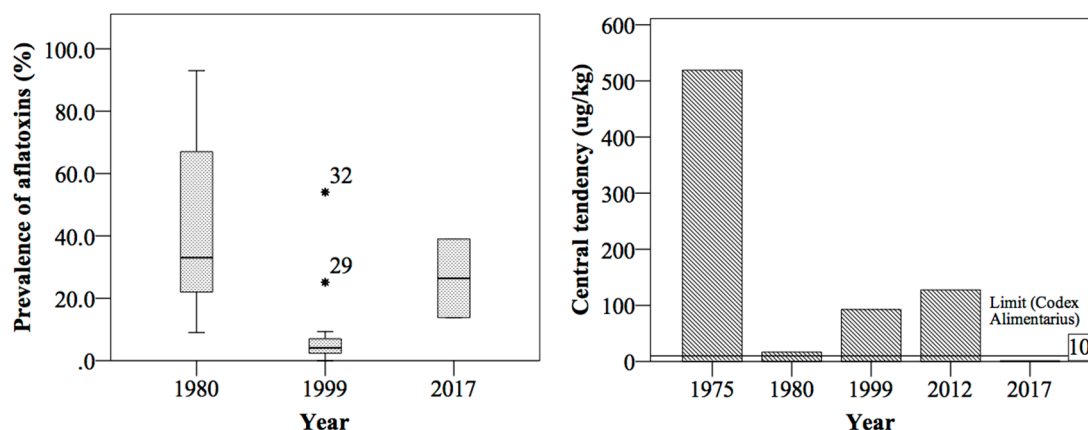
### 33 **2. Impact**

#### 34 *2.1. Risk analysis*

35 This analysis will be focused on the potential exposure to aflatoxins in Mozambique, based on  
36 the food, prevalence of contamination and toxin levels, considering space and time. There is very  
37 little information on the demographic profile of who actually consume the food or the safety  
38 measures, practices or behaviors capable of affecting the exposure. A more accurate risk analysis  
39 would also require information on the quantity and frequency of intake for each food, and the  
40 Mozambican culture and habits are very heterogeneous, sometimes within the same region. For  
41 instance, Linsell and Peers [11] admitted their incapacity to assess the intake of a home brewed beer  
42 made from cereals or groundnuts, "often taken irregularly as snacks". Thus, this assessment is just a

43 kick-start for future researchers willing to know more about the exposure of Mozambicans to  
44 aflatoxins.

45 The records show considerable variation in aflatoxin contamination among the samples  
46 analyzed throughout the years ( $p < 0.001$ ) (Figure 1). It apparently declined since 1980, with the  
47 lowest level in 1999.



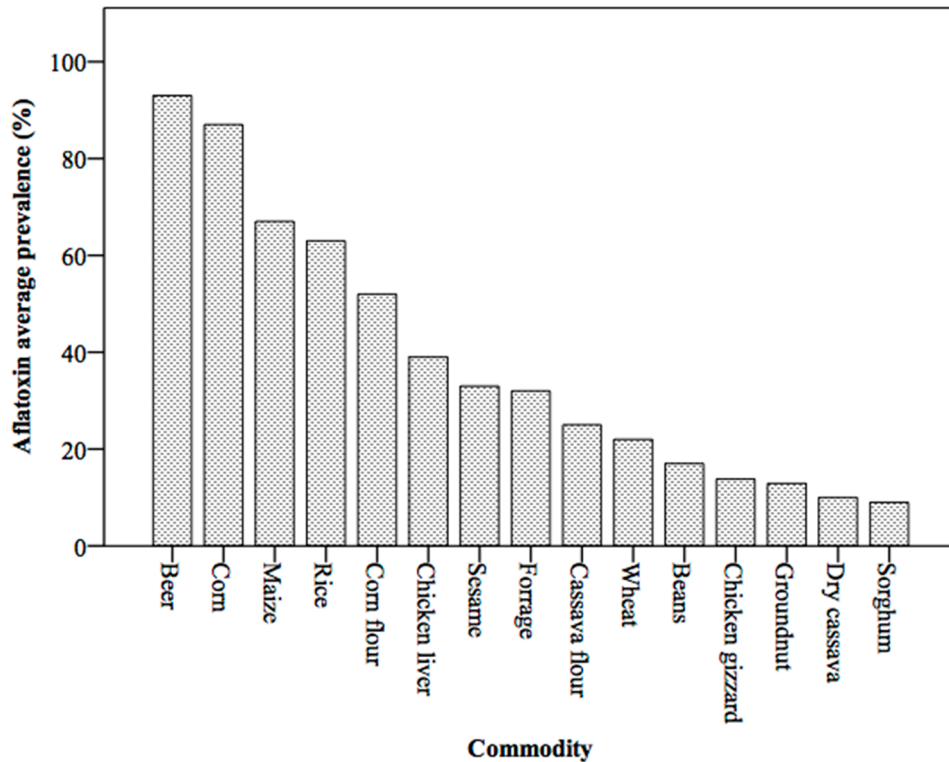
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49 Figure 1. Prevalence (left) and levels (right) of aflatoxins over time. Based on Casadei [12], Van  
50 Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These studies were  
51 carried from 1985 to 2017 and included in total 934 samples including agricultural crops, chicken  
52 livers and gizzards.

53 It could imply that the risk of contamination was highly reduced but it is rising again. However,  
54 it is arguable because there was an overall change of commodities analyzed, especially an increased  
55 focus on groundnuts and other products economically prioritized. It is hard to know if the risk of  
56 contamination is decreasing over time because there is a generalized effort to reduce food insecurity  
57 [14,15], at some time not allowing the rejection of contaminated food [12], on one hand, and the  
58 dispersion seems to reflect the level of product variety. For example, groundnut was the sole  
59 product analyzed in 1999.

60 Regarding the aflatoxin levels, there were also notable variations in central tendency, though a  
61 Kruskal-Wallis test did not reveal significant differences among them ( $p = 0.478$ ). This lack of  
62 difference was certainly due to the samples' high level of dispersion, specially the ones from 1975.  
63 Anyway, the central tendency was almost consistently (except in 2017) the above the maximum limit  
64 recommended by the Codex Alimentarius ( $10 \mu\text{g}/\text{kg}$ ). The highest record is from 1975, decreasing  
65 brusquely in the ensuing five years. Then it grew until 2012 and then decreased again. Samples from  
66 2017 are from chicken giblets, different from the crops directly contaminated in the field. Thus, direct  
67 analyses of groundnuts or maize will probably still show high levels of contamination.

68 Figure 2 displays the percentage of samples positive for aflatoxins regarding different  
69 commodities. Beer was surprisingly the most contaminated. This result conflicts with the perceived  
70 beer factories' high quality standards. However, this information was obtained by Casadei [12]  
71 several years ago and it is likely to have changed due to international demands because  
72 Mozambique exports beer. Corn and maize were among the most widely contaminated, as expected.  
73 However, groundnuts presented a considerably low prevalence if compared with other  
74 commodities. Maybe its hard shell and other forms of resistance lead the fungus to produce high  
75 quantities of chemicals in order to invade.



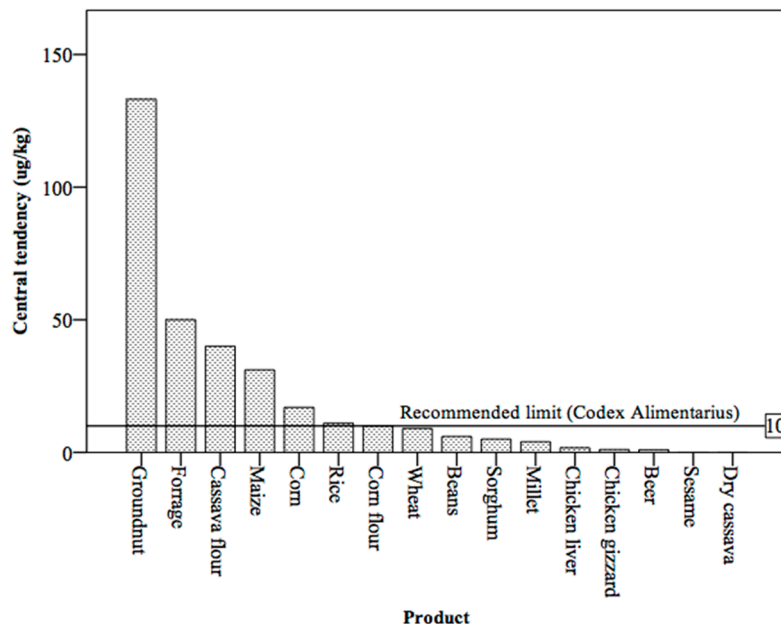
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78 Figure 2. Average aflatoxin prevalence among the samples. Based on Casadei [12], Van  
 79 Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These studies were  
 80 carried from 1985 to 2017 and included in total 934 samples including agricultural crops, chicken  
 livers and gizzards.

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The foods showed varying levels of contamination (Figure 3).



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Figure 3. Aflatoxin levels in different commodities. The Codex Alimentarius has set the reference  
 limit for peanuts. It can be regarded as a good threshold to make a rough risk evaluation for food,  
 probably not for feed. Feed waste was excluded from the graph because of its overwhelming value  
 (443 g/kg), outside the order of magnitude in relation to the other foods. Based on Casadei [12],  
 Van Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These studies were

88 carried from 1985 to 2017 and included in total 934 samples including agricultural crops, chicken  
89 livers and gizzards.

90 Feed waste was the most contaminated commodity, but it does not pose a direct threat to  
91 health, as there is no intention to use it as food or feed. There were six commodities above the limit  
92 recommended by the Codex Alimentarius Commission [16], while most foods did not seem to pose a  
93 significant threat to health.

## 94 2.2. Human and animal health

95 There is a wide literature on the impact of aflatoxins in human health. Thus, this review will  
96 refrain from echoing most of it, providing solely the little contribution from Mozambican aflatoxin  
97 research. Not much has been written since the studies in Inhambane over 40 years ago [9] and other  
98 related. Instead, several authors have been repeating their results, frequently neglecting the  
99 timeframe. Still, there are “side analyses” in few animal studies giving a hint on how the toxins have  
100 been affecting the health in Mozambique.

101 Aflatoxins are a known component of primary liver cancer’s etiology [17], but they also affect  
102 children’s growth and livestock [18]. Sineque, *et al.* [8] tried to see the relationship between liver  
103 weight and AFB<sub>1</sub> levels in chicken but they did not seem correlated. However, Anjos, *et al.* [19]  
104 found signs of aflatoxin toxicity in chicks. According to them, it not only causes organ swelling but  
105 also growth retardation, liver lesions, reduction in serum proteins and death.

106 The incidence of liver cancer can reach 40% in some African regions [20], and it is endemic in  
107 Mozambique, causing 65-70% of all cancer deaths in males and 30-55% in females [21]. However, it  
108 has a late-stage effect in the development of hepatocellular carcinoma, especially for people carrying  
109 hepatitis (HBV) [5].

110 Mozambique certainly faces the same challenges as any other tropical country with crops  
111 infested by aflatoxigenic *Aspergillus*. The country only needs to verify at what extent there are  
112 similarities or not between the local and international profiles because there is plenty of information  
113 from all over the world. The methods are all developed and glimpses on what to expect also  
114 available. Now it is simply a matter of initiative.

## 115 2.3. Etiology

116 By now, it is clear that maize, cassava and groundnuts are heavily contaminated with  
117 aflatoxins, in incidence, prevalence and levels. This poses a problem because they are the main  
118 source of agricultural income to the country, and staple food for the population [22].

119 Aflatoxins are particularly important for the peanut industry [18], and this is attributable to  
120 their potential to limit consumption by humans and livestock [23]. In Mozambique, groundnut is an  
121 important source of cooking oil [24]. Moreover, the country produces the highest volume of  
122 groundnuts in Southern Africa, mainly in Nampula and Cabo Delgado Provinces [7,25], and it is the  
123 most cultivated legume [26] locally. The high toxin content led South African importers to stop  
124 purchasing Mozambican groundnuts [7,17]. These international trade issues are happening all over  
125 Africa, especially because of European Union’s demands [6]. From groundnuts only, Mozambique  
126 might lose up to US \$4.000.000 in revenue [27].

127 Maize and cassava also require some attention. These commodities require quality certification  
128 for export, and the presence of aflatoxin is a strong barrier. Maize is the country’s most important  
129 staple food. Bandyopadhyay and Dubois [28] said Mozambique produces 1.5 million tons per year,  
130 mostly in small farms for subsistence. Cassava raises even more concerns as it produces cyanogenic  
131 glycosides.

132 Anjos, *et al.* [19] said the most heavily infested grains are primarily sold for poultry feeding.  
133 This raises another issue with economic implications. Diverting the risk to animals might result in  
134 considerable losses. Indeed, that is exactly how AFB<sub>1</sub> was discovered in first place [1,2]. However,  
135 the extent of such losses in Mozambique, or at least the risk, has yet to be investigated.

### 136 3. Resources

#### 137 3.1. Facilities

138 Earlier analyses were frequently performed in foreign laboratories but the local has been  
139 improving substantially. The capacity is expected to increase. The Directory of Calibration and  
140 Assay Laboratories in Mozambique [29] mentioned only two major institutions with the capacity for  
141 aflatoxin analyses:

- 142 • National Laboratory of Water and Food Hygiene (LNHAA);
- 143 • Directorate of Animal Sciences. The Mozambican Institute for Research on Agriculture (IIAM).

144 They are both in Maputo area and only LNHAA is indicated as accredited. Mondlane, *et al.* [30]  
145 used both laboratories to analyze poultry feed, and Sineque, *et al.* [8] used IIAM's to study AFB<sub>1</sub> in  
146 livers and gizzards. According to Baquete and Freire [31]. LNHAA uses more TLC for aflatoxin  
147 analysis but the laboratory also has HPLC. The other laboratory uses ELISA.

148 The International Institute for Tropical Agriculture (IITA) opened research facilities in the city  
149 of Nampula [32]. They include a laboratory where two *Aspergillus* bio-control products are prepared.  
150 They are thought to reduce the mold infestation of groundnut and maize fields and ultimately lower  
151 the aflatoxin levels to meet international commercial standards.

152 Harmsen, *et al.* [18] publish a report describing a new laboratory for soil studies in the Tertiary  
153 Polytechnic Institute of Manica. It had no equipment for aflatoxin analysis, but they manifested the  
154 intention to include such gadgets in the project. They specifically mentioned HPLC, but the report  
155 admitted some alternatives.

#### 156 3.2. Projects

157 The National Laboratory for Food and Water Hygiene (LNHAA) is arguably the best equipped  
158 in the country for aflatoxin screening projects. According to Casadei [12], this institution actively  
159 participated in major programs for quality control or the results and evaluation of analytic methods  
160 for aflatoxins. Some collaborators are AOAC (Association of Official Analytical Chemistry) and  
161 IARC (International Agency for Research on Cancer). We shall expect stronger alliances in this  
162 increasingly globalized world, with countries cooperating to face similar issues.

163 Baquete and Freire [31] told about a plan to be developed between LNHAA and the Faculty of  
164 Agriculture from Eduardo Mondlane University, called Groundnut Improvement Project, to  
165 develop practical guidelines from small farmers and traders. The current literature has no  
166 information on the follow up or if it was implemented, but the idea is constructive and would  
167 certainly make a difference. Yet, it probably has some connection with the bio-control project  
168 implemented by IITA in Nampula, because the university was also involved, and there is also  
169 intention to include an extension component.

170 The multi-toxin screening by Warth, *et al.* [13] in samples from Mozambique and Burkina Faso  
171 does not seem to involve major authorities from any of these countries. Instead, it took place in  
172 Nigeria in collaboration with the Austrian Development Agency. Yet, the project aims to introduce  
173 mycotoxin control technologies in Mozambique, Tanzania and Burkina Faso. It is likely to include  
174 entities from these countries in the future.

#### 175 3.3. Personnel

176 Food science is emerging in Mozambique, though subjects such as agriculture, biology,  
177 chemistry and medicine have covered various related topics. The older generation of food scientists  
178 acquired their qualifications in different countries, and some of the newer are still doing the same.  
179 However, some tertiary institution started licentiate and masters programs on food safety,  
180 technology and nutrition [33-35].

181 Projects such as the IITA's *Aspergillus* bio-control initiative can play a significant role in capacity  
182 building of national partners [28], and this can be a good kick-start to motivate young students to  
183 endeavor in aflatoxin research and mitigation.

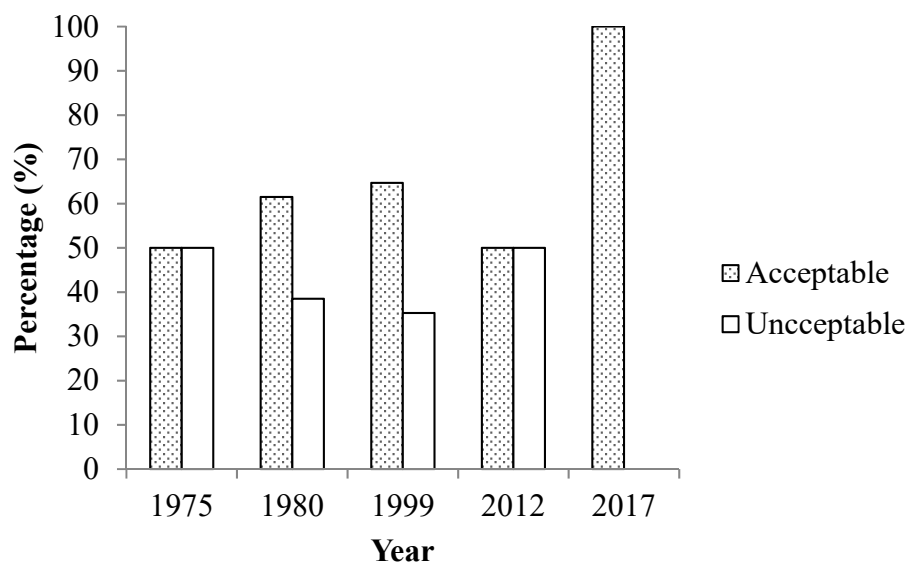
#### 184 4. Levels and regulation

185 Many countries have distinct limits and it is changing trade patterns [36], and most are between  
 186 4 and 20  $\mu\text{g}/\text{kg}$  for maize and peanuts [37]. African countries [37], including Mozambique [8], do not  
 187 have adequately protective regulations for AFB<sub>1</sub>. Yet, the regulations from the developed countries  
 188 impact the emerging economies and its influence in the trade is increasingly unavoidable. For  
 189 example, the European Union new Standard was about to reduce US \$670 million in exports from  
 190 African countries [38].

191 There is a law for Consumer's Protection recently approved by the Government of  
 192 Mozambique [39] but it lacks the depth to regulate mycotoxin contamination. Furthermore,  
 193 Mozambique is a member of the Food and Agriculture Organization (FAO). World Health  
 194 Organization (WHO) and the World Trade Organization (WTO). These organizations have been  
 195 directing efforts to ensure the best feasible manufacturing and trade practices among their members.

196 A joint FAO/WHO Expert Committee on Food Additives (JECFA) established limits for  
 197 aflatoxins through the Codex Alimentarius Commission [16]. The current limit for aflatoxin type B is  
 198 10  $\mu\text{g}/\text{kg}$ , only for peanut and related nuts. It is still subject to some controversy because some  
 199 developing countries advocate they should be higher while the European Union (EU) suggested a  
 200 lower limit. Nevertheless, it is so far the best-agreed global threshold to evaluate the risk of  
 201 exposure. It is possible to give a glance at the acceptability of some products from Mozambique  
 202 considering the recommendation from the codex and the current information on the country's  
 203 aflatoxin levels.

204 Most food fell within the acceptable range, though sometimes the values over 10  $\mu\text{g}/\text{g}$  were  
 205 present in half of the samples (Figure 4). All samples from 2017 appeared to be within the acceptable  
 206 range but those were all from animal products, presumably low in aflatoxins. Ecologically, animals  
 207 are consumers, and farm animals are usually primary consumers, if the feed is made from  
 208 vegetables. Since the toxin reaches the livestock or poultry through feed, animal products are not  
 209 expected to have the same aflatoxin levels as the plants. These results are alarming because the  
 210 results from 1975 and 2012 are almost 40 years apart but the situation remains, disregarding the most  
 211 recent values. This is not encouraging for foreign companies willing to purchase commodities from  
 212 Mozambique.



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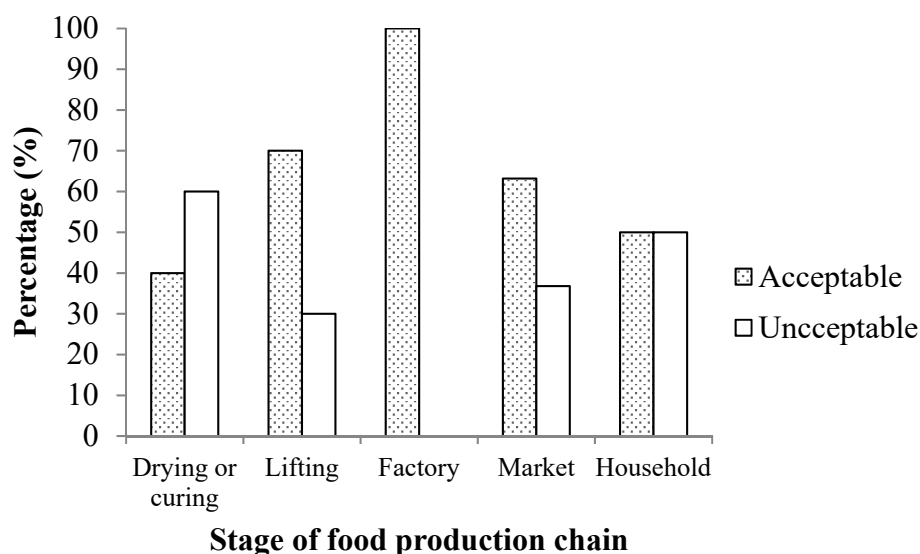
214 Figure 4. Evaluation of the food analyzed over time based on the Codex Alimentarius limit. Based on  
 215 Casadei [12], Van Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These  
 216 studies were carried from 1985 to 2017 and included in total 934 samples including agricultural  
 217 crops, chicken livers and gizzards.

218 Regarding the different districts, there were variations from 0% to 100% of acceptability (Table  
 219 1). In this case, the sample size probably influenced the results, as one can see bold divisions such as  
 220 all, half or one third. Nevertheless, these values can provide a rough idea of where to direct the  
 221 highest concerns. Most samples (67.4%) are acceptable, but there are some cases of absolute  
 222 unacceptability (Amendo and Muecate). Mugovola also had most samples with contamination  
 223 above the limit (66.7%). Erati, Inhambane, Murrupula, Nacaroa (all 50%) and Nampula (40%) also  
 224 require attention.

225 Table 1. Acceptability level of samples from different districts considering the Codex Alimentarius.  
 226 Note: the overall value do not accurately reflect the ones on the table because it includes some data  
 227 not specific for one or another district but from several areas of Mozambique. Based on Casadei [12],  
 228 Van Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These studies were  
 229 carried from 1985 to 2017 and included in total 934 samples including agricultural crops, chicken  
 230 livers and gizzards.

Area	Codex Evaluation (%)		Total (%)
	Acceptable	Unacceptable	
Amendo	0.0	100.0	100.0
Eрати	50.0	50.0	100.0
Inhambane	50.0	50.0	100.0
Maletna	100.0	0.0	100.0
Maputo	100.0	0.0	100.0
Mecuburi	100.0	0.0	100.0
Muabassa	100.0	0.0	100.0
Muecate	0.0	100.0	100.0
Mugovola	33.3	66.7	100.0
Murrupula	50.0	50.0	100.0
Nacala	100.0	0.0	100.0
Nacaroa	100.0	0.0	100.0
Nampula	60.0	40.0	100.0
Ribaue	100.0	0.0	100.0
Overall	63.2	36.8	100.0

231 Just after harvest, most samples are above the limit (Figure 5). There is certainly selection of the  
 232 less moldy specimens and it improves substantially the lot's quality.  
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Figure 5. Percentage of food acceptability through the chain of production considering the Codex Alimentarius. Based on Casadei [12], Van Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These studies were carried from 1985 to 2017 and included in total 934 samples including agricultural crops, chicken livers and gizzards.

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There is an agreement that level of contamination is not a linear function of the amount of mold infestation [40], but this logic might not apply to the field. The argument is based on the fact that even when the fungus is eliminated the toxins remain in the commodity. Such processes of elimination are post-harvest and there are cases when the mold is actually there and then it is removed. There is no reason why the fungi would parasite the commodity in the field and then abandon it without some sort of intervention. In such cases, infestation is more likely to be more tightly associated with contamination. From that moment on, the level of acceptability seems high to moderate. The peculiar result from the factory is the already discussed about animal products.

All samples but groundnut and maize were fully acceptable or unacceptable (Table 2). It is again probably an issue of sample size, leading to a higher standard error. Nonetheless, assuming it as the best lead available, most samples are within the recommended range, the unacceptable being cassava flour, corn, feed waste and forage. The latter two are not an issue for human health because they are not foodstuffs. Groundnuts were mostly acceptable (60%) while maize was unacceptable (33.3%). The former appears to have relatively low heavily contaminated samples, while the latter has more of less contaminated specimens.

In summary, many crops are fit for export if the Codex Alimentarius is enforced, but some require some attention if they are also to be internationally traded. The trend has never changed since the independence and the area where the commodities are grown matters. Good agricultural practices can be of great help and simple procedures such as selection of non-infested grains can impact the final products. However, every step of the production chain, including the consumers' behavior, is reflected in the fitness of the food to be consumed and acceptability considering the Codex Alimentarius.

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Table 2. Codex acceptability regarding the commodities. Based on Casadei [12], Van Rensburg, *et al.* [5], van Wyk, *et al.* [7], Warth, *et al.* [13] and Sineque, *et al.* [8]. These studies were carried from 1985 to 2017 and included in total 934 samples including agricultural crops, chicken livers and gizzards.

Product	Codex evaluation (%)		Total (%)
	Acceptable	Unacceptable	
Beans	100.0	0.0	100.0
Beer	100.0	0.0	100.0
Cassava flour	0.0	100.0	100.0
Chicken gizzard	100.0	0.0	100.0
Chicken liver	100.0	0.0	100.0
Corn	0.0	100.0	100.0
Corn flour	100.0	0.0	100.0
Dry cassava	100.0	0.0	100.0
Feed waste	0.0	100.0	100.0
Forage	0.0	100.0	100.0
Groundnut	60.0	40.0	100.0
Maize	33.3	66.7	100.0
Millet	100.0	0.0	100.0
Rice	100.0	0.0	100.0
Sesame	100.0	0.0	100.0
Sorghum	100.0	0.0	100.0
Wheat	100.0	0.0	100.0
Overall	63.2	36.8	100.0

## 265 5. Final remark

266 Mozambique is among the countries most affected by AFB<sub>1</sub>, both in health and economy, and  
 267 this should be a cause for the country to lead in this line of research. There are currently more  
 268 facilities than ever before, though screening studies require sophisticated and onerous material.  
 269 Furthermore, there are alternatives to chemical analysis, probably more feasible yet as relevant. Why  
 270 not, for example, trying to search for ways to control for known aflatoxigenic *Aspergillus* strains? Or  
 271 just to analyze its growth pattern or how environmental factors affect the mold? Which other crops  
 272 than groundnuts, maize and cassava are being affected? Why not to perform studies on economic  
 273 losses? There is no need for much money in order to expand the knowledge about aflatoxin in  
 274 Mozambique. Different sectors could try to study the best way to tackle this issue.

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277 **Conflicts of Interest:** The authors declare no conflict of interest

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