

1 **Postharvest handling practices and mycotoxin occurrence along the dried *berbere* chilli**
2 **pepper value chain: A case study from Northern Ethiopia**

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11

12 **Abstract**

13 *Berbere*, the hot pepper spice, is a central ingredient in Ethiopian cuisine. This study aimed to
14 deepen understanding of the agricultural and postharvest handling practices and incidence of
15 aflatoxins and ochratoxins at different stages of the domestic *berbere* (*Capsicum frutescens*)
16 value chain in the Amhara region of Northern Ethiopia. The main actors in the *berbere* value
17 chain in Ethiopia are farmers, assemblers, wholesalers, retailers, industrial processors, and
18 domestic consumers. A series of semi-structured questionnaires were developed and used to
19 interview 90 stakeholders from across these different value chain stages to learn about their
20 *berbere*-related activities and challenges. Additionally, a random sampling method was used
21 to collect 100 *berbere* samples (80 samples of whole dry *berbere* pods and 20 samples of
22 *berbere* powder) from across the different focal value chain stages (harvesting, storage,
23 processing, and retail) for analysis to determine whether aflatoxin and ochratoxin were present
24 above the maximum recommended thresholds. In the Amhara region, most farmers typically
25 cultivate *berbere* on areas of 0.5 to 1 ha of farmland. The *berbere* harvest is done manually,
26 and the chilli pods are then spread directly on the ground to sun dry. Such drying practices
27 increase the risk of contamination of the produce by dust, debris, and soil-borne fungi.
28 Assemblers purchase *berbere* from the farmers, the pods are then sorted by variety and packed
29 in woven polypropylene bags which are then heaped in store rooms. All the *berbere* samples,
30 from each stage of the value chain analysed were found to test positive for mycotoxins,
31 exhibiting levels of >20 ppb and >2 ppb of total aflatoxin and ochratoxin, respectively. The
32 promotion of a range of integrated pest and postharvest management actions with the different
33 value chain actors are recommended to improve the quality and safety of *berbere* within these
34 important domestic value chains.

35 **Keywords:** *Farmers handling, Spice mix, Beriberi, Aflatoxin, Ochratoxin.*

36 **1. Introduction**

37 Chilli pepper (*Capsicum frutescens*) is a widely cultivated crop belonging to the Solanaceae
38 family and is believed to have originated from South America (Chakrabarty & Islam, 2017;
39 Boseland & Votava, 2000). It was introduced to Asia and Africa in 1493, and globally it has
40 become an important crop with around 4,255,050 tonnes of dried chilli produced in 2019
41 (FAOSTAT, 2021). India is the leading producer of dry hot red pepper, while Ethiopia is ranked
42 as the fourth-highest-producing country in the world, with an annual production of 313,115
43 tons in 2019 (FAOSTAT, 2021). However, despite Ethiopia being a leading producer of chilli
44 pepper, the country ranks low with regards to chilli pepper exports due to issues related to
45 product quality and food safety (Boseland & Votava, 2000). In general, most (96%) of the
46 spices produced in Ethiopia are currently consumed domestically, and chilli pepper accounts
47 for over 80% of Ethiopia's total spice production (Sendrowicz & Dubellar, 2020). Over 1.1
48 million farming households in Ethiopia are estimated to be engaged in the farming of spices
49 (Sendrowicz & Dubellar, 2020).

50 In Ethiopia, hot pepper is known as *berbere* (here after the term *berbere* is used when referring
51 to Ethiopian dry red hot chilli pepper). Within Ethiopia, *berbere* is commonly cultivated in
52 locations with altitudes ranging from 1400 to 1900 m above the sea level, and a mean annual
53 rainfall and temperature range of 600 to 1200 mm, 25 to 28°C, respectively. The production of
54 *berbere* by smallholder farmers is concentrated in Amhara, Oromia, and Southern Nations,
55 Nationalities and Peoples' (SNNP) Region states of Ethiopia (Ababayehu et al., 2014). The
56 *berbere* crop is typically rotated with maize, which is grown as a primary staple food crop for
57 home consumption. *Berberere* is usually planted in April/May when the rainy season starts and
58 harvested from October to February (dry season) (Boseland & Votava, 2000) (Gobie, 2019).
59 In Ethiopia, *berbere* cultivation is practised at small-scale levels using traditional practices.
60 The harvest and postharvest operations include traditional harvesting, drying, sorting, storage,
61 and milling practices.

62 In many Ethiopian dishes, highly pungent dried *berbere* powder is an important ingredient. The
63 fine-powdered pungent product known as "*berbere* powder/ *mitmita* (extra hot version of the
64 powder)", imparts both flavour and colour to the traditional Ethiopian sauce "*Stew or Wol*".
65 The average daily consumption of *berbere* by Ethiopian adults is estimated at 15 g, which is
66 higher than tomatoes and most other vegetables (Gobie, 2019).

67 The different *berbere* varieties have a range of pod colours from pale to deep red. Usually,
68 *berbere* pods are transformed into *berbere* powder and oleoresins. Both are used to impart
69 colour and flavour to different foods. Capsaicin, the pungent alkaloid is the major bioactive
70 compound in *berbere* (Lu et al., 2020). The capsaicin concentration in *berbere* reportedly

71 ranges from 0.1 to 3.2g/100g dry weight (Arimboor et al., 2015). The capsaicin in red *berbere*
72 is reported to have health benefits such as cardio-protective influence, anti-lithogenic effect,
73 anti-inflammatory and analgesia, thermogenic influence, and beneficial effects on the
74 gastrointestinal system (Srinivasan, 2016). Chopan & Littenberg, (2017) reported that
75 consumption of hot *berbere* was associated with reduced mortality and concluded that, *berbere*
76 is beneficial for the human diet. Other work suggests that *berbere* can be used to alleviate
77 human dietary micronutrient deficiencies (Olatunji & Afolayan, 2018).

78 The presence of fungal toxins in *berbere* is viewed as one of the major quality and safety
79 challenges as they are known to be responsible for a wide range of health problems. Fungal or
80 mycotoxins are naturally occurring toxic low molecular weight metabolites produced by certain
81 fungal species and can be found in food. Warm and humid environments are known to
82 encourage the growth of mycotoxin-producing fungi and certain food crops such as cereals,
83 nuts, spices, dried fruits, and coffee beans are particularly affected by the growth of these fungi
84 in humid environments (Fufa & Urga,1996; Patriarca & Pinto, 2017). Consumption of
85 mycotoxin-containing products can cause various health-related impacts ranging from acute
86 poisoning to longer-term chronic effects on immunity, growth and different cancers (Fufa &
87 Urga,1996; Liew & Mohd-Redzwan, 2018). Links between gall bladder cancer and the
88 consumption of aflatoxin-contaminated *berbere* were reported by Tsuchiya et al. (2011).

89 To our knowledge, despite the importance of *berbere* in Ethiopian domestic food systems,
90 limited documented information on the postharvest handling and incidence of aflatoxins and
91 ochratoxins exists for *berbere* in Northern Ethiopia. This study aims to address this knowledge
92 gap in order to help inform the development and testing of practical strategies for reducing
93 quantity and quality losses of *berbere* at and after harvest and to support and strengthen the
94 livelihoods of the different actors involved in the *berbere* value chain.

95 **2. Materials and Methods**

96 **2.1 Study area**

97 The study location was Bure district (*woreda*) of Amhara National Regional State of Ethiopia.
98 Bure is located 400 km Northwest of Addis Ababa and 148 km southwest of Bahir Dar city.
99 Bure *woreda* receives a relatively high rainfall amount with a balanced distribution pattern.
100 The mean annual rainfall is reported to range from 1386 to 1757 mm. The altitude ranges from
101 713 to 2604 meters above sea level. Long-term annual mean temperature ranges from 14°C to
102 24°C. Maize, wheat, teff, finger millet and barley are the main cereals produced in the region.
103 *Berberere* is mainly produced under rain-fed conditions in the low and mid-altitude areas of Bure
104 *woreda* (ILRI 2007). Farmers in the lowland area grow *berbere* on an average of 0.5 to 1 ha of

105 land annually. Given this large area of land planted to *berbere*, farmers use a direct sowing
106 method (Shimelis, 2021).

107 **2.1.1 Selection of Study Location:**

108 Bure district, West Gojjam zone, Amhara region was purposively selected as the study location
109 due to it being within the main *berbere* production areas. In Bure district, red pepper is a major
110 cash crop that is mainly produced by smallholder farmers.

111 **2.2 Selection of survey participants**

112 **2.2.1 Berbere farmers**

113 Three kebeles, Alefa, Dedun, and Wadero, within Bure district were purposively selected as
114 the majority of farmers engaged in *berbere* cultivation live within them. These three kebeles
115 cumulatively contain 12 sub- kebeles (a sub-unit of a kebeles, these are locally recognized units
116 although not official units). Five sub- *kebeles* (5th, 8th, 9th, 10th, and 11th) were randomly
117 selected. From each selected sub- *kebeles*, every sixth house was selected for interview. In sub-
118 *kebeles* 5, 8, and 9, interview data was collected from six *berbere* farming households as the
119 population is similar (i.e., each have 38 to 40 households which cultivate *berbere*). In sub-
120 *kebele* 10, interview data was collected from 7 households as it contains a total of 46
121 households which cultivate *berbere*. In sub- *kebele* 11, data was collected from 5 households
122 as the population there is smaller, with a total of 34 households which cultivate *berbere*.

123 **2.2.2 Berbere assemblers**

124 The snowball sampling method was used to select assemblers. Assemblers were initially
125 identified in the market area. Contact information of other assemblers was then collected from
126 the identified assemblers. Interview data was collected from a total of 10 assemblers randomly
127 selected from the collected contacts.

128 **2.2.3 Berbere wholesalers**

129 Around 32 wholesalers are based in the Bahir Dar City market area (kebele 4). A systematic
130 sampling approach was used to select the wholesaler sample. Every third wholesaler on the list
131 of wholesalers was selected and a total of 10 were interviewed.

132 **2.2.4 Industrial berbere processors**

133 The snowball sampling method was used to select industrial processors. Initially, one industrial
134 processor was identified through contacts, and that person then connected the team to other
135 processors and so on. Data was collected from 10 industrial processors located in Bahir Dar
136 City.

137

138

139

140 **2.2.5 Domestic berbere processors**

141 Random purposive sampling was used to identify 10 domestic processors within Bahir Dar
142 City, where households prepare *berbere* powder, a total of 10 domestic processors were
143 interviewed.

144 Information was collected on socio-demographical aspects; *berbere* farming practices,
145 harvesting, post-harvest handling and preservation and processing. The survey was done
146 between 29th May to 1st July 2020.

147

148 **2.3 Dried berbere pod/powder sample collection**

149 During the survey, a total of 100 samples were collected from across the different stages of the
150 value chain. Of the 70 samples of red *berbere* pods, 30 came from the farmers, 10 from the
151 assemblers, 10 from the wholesalers, 10 from the retailers and 10 from the industrial
152 processors. Of the 30 samples of red *berbere* powders, 10 came from industrial processors, 10
153 from domestic processors and 10 from the market. *Berbere* pods from the farmers were
154 collected from the different places within their storage facilities to form a representative sample
155 per farmer (each composite sample was a combination of three samples). In the case of *berbere*
156 stored in bags, pods were collected from different bags to form the sample. In the case of
157 *berbere* powder, sub-samples were selected from different areas of the bag/ container and
158 different bags if more than one bag existed. Immediately following collection, all the samples
159 were placed into three layers of polythene bags and sealed to prevent air entry and then stored
160 in the refrigerator at 4°C until further analysis was done (within two months). The samples
161 were stored under refrigerated conditions to avoid further growth of any fungi which were
162 present.

163

164 **2.4 Determination of aflatoxin and ochratoxin presence**

165 The ELISA kit method was used to determine the presence of aflatoxin and ochratoxin in each
166 sample, and whether they exceeded the maximum tolerable levels. Each *berbere* pod sample
167 was ground for 10 minutes into a coarse powder using the laboratory mortar and pestle
168 (Porcelain Mortar & Pestle, India). After each sample was ground, the mortar and pestle were
169 cleaned with ethanol twice before processing the next sample. The *berbere* powder samples
170 collected from stakeholders were used directly without any further grinding. The powders were
171 used for the determination of both toxins following the extraction process described in 2.4.1.1
172 and 2.4.2.1 which is as per the instructions given by the ELISA kit manufacturer without any
173 modifications.

175 **2.4.1 Aflatoxin determination**

176 VICAM-AflaCheck® (VICAM, USA) (Manufacture catalogue numbers 100000173, 175, 812,
177 813 and 827) was used for the qualitative determination of the aflatoxins in *berbere* samples.
178 AflaCheck® Test Strips are provided for fast accurate determination of the aflatoxins at 10 ppb
179 and 20 ppb levels only. The two steps described in 2.4.1.1 and 2.4.1.3 were followed to
180 determine aflatoxin presence in the samples using the kit.

181 **2.4.2 Extraction of the sample for aflatoxin determination**

182 The extraction procedure was carried out following the instructions provided by the ELISA kit
183 supplier. Five grams of the ground sample was added to the extraction tube provided by the
184 manufacturer, and a new tube was used for each sample. Exactly 10 ml of 70% Methanol was
185 added into the extraction tube. The tubes with the contents were mixed well for 1 minute using
186 a vortex at high speed. The samples were then allowed to settle for 3 minutes as per the ELISA
187 kit instructions. The supernatant was used for the determination of aflatoxin.

188 **2.4.3 Sample test for aflatoxin**

189 Exactly 250µL of the supernatant solution (sample extract) was taken into the fresh dilution
190 tube (Eppendorf Tube) using a test pipette (provided by the manufacturer) and 250µL of
191 distilled water was added using a fresh test pipette. After closing the cap of the tube, the
192 solution was mixed using manual shaking. The test strip (ELISA strip) was then inserted into
193 the Eppendorf tube and left to stand for five minutes after which the results were read.
194 Depending on the development of the coloured lines on the strip the test was determined as
195 positive or negative. For each sample, the strip was also checked for the development of the
196 control line. If the control line does not develop the test is considered invalid and another new
197 strip would then be used to check the same sample. The sample extraction and determination
198 are presented in the Supplementary Information Figure 1.

199 **2.4.4 Ochratoxin determination**

200 For determination of the Ochratoxin, the Ochra-V Aqua® cassette from VICAM, USA was
201 used. For this test the detection limit is 2ppb. The catalogue numbers are 176004087 and
202 10000345.

203 **2.4.5 Extraction of the sample for ochratoxin determination**

204 As per the ELISA kit instructions, 5±0.1g of the ground sample was placed in the extraction
205 tube provided by the manufacturer, a new tube was used for each sample. Then 25 ml of Aqua
206 premix solution which was supplied as part of the kit was added and gently mixed by vortex at

207 minimum speed for 2 min. The sample was filtered into a clean extraction tube supplied in the
208 kit using a fresh piece of fine filter paper for each sample.

209

210 **2.4.6 Sample test for Ochratoxin**

211 Clear filtrate of 100 μ L sample extract was added to the Ochra-V strip cassette by dropping 1
212 drop/second vertically into the sample well. The test cassette was allowed to develop for 5
213 minutes on a flat surface. If no development of the control line occurred, the test was considered
214 invalid, and another new cassette was used for the determination of Ochratoxin in the same
215 sample. The sample extraction and determination procedures are depicted in Supplementary
216 Figure 2.

217 **2.5 Determination of water activity (aw) in the samples**

218 A Lab Master-water activity instrument (Novasina AG, CH-8853 Lachen) was used to
219 determine the water activity of the *berbere* samples. The ground samples were placed in a
220 plastic sample cup, completely covering the bottom of the cup. The sample cup was then placed
221 in the sample chamber and the door of the chamber was closed carefully. Water activity was
222 reported directly from the display panel.

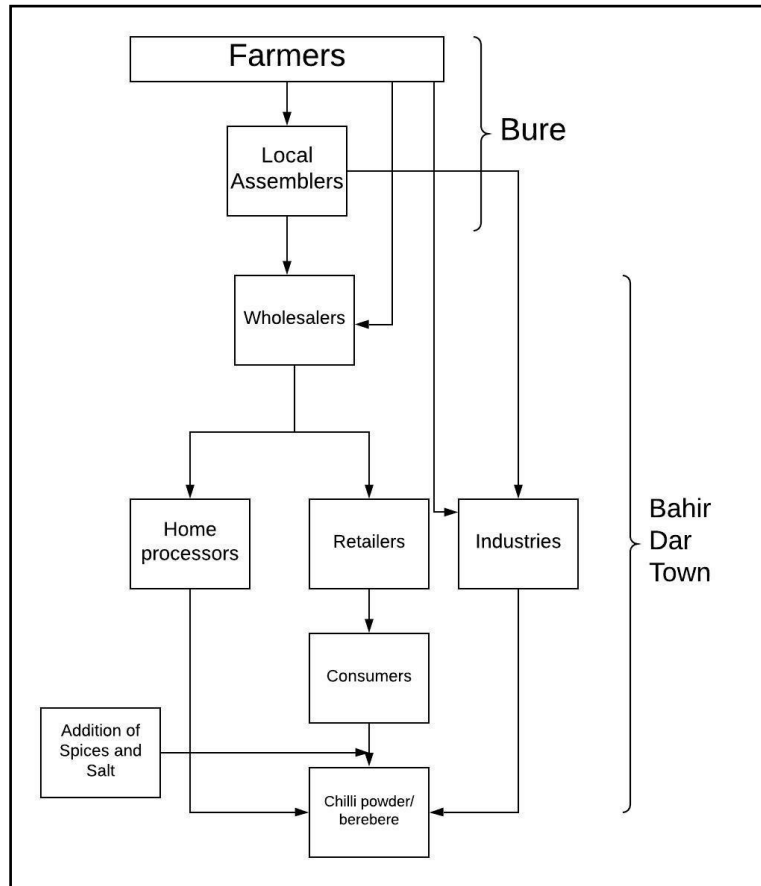
223 **2.6 Data analysis**

224 The survey data was analysed using the statistics package for social science (SPSS) IBM Ver.
225 18. The main analyses performed were descriptive statistics such as the frequency, percentages,
226 and ranges.

227

228 **3. Results**

229 The structure of the berbere value chain in the Amhara region of Ethiopia is shown in Figure
230 1. The surveyed farmers and assemblers in the berbere value chain were in Bure district. While
231 the other major berbere value chain actors, the wholesalers, retailers, industrial processors, and
232 home processors were based in Bahir Dar city.



233
 234 **Figure 1.** The value chain for *berbere* red-hot pepper in Bure district and Bahir Dar city,
 235 Ethiopia

236 **3.1 *Berber* agricultural and handling practices at the farmer’s level**

237 **3.1.1 Socio-demographic characteristics of farmer respondents**

238 The 30 farmer respondents belonged to three *kebeles* (wards or villages), namely Aleafa, Debun
 239 and Wadero within the Western Gojam zone. The respondents were distributed across five
 240 *woredas* (districts) 5, 8, 9, 10, and 11. Aleafa has previously been reported to be one of the
 241 highest *berbere*-producing *kebeles* in Bure (Tesheshigo, et al., 2019) (Table 1).

242 The mean age of respondents was 40 years, and the range was from 19 to 70 years. Of the 30
 243 farmer respondents, 40% were female. Respondents’ education status varied from basic
 244 education (73% of respondents) to 11th grade (3% of respondents) (Table 1).

245 **Table 1.** Socio-demographic characteristics of respondents in Bure district, Amhara region,
 246 Ethiopia

Parameter	Number of respondents	Percentage (%)
Respondent's Kebele		
Alefa	10	33.33
Debun	10	33.33

Wadero	10	33.33
Woreda number	6	20
Woreda-5	6	20
Woreda-8	6	20
Woreda-9	7	23.3
Woreda-10	5	16.6
Woreda-11		
Age		
<20 Years	1	3.33
20-30 Years	8	26.66
31-40 Years	8	26.66
41-50 Years	5	16.66
51-60 Years	5	16.66
> 61 Years	3	10.0
Level of education		
No education	0	
Basic education	22	73.33
5-10 grade	7	23.33
>10 grade	1	3.33

247

248 **3.1.2 Berbere cultivation practices**

249 Among the respondents, the mean area of *berbere* cultivated was 0.65 ha and ranged from a
250 minimum of 0.25 ha to a maximum of 2 ha. Of the respondents, two-thirds were cultivating
251 *berbere* on ≥ 0.5 to ≤ 1 ha of land. In the current study, 80% of respondents practised rain-fed
252 *berbere* production only, with the remaining respondents practising both rain-fed and irrigated
253 production. Almost all the respondents cultivated *berbere* both for sale and for domestic
254 consumption purposes.

255 The majority (80%) of the respondents were cultivating the Danbure variety of *berbere*, the
256 rest were cultivating the Mareko Fana variety, and only one respondent reported cultivating
257 both varieties. Most of the respondents (93.3%) plant their *berbere* in June, and the others in
258 July. Harvesting of *berbere* occurred 5 to 6 months later in November and December (Tables
259 2 and 6).

260 Just over half of the farmer respondents (53%) reported facing problems of pests and diseases
 261 during *berbere* cultivation (Table 2). These farmers perceived these problems to all be fungal
 262 diseases, but none of them knew of or had any control measures to counteract these problems.
 263 Weeds in their *berbere* fields were another problem reported by 50% of the farmer respondents.
 264 The weed species mentioned translate to grasses mainly, as well as *Guizotia scabra* and *Rumex*
 265 *nervosus*. Around 70% of the respondents practice crop rotation. Prior to cultivating *berbere*,
 266 the farmers reported having grown cereals on the land. However, the remaining 30% of
 267 respondents grow *berbere* continuously on the same piece of land.
 268 The majority (90%) of respondents reported rainfall occurring during the time of *berbere*
 269 flowering (Table 3). Around 90% of respondents reported that rainfall also occurred when
 270 *berbere* pods were ready for harvest and during harvesting. Around 70% of respondents
 271 reported rainfall occurring during the drying process. All respondents reported rainfall in at
 272 least one of the following three important stages of *berbere* growing and handling (flowering,
 273 ready for harvesting, drying).

274

275 **Table 2.** Agricultural characteristics of *berbere* farmers in Bure district, Amhara region,
 276 Ethiopia

Parameter	Number of respondents	Percentage (%)
Land size under <i>berbere</i> cultivation		
0.25-0.5	3	10
0.6-1.00	20	66.6
1.00-1.5	8	26.6
1.6-2.0	1	3.33
>2.00	1	3.33
<i>Berbere</i> variety cultivated		
Danbure	24	80
Mareko	6	20
In which month is <i>berbere</i> planting done?		
June	28	93.3
July	2	6.66
In which month is <i>berbere</i> harvesting done?		
November	15	50
December	15	50

Is your <i>berbere</i> cultivation rain-fed or irrigated?		
Rain-fed	24	80.0
Irrigated	0	
Both	5	20.0
Does your <i>berbere</i> crop suffer any insect/pest/disease damage in the field during production?		
Yes	16	53.3
No	13	43.33
Not sure	1	3.33
Do you use any pest control measures?		
Yes	0	
No	28	93.3
Not sure	2	6.66
Do you typically have weeds growing in the field during <i>berbere</i> production?		
Yes	15	50.0
No	14	46.6
Not sure	1	3.33
If yes, name the type of weed		
<i>Rumexnervosus</i>	8	26.66
<i>Guizotiascabra</i>	11	36.6
Grass	8	26.66
Do you use any weed control measures?		
Yes	1	3.33
No	29	96.6
Not sure	0	
Do you use any chemicals to control weed?		
Yes	0	
No	30	100
What type of crop was cultivated in the same field before you planted this red <i>berbere</i> crop		
Same crop	9	30
Cereals	21	70
Others	0	

277

278

279 **Table 3.** Farmers' perceptions of rainfall occurrence during the in-field and postharvest *berbere*
 280 stages in Bure district, Amhara region, Ethiopia

Parameter	Number of respondents	Percentage (%)
Was there rainfall during the flowering time of this <i>berbere</i> crop?		
Yes	27	90
No	2	6.66
Do not remember	1	3.33
Did any rainfall occur when this <i>berbere</i> crop was ready for harvesting?		
Yes	27	90
No	2	6.66
Do not remember	1	3.33
Did any rainfall occur when you were harvesting this <i>berbere</i> crop?		
Yes	26	86.6
No	4	13.33
Do not remember		
Did any rainfall occur when you were drying this <i>berbere</i> crop?		
Yes	22	73.33
No	8	26.6
Do not remember	0	

281

282 **3.1.3 Drying of the *berbere* pods**

283 Regarding the drying of the matured pods after harvesting, around 13% of the farmers practice
 284 drying in the field, while 60% dry the pods at their homesteads, and 27% of the respondents
 285 use both the field and home for drying (Table 4). Virtually all the farmers surveyed (97%) sun-
 286 dry their *berbere* by spreading it directly on the ground (Figure 2A). Only one farmer used a
 287 concrete floor for the drying, and none of the farmers use plastic sheets for drying their *berbere*

288 on. The farmers typically sun-dry the *berbere* for a duration of between one week and 30 days,
289 with the majority (70%) stating they dry for four weeks with the duration being dependent on
290 the environmental conditions and the initial moisture content of the pods.

291 To determine whether the *berbere* is sufficiently dry, manual touching is used by 93% of the
292 respondents, while the other farmers use visual observations (Table 4). Of the farmers, 53%
293 reported that they observe fungal infection/ disease on the pods before harvesting. Only 33%
294 of the farmers were aware of the importance of proper drying for maintaining the quality of the
295 *berbere* and avoiding fungal growth during storage (Table 4).

296 The majority of farmers reported using pod dryness as an indicator of maturity and harvesting
297 readiness (Table 4). While 23% of respondents reported that they considered and followed a
298 calendar-based calculation of the number of days since planting to determine maturity and
299 harvesting.

300 ***3.1.4 Sorting and grading of berbere pods***

301 Sorting of the *berbere* before drying was practised by just 37% of the farmers (Table 4 and
302 Figure 2B). The farmers who sort their pods before drying, separate the spoiled, broken,
303 fungus-attacked pods and plant debris from the good quality pods. However, following the
304 drying process, 70% of the farmers sort their pods. During this post-drying sorting process, the
305 spoiled, broken, pale or white-coloured *berbere* pods are sorted out, and plant waste and dust
306 are removed. The farmers sort the pods to achieve uniform quality of their *berbere* during
307 storage. Two farmers explained that the poor quality *berbere* they have sorted out would be
308 used for their domestic/home consumption while the good quality *berbere* would be for sale.

309

310

311



312
 313 **Figure 2.** *Berberere* handling practices at the farmer and assembler stages in Bure district,
 314 Ethiopia (A) *Berberere* pods spread on the field for sun drying. (B) Sorted-out colour-less *berbere*
 315 at a farmer's homestead. (C) *Berberere* is stored in the traditional storage structure in a farmer's
 316 home. (D) Farmers take the *berbere* to an assembler's aggregation point by horse cart. (E)
 317 *Berberere* weighing at the assembler's level. (F) Storage of *berbere* in woven polypropylene
 318 sacks at the assembler's level. (G) Bulk storage of *berbere* pods heaped in a room at the
 319 assembler's level. (H) Sorted *berbere* pods on plastic sheeting at the assembler's level.

320
 321 **Table 4.** Farmers' *berbere* harvest and drying practices in Bure district, Amhara region,
 322 Ethiopia

Parameter	Number of respondents	Percentage (%)
How do you usually tell when your <i>berbere</i> is ready for harvesting?		
Calendar (month) calculation	7	23.33
When pods dry up	24	80.0
Other specify (colour)	1	3.33

Do you dry the freshly harvested <i>berbere</i> in the field, at home, or somewhere else?		
Field	4	13.3
Home	18	60.0
Both	8	26.6
Others		
Where do you dry the hot pepper/<i>berbere</i>?		
Berbere pods are spread directly on the ground	29	96.6
Berbere pods are spread on a plastic sheet or covered on the ground	0	
Berbere pods are spread on a concrete drying area	1	
Others (specify):	0	
How do you dry the hot pepper/<i>berbere</i>?		
Sun drying	30	100
Solar drying (Developed Driers)	0	
Artificial dryers/hot-air Mechanical dryers	0	
Others	0	
How long did the <i>berbere</i> take to dry to be ready for storage?		
One Week	3	10
Two Weeks	6	20
Three Weeks	1	3.33
Four weeks	20	66.66
How do you tell that the <i>berbere</i> pods are dry enough?		
Just by looking at them	2	6.66
By touching them	28	93.33
Using Moisture testers	0	
By indigenous knowledge / (specify what)	0	
Any other methods used	0	
Do you see any fungal infection on the hot pepper pods in the field, before harvest?		
Yes	16	53.5
No	14	46.6

Are you aware that proper drying could control mould /fungal development and maintain the quality of your *berbere*?

Yes	10	33.33
No	20	66.66

Do you do any sorting/ cleaning BEFORE drying the *berbere*?

Yes	11	36.66
No	19	63.33

Do you do any sorting and grading AFTER drying the *berbere*?

Yes	21	70
No	9	30

323

324 **3.1.5 Farmer-level storage of *berbere***

325 Around 63% of the farmers store their dried *berbere* in a *gotera*, a traditional storage structure
 326 constructed from locally available wooden sticks (Table 5 and Figure 2C). About 13% of the
 327 farmers store their *berbere* on the ground in a heap, 13% store it in polypropylene bags and
 328 10% of the farmers use jute bags. The polypropylene bags have a capacity of 20-30 kg; while
 329 the jute bags are typical of 50 kg capacity, the *goteras* range in size with capacities from 150-
 330 2000 kg. Those farmers who heap the *berbere* on the floor reported doing so for 300-1000 kg
 331 of the dried crop. On average the farmers reported storing 270 kg of dried *berbere* annually,
 332 however, this ranged widely among respondents from a minimum of 100 kg to 1000 kg.

333 Around 57% of the farmers store their *berbere* at their homes, while 27% use co-operatives or
 334 society warehouses for storage. Some respondents (17%) store their *berbere* in the field. The
 335 mean storage duration of dried *berbere* by farmers was 3.9 months (Table 5). But storage
 336 durations ranged between farmers, with 10% of the farmers storing their *berbere* for six
 337 months, 33% for five months, 20% for four months, 17% for three months, 17% for two months
 338 and 3% for one month. The majority (67%) of the respondents reported that they had not found
 339 any signs of fungal growth on their stored *berbere*, while 27% had, and 7% were not sure. In
 340 the current study, none of the responding farmers knew about or was aware of mycotoxins.
 341 None of the farmers reported pest or disease issues which caused losses after harvest or during
 342 storage of their *berbere* (Table 5). None of them use any storage protectants or other practices
 343 on their stored *berbere* to maintain its quality.

345 **Table 5.** Farmers' *berbere* storage practices and mycotoxin awareness in Bure district, Amhara
 346 region, Ethiopia

Parameter	Number of respondents	Percentage (%)
What type of packaging /storage systems do you use to store dry <i>berbere</i>?		
Polypropylene bags	4	13.33
On-ground/ heap	4	13.33
Jute bags	3	10.00
Gotera	19	63.33
Hermetic bags	0	
What quantity of <i>berbere</i> can each storage structure/ unit hold? (kg)		
<100	7	23.3
100-500	15	50.0
500-100	2	6.66
>1000	6	20
How much <i>berbere</i> do you store normally (on an annual basis, per year)? (kg)		
<100	0	
100-500	25	83.3
500-100	5	6.66
>1000	0	
Where is your <i>berbere</i> storage facility located?		
On the field	5	16.66
Within the home	17	56.66
Both (on the field and within the home)	0	
Depends on others to store it	6	20
For how many months (s) do you store your <i>berbere</i>?		
One month	1	3.33
Two months	5	16.66
Three months	4	13.33
Four months	7	23.33

Five months	10	33.33
Six months	3	10.00
Do you find any signs of fungal growth in the stored <i>berbere</i>?		
Yes	8	26.6
No	20	66.6
I don't know	2	6.66
Do you know anything about mycotoxins?		
Yes	0	
No	30	100
Do you face other problems such as insect/pest damage which cause damage or losses after harvest or during storage of your dry <i>berbere</i>?		
Yes	0	
No	30	100
Do you add any chemicals/preservative to your stored <i>berbere</i> to reduce postharvest losses?		
Yes	0	
No	30	100
Do you do any other practices to help maintain the quality of your stored <i>berbere</i>?		
Yes	0	
No	30	100
Purpose of hot red chilli production		
Home Consumption	0	
Sales	1	3.33
Both	29	96.99
What do you want to know more about regarding your <i>berbere</i>?		
Factors of deterioration,	30	100
Extending the shelf life/storage	30	100
Quality and weed management	30	100

348

349

350 **3.1.6 Postharvest information pathways for *berbere***

351 When farmers were asked who had taught them to dry and store their *berbere*, elders were the
352 most commonly cited source (67% of respondents), followed by friends (53%), agricultural
353 officers (7%), and own observations (3%) with many having learnt from both their elders and
354 friends.

355

356 **3.1.7 Farmers' further knowledge demands for *berbere***

357 The main topics the farmer respondents wanted to know more about regarding their *berbere*
358 activities were the causes of the deterioration, methods for extending the shelf life/storage
359 duration while retaining the quality and weed management.

360

361 **3.2 *Berber* handling practices at the Assembler level**

362 The ten assemblers interviewed were residents of Bure city which has good road access to the
363 other parts of the country. Farmers from a range of different villages bring their *berbere* to the
364 assemblers located in Bure (Figure 2D&E). In the study area, all the assemblers are males, and
365 those interviewed ranged in age from 27 to 60 years. The maximum education qualification
366 obtained by any of the assemblers interviewed was 12th grade (equivalent to high school) (10%
367 of respondents). While 40% of the assemblers had studied to standard 10 (10 years of basic
368 education), and 20% had achieved a basic school education. The *kebeles* which the assemblers
369 based in Bure city purchase and collect *berbere* from include *Zalema, Debun, Wadera, Alefa,*
370 *Beso, Arebajero, Medro, Gelebed, Gulum, Wadero, Dejen, Bure, Tengea and Tataya.*

371 All the assemblers in the study area collect the *berbere* pods after they have been sun-dried by
372 the farmers. If *berbere* were purchased fresh from farmers, then the assemblers would
373 undertake sun-drying of the *berbere* after spreading it directly on the ground for 7 to 20 days.
374 Just two assemblers said they might place the *berbere* on a plastic sheet to dry.

375 The purchased *berbere* may be stored by the assemblers for periods ranging from 15 days to 2
376 years. However, only very small quantities of the *berbere* are stored for longer durations as the
377 majority (60%) of assemblers sell their *berbere* immediately to the next-level purchasers.
378 About 60% of the assemblers pack their *berbere* in jute bags, while 20% pack the product in
379 woven polypropylene bags (Figure 2F). The other 20% of assemblers store their *berbere* in
380 heaps on the floor of a store room (Figure 2G). All the storage structures are simple rooms
381 constructed with locally available materials (i.e., wood and mud walls, metal sheets, plastic
382 sheets) without any controls for temperature or relative humidity. One assembler reported using

383 both jute and hermetic bags to store *berbere* in. Different varieties of *berbere* are stored in
384 separate containers from each other.

385 All the assemblers sort the *berbere* before purchasing it from the farmers. The majority remove
386 dirty, discoloured or white coloured, spoiled, broken pods and any plant debris (Figure 2H).
387 Around 30% of the assemblers clean and grade the *berbere* pods into first, second and third
388 quality classes. The properties the assemblers check for when grading the *berbere* are colour,
389 larger-sized pods, dryness (moisture content), pungency, and absence of contaminants and
390 impurities. It was observed during the survey that sorting by assemblers typically takes place
391 manually with the *berbere* spread on the ground.

392 About 70% of the assemblers reported observing fungal infection on their *berbere* during
393 storage. The majority of the assemblers confirmed that after two or three months of storage if
394 there is high humidity in the environment, they observe fungal growth. Two respondents
395 explained that when *berbere* was stored for one year or more, it suffered from spoilage. None
396 of the assemblers used fungicides to extend the shelf life of *berbere*. None of the assemblers
397 possessed any cold storage, nor any devices for determining the colour, moisture content and
398 other quality properties.

399 None of the assemblers had any knowledge or awareness about mycotoxins. Around 70% of
400 the assemblers did not use any formal quality control determination procedures at the time of
401 purchasing the *berbere* from the farmers. While 30% relied on their own experience to judge
402 quality-related factors such as moisture content, absence of impurities, pod size and good pod
403 colour. Around 90% of the assemblers practice re-drying of the *berbere* pods in the sun for one
404 to seven days in the middle of the storage period. Only 20% of the respondents used plastic
405 sheets for drying the *berbere*, while the others spread the *berbere* directly on the ground.

406 **3.3 *Berberere* handling at the wholesale level**

407 The wholesalers purchase *berbere* directly from the farmers as well as from the assemblers in
408 the different *berbere* production locations around Bahir Dar city, such as Bure, Chagni, Zegem,
409 and Wade locations. The wholesalers are located in the Bahir Dar city vegetable market and
410 sell their *berbere* to retailers, local industry people and households for domestic processing
411 (Figure 3A). Of the ten wholesalers who were interviewed, eight were male. Most (60%) of
412 these wholesalers did not have any formal education; although two of the ten wholesalers
413 interviewed were diploma holders. The wholesalers interviewed reported determining the
414 quality of the *berbere* at the assembler's or farmer's stage by both visual and manual handling
415 inspection of the *berbere* pods before purchasing them. These wholesalers typically purchase
416 between 300 and 4,000 kg of *berbere* at a time.

417 While two of the wholesalers interviewed reported selling on the *berbere* they purchase almost
418 immediately. The majority reported typically storing it for three to eight days before starting to
419 sell it, this was usually due to them trying to clear their old stock before they started selling the
420 newly acquired produce. No device or formally agreed methods are used by the wholesalers
421 for determining the moisture content or the quality of the *berbere*. The *berbere* is typically
422 stored by the wholesalers in sheds without any temperature or relative humidity control
423 (Figures 3B and C). At the wholesale stage in the value chain, the *berbere* is typically packed
424 in woven polypropylene bags, with each of the bags having a hole in it which has been made
425 by and is used for taking samples from it to check the quality of the *berbere* (Figure 3D). These
426 holes are not closed again after sampling.

427 No practice of adding moisture to the *berbere* to increase its weight was reported during the
428 current survey. The wholesalers reported observing fungal growth on the *berbere* pods they
429 had procured, and that in the rainy seasons there is an increase in the moisture content of the
430 *berbere* and the pod colour may change with white or black spots being observed due to the
431 growth of fungus on it. The wholesalers reported that when high levels of spoilage occur they
432 then discard the *berbere*, but if the spoilage is limited, they sort out the spoiled pods and sun-
433 dry the rest in the open (Figures 3E and F). All of the wholesalers interviewed, reported
434 manually sorting the *berbere* before selling it on. They do this to sort out pods with white on
435 them, broken and small pods and plant debris. The majority of the wholesalers (70%) said they
436 sun-dry their *berbere* stocks for one to three days if they observe fungal spoilage. Just over half
437 of them report using plastic sheets to sun-dry the pods on, while the rest dry the *berbere* directly
438 on the ground.

439 **3.4 *Berberere* handling at the retailer level**

440 Within Bahir Dar city, there are several small retail markets located in different locations
441 (Figure 3 G). About 70% of the retailer respondents were female. With the youngest respondent
442 being 29 years and the oldest 60 years old. Two of the ten retailers interviewed had no formal
443 education, the rest had attended school between 5th and 12th grade, and one had a Bachelor's
444 degree. All of the retailers purchased their *berbere* from wholesalers, with two of them also
445 occasionally purchasing directly from farmers. Retailers tend to purchase 50 to 200 kg of
446 *berbere* at a time.

447 About 30% of the retailers reported that they start selling the *berbere* immediately after they
448 have procured it. While a few typically store their *berbere* for between 1 day and a week before
449 starting to sell it. The retailers interviewed do not have any devices or methods for objectively
450 determining the moisture content. In the case of *berbere* quality determination at the retailer
451 level, 100% of the retailers said they check the sample by touch, and use visual observation

452 and direct interaction with the wholesalers regarding the quality, origin and other issues.
453 Around 30% of the retailer respondents had not observed the presence of fungal growth on the
454 *berbere*. While 70% of retailers said they sort out the spoiled *berbere* and discard the spoiled
455 pods. About 40% of the retailers reported that they do sun-dry the *berbere*, typically for 1-2
456 days if the moisture content was perceived to be high. This sun-drying is typically done on
457 plastic sheets (Figure 3H).

458 The majority of the retailers sell their products to domestic processors or local retail shops,
459 with a few selling to industrial processors. All the retailers explained that the customers inspect
460 the quality of the *berbere* before they purchase, and concentrate on the colour, whether it is
461 free of contaminants, the pod size, the absence of white discoloured *berbere*, and whether it
462 has a good pungency. Only a few consumers enquire about the variety and origin of the *berbere*.
463 Most of the retailers sell their *berbere* divided into three quality categories: high, medium, and
464 low. While 40% just sell a single quality of the *berbere*.

465 **3.5 *Berberere* handling during domestic household processing into powder**

466 The age of the domestic household processors interviewed ranged from 26 to 54 years. All of
467 them were female and educated, with the minimum education level being 12th standard and the
468 maximum being a Master's Degree, 50% of the respondents were Bachelor's degree holders.
469 All the respondents purchase *berbere* pods from Bahir Dar local market to prepare the *berbere*
470 powder. Most of the respondents (60%) purchase *berbere* pods in dried form, while the others
471 (40%) purchase them in fresh form. All the respondents consider the quality of the pods before
472 purchasing. In the case of dried *berbere*, visual observation and manual touching were used to
473 inspect the quality, and the respondents looked for a whole undamaged pod with thick skin and
474 no discolouration (white patches), no plant debris, and with good pungency. Fresh pods were
475 also inspected visually and manually for colour, weight and absence of spoilage. No
476 instrumentation for checking moisture content was used.

477



478

479 **Figure 3.** Berbere handling at wholesalers and retailer levels in Bahir Dar city, Ethiopia.

480 (A) Berbere pods on display at the wholesale market level. (B, C) Storage of the berbere pods
 481 in stacks of woven polypropylene bags. (D) Example of holes in damaged bags of stored
 482 berbere following sampling to check the quality of the stored produce. (E) Sorted berbere at
 483 the wholesale level. (F) Berbere is sun-dried on plastic sheeting at the wholesale level. (G)
 484 Retail marketing of berbere alongside other spices and vegetables. (H) Drying of the berbere
 485 at retailer levels.

486

487 The respondents purchase between 10 kg and 30 kg to prepare *berbere* powder depending on
 488 their family size and consumption patterns. Most of the respondents (90%), store the *berbere*
 489 powder they have prepared for up to one year. All the respondents prepare the *berbere* just
 490 once per year, typically between November and January, and then they use it for the rest of the
 491 year. Around 70% of these respondents had observed fungal growth on and inside the *berbere*
 492 pods, describing it as discolouration, or the presence of a black powder/dust inside the pod.
 493 Where fungal growth was observed, the respondents sort the sample and remove the infected
 494 portion and still use the remaining part. If the incidence of fungal growth is high, 40% of
 495 respondents said they discard the *berbere*. While one respondent reported that if spoilage is

496 high they may return the *berbere* to the seller. All the respondents sort the purchased *berbere*
497 and remove any white and black discoloured pods, plant debris or dirt. Of the respondents, 80%
498 said they had no knowledge about mycotoxins. The 20% of respondents who were aware of
499 mycotoxins, explained that mycotoxins are responsible for cancers and other negative health
500 impacts. The respondents who knew about mycotoxins and their effects were those with
501 Masters' level education.

502 All the respondents mix other local spices with the *berbere* and use small-scale commercial
503 milling establishments to prepare *berbere* powder. The type and proportions of the spices
504 depend on their experience and traditional practices. The other spices used are garlic, ginger,
505 rue (*Ruta chalepensis*), cinnamon (*Cinnamomum zelanii*), kurunfid (*Aframomum corrorima*),
506 clove, and black pepper. The wet spices such as ginger and garlic are crushed separately and
507 dried in the sun and added to *berbere* pods before grinding (Figure 4A and B). Salt is also
508 added by all the respondents. All the respondents reported that the spices are added for taste
509 purposes. Additionally, around 30% of respondents said the other spices are also added as
510 preservatives, 20% of respondents said the other spices have certain health benefits, and 20%
511 of respondents said they help reduce the hotness/ burning effect of the *berbere* powder.

512 About 70% of the domestic *berbere* processing respondents reported some spoilage of the
513 processed *berbere* powder occurs as the storage duration increases, with spoiled *berbere*
514 powder forming lumps and the taste and colour of it changing. To avoid spoilage, the
515 respondents said they pack the *berbere* powder in airtight containers, to reduce its exposure to
516 air and moisture. A few respondents explained that if spoilage occurs, the spoiled portion will
517 be removed and the remaining portion will be used. While other respondents said they would
518 discard all the *berbere* powder if any spoilage occurred. Around 70% of the respondents
519 reported that they do not practice any re-drying of the *berbere* powder during the storage
520 period. While a few respondents said that if they find any changes, the *berbere* powder will be
521 sun-dried for one day (10% of respondents), or that they re-dry the *berbere* powder for 2-3
522 days in the sunlight after it has been stored for about 6 months (20% of respondents), and this
523 is particularly the case in the rainy season.

524

525 **3.6 *Berber* handling at the industrial processing level**

526 All the industrial processors surveyed were small-scale/ cottage industries. Fifty per cent of the
527 industrial processors interviewed were male. Most (70%) of the industrial *berbere* powder
528 processors procure their raw material (*berbere* pods) from wholesalers in the Bahir Dar city
529 local market. The remaining 30% of respondents collect *berbere* pods directly from assemblers
530 and farmers in different production locations depending on their contacts.

531 Regarding the type of *berbere* procured, 60% of the respondents purchased dry *berbere* pods
532 while the remaining 40% purchase fresh pods. Those who purchase fresh *berbere* pods do so
533 in amounts of around 150-200 kg each time. This may be because fresh *berbere* requires quite
534 a large space for drying so, they cannot procure higher amounts. The industrial processors who
535 purchase the dry *berbere*, procure batches of 300 to 3000 kg. These amounts depend on the
536 processor's storage, processing and human resource capacities.

537 The processors store the *berbere* for between 2 and 15 days. Prior to starting to process
538 the *berbere* pods, the industrial processors sort them to remove the white-coloured pods, tips/
539 broken pieces, dust, and plant debris. Despite these practices, the majority (70%) said they did
540 not use any quality control or quality management systems. The remaining 30% use a quality
541 control process involving visual checking of dryness and cleanliness at each step but no use of
542 any analytical instruments. After purchasing the *berbere* pods, all the processors sun-dry them.
543 The duration of the sun-drying ranges from two days to one week depending on the moisture
544 content of the *berbere* pods and weather conditions.

545 During the processing of the *berbere* powder around 90% of respondents add different spices
546 and salt in different concentrations. After milling, the colour, smell, taste, smoothness/fineness
547 and visual appearance of the *berbere* powder will be checked. Following milling, the *berbere*
548 powder is packed in 250 g, 500 g or 1 kg packets for sale in the local markets (Figure 4D). The
549 quantities of *berbere* produced varied between these industrial processors from 100 kg/week
550 to 300 kg/day. Following the packing of the *berbere* powder product, it is released to the market
551 within one to three weeks depending on the demand.

552



553
554

555 **Fig. 4.** Berbere postharvest handling at the domestic processors' level.
556 (A) Berbere pods spread on plastic sheets at a domestic processor. (B) Sun-drying of berbere
557 and different spices on plastic sheets at the domestic processor level. (C) The mill used for
558 berbere powder preparation (used by both industrial and domestic processors). (D) A packet
559 of berbere powder on sale in the market. (E) Lose berbere powder on sale at the market.

560 3.7 Water activity and mycotoxins occurrence

561 The seasonal calendar for the production and processing of *berbere* in Bure district, Amhara
562 region, Ethiopia is presented in Table 6. The incidence of aflatoxin and ochratoxin and water
563 activity (aw) in the *berbere* pod and powder samples collected during the survey are presented
564 in Table 7. All the samples tested positive for both aflatoxins and ochratoxins, meaning they
565 contained levels higher than the maximum tolerable levels (>20 ppb and >2 ppb, respectively)
566 set for both aflatoxin and ochratoxin in *berbere* by different regulatory bodies (Supplementary
567 Information Table 2). The aw of the *berbere* samples collected from farmers ranged from 0.59-
568 0.75 corresponding to moisture contents of 10-18% (Supplementary Information Table 1).

569 In the case of assembler samples, all samples tested positive for the presence of aflatoxins and
570 ochratoxins. Water activity of the samples at assembler-level ranged from aw 0.58-0.71.

571 Typically, aw value for dehydrated vegetables should be $aw < 0.60$ (mould growth starts when
 572 aw is above 0.60). This shows that several of the tested samples had a higher aw than
 573 recommended to control mould growth.

574 All the samples tested at wholesaler level also showed the presence of the studied mycotoxins.
 575 The water activity of 0.55-0.72 was reported for the *berbere* pod samples collected from the
 576 wholesale level. No control over the temperatures and relative humidity occurs in the
 577 wholesaler's storage warehouses and units. Nor are there any pest control, fungal control, or
 578 rodent control activities which occur for *berbere* after harvest. Poor hygiene during handling
 579 and storage was observed. Re-drying of *berbere* at the market places was reported, these
 580 crowded places are dusty and may contain fungal spores which may cause contamination
 581 during the re-drying of the *berbere*.

582 **Table 6.** Seasonal calendar for production and processing of *berbere* in Bure district, Amhara
 583 region, Ethiopia

<i>Months</i>	<i>Mar</i>	<i>Apr</i>	<i>May</i>	<i>Jun</i>	<i>Jul</i>	<i>Aug</i>	<i>Sep</i>	<i>Oct</i>	<i>Nov</i>	<i>Dec</i>	<i>Jan</i>	<i>Feb</i>
<i>Seasons</i>	Autumn (<i>Belg/</i> short rainy season)			The rainy season (<i>Meher</i>)			Harvest season (<i>Tseday</i>)			Dry season (<i>Bega</i>)		
<i>Precipitation</i> (<i>mm</i>)	74.2	83.9	70.2	56.7	237.5	255.7	111.1	41.8	27.6	19.7	19.7	18.7
<i>Temperature</i> •C (<i>min/max</i>)	13/ 27	15/ 28	15/ 29	16/ 30	16/ 28	15/ 27	15/ 27	12/ 26	11/ 25	10/ 24	11/ 25	11/ 27
<i>Chilli</i> <i>planting</i>				■								
<i>Harvesting</i> <i>of fresh chilli</i>									■			
<i>Household</i> <i>processing</i>									■			

584

585 All of the samples collected from the retailers also tested positive for both mycotoxins. The
 586 water activity was similar to that in the wholesaler's samples at 0.51-0.75. The retailers reported
 587 noticing fungal infection in *berbere* pods.

588 Similarly, all the dry pods and processed *berbere* powder at the industrial processor level tested
 589 positive for both toxins. The pods had a water activity of 0.41-0.73 and the prepared *berbere*
 590 powder 0.42-0.53. The reduced water activity in the powder is attributed to the application of
 591 the sun-drying method. The water activity in the *berbere* pods is similar to that in the *berbere*
 592 samples from the farmers, assemblers, wholesalers and retailer levels.

593 A relatively low water activity of 0.49-0.61 was observed in the *berbere* powder collected from
 594 the market compared to the *berbere* pods (aw 0.41-0.73). However, all the market samples
 595 were positive for both toxins. The *berbere* powder collected from the market is packed in
 596 individual low-density polyethylene packets with limited samples available loose or packed in
 597 large plastic bags and exposed to the open environment/dust (Figure 4E).

598 When samples of *berbere* powder were taken from homes, the water activity in them was 0.42-
 599 0.59, and all the samples tested positive for the presence of both toxins. The usage of the spices,
 600 poor hygiene, usage of community mills (Figure 4C) and the *berbere* pods already positive for
 601 both toxins at all stages from farmer to retailer levels contribute to the presence of mycotoxins
 602 in *berbere* powder at the processor stage.

603

604 **Table 7.** Presence of aflatoxins, ochratoxins and water activity in *berbere* samples collected
 605 from different stages of the value chain in Bure district, Amhara region, Ethiopia

Sample location	No. of samples	% of samples testing positive for		Range of water activity (Aw)
		Aflatoxin	Ochratoxin	
Farmers	30	100	100	0.59-0.75
Assemblers	10	100	100	0.58-0.71
Wholesalers	10	100	100	0.55-0.72
Retailers	10	100	100	0.51-0.75
Industrial processors	20	100	100	0.41-0.73(Pods) 0.42-0.53 (Powder)
Berbere powder in market	10	100	100	0.49-0.61

7	Berbere powder from homes/ domestic	10	100	100	0.42-0.59
	Total samples	100	100	100	

606

607 **4. Discussion**

608 **4.1 Farm-level *berbere* production**

609 This study revealed several important aspects of *berbere* cultivation among the responding
610 farmers. The mean area of *berbere* cultivated per household was 0.65 ha, and ranged from 0.25
611 ha to 2 ha. These findings align with a previous study by Abebe & Abera (2019) which reported
612 that *berbere* cultivation in Bure is typically carried out on land areas of less than one hectare.
613 The majority of respondents rely on natural rainfall and practice rain-fed *berbere* production,
614 while a smaller proportion practiced both rain-fed and irrigated production.

615 The preferred variety of *berbere* grown by the respondents was the Danbure variety, with only
616 a few cultivating the Mareko Fana variety. This finding contrasts with an earlier report by
617 Tesheshigo et al. (2019), which indicated that Mareko Fana was the main *berbere* variety
618 cultivated in Bure. The study by Tesheshigo et al. (2019) also provided valuable insights into
619 the harvesting timeline (150 days after planting), marketable yield (6.3 t/ha) and average plant
620 height (69.33 cm) of the Mareko Fana variety. Work by Gobie (2019) also found harvesting
621 took place 5 to 6 months after planting but was directly dependent on the climate, agriculture
622 practices performed, and the variety grown.

623 Pests and diseases were reported as a significant challenge by more than half of the farmers.
624 Bacterial spot (*Xanthomonas campestris*), powdery mildew (*Leveillula Taurica*), and wilt/root
625 rot (*Fusarium oxysporum*) have been identified as the predominant disease problems in *berbere*
626 cultivation in the Bure district (Abebe & Abera, 2019).

627 Weed infestation was also reported by 50% of the respondents, which is consistent with the
628 understanding that weeds can negatively impact *berbere* plant growth and yield (Adenubi &
629 Sanni, 2020). Many weed management methods exist, including manual removal and chemical
630 spraying, however, availability of such chemicals in low-income countries such as Ethiopia is
631 often limited. Tesfa et al., (2017) reported that in some locations farmers believe that weeding
632 immediately following a rain shower or on cloudy days will favour a powdery mildew
633 outbreak. However, limited availability of herbicides and sprayers in low-income countries
634 such as Ethiopia add labour challenges for farmers needing to control weeds.

635 Crop rotation was practiced by around 70% of the respondents, indicating their awareness of
636 the benefits of alternating crops on the same piece of land. However, the remaining 30%
637 reported continuous cultivation of *berbere* on the same land. It has been suggested this
638 continuous cultivation may be influenced by the increased market price of the commodity
639 (Shumeta, 2012).

640 Rainfall played a crucial role in the various stages of *berbere* cultivation. The majority of
641 respondents reported rainfall during flowering, pod maturation, and harvesting. This aligns
642 with the findings of Djomo et al. (2020) and Dahiru & Tanko (2018), who highlighted the
643 impact of climate variations and rainy seasons on crops, including *berbere*. Rainfall during the
644 drying process was reported by 70% of the respondents, this can lead to prolonged drying
645 periods and quality deterioration of *berbere* pods (Hempattarasuwan et al., 2020).

646 **4.2 Farmers' drying, sorting and storage of *berbere***

647 Sun-drying was the predominant drying method employed by virtually all the farmers. A
648 moisture content of less than 10% is recommended for optimal shelf-life of *berbere* pods
649 (Prakash & Eipeson, 2003). Sun-drying *berbere* pods under uncontrolled conditions can due to
650 prolonged sunlight exposure, lead to challenges such as bleaching and dull colour formation.
651 Additionally, sun-drying directly on the ground may result in contamination with foreign
652 matter or infestation by insects and fungi, which thrive in moist conditions, thereby reducing
653 the quality and safety of the *berbere* (Prakash & Eipeson, 2003; Fudholi et al., 2013).

654 In recent years, various types of solar and artificial dryers have been developed across the world
655 (Paul & Singh, 2013). Artificial dryers were shown by Gupta et al., (2018) to be better at
656 maintaining *berbere* pod quality than traditional sun-drying methods. However, such advanced
657 drying equipment is not readily available or affordable to farmers in the study area.

658 It is worth noting that 53% of the farmers reported observing fungal infections or diseases on
659 the pods while still in the field before harvesting, supporting research by Abebe & Abera (2019)
660 that highlighted the prevalence of fungal diseases in *berbere* in the West Gojjam zone of
661 Ethiopia. Regarding sorting, the majority of the farmers (70%) practiced post-drying sorting.
662 During this stage, spoiled, broken, and pale or white-coloured *berbere* pods were sorted out,
663 while plant waste and dust were removed. This practice of post-drying sorting is similar to
664 practices observed among chilli farmers in India (Pruthi, 2003).

665 Most of the farmers used *goteras* for *berbere* storage, as is commonly done for preserving
666 spices in Ethiopia (Tesfa et al., 2017). While in many places' plastic bags (polyethylene) are

667 commonly used for packing dried *berbere* (Yanti et al., 2018), in India, jute bags or gunny bags
668 are used for chilli pepper storage (Peter et al., 2003). The environmental conditions, specifically
669 temperature and humidity, within the storage containers play a crucial role in maintaining
670 *berbere* quality. However, insect pests can cause damage during storage, and fumigation has
671 been suggested as a control measure (Yogeesbh & Gowda, 2003). The mean storage duration
672 reported by the farmers in this study was 3.9 months, but individual farmers stored *berbere* for
673 varying durations. Fungal growth during storage is a common issue when the moisture content
674 of the *berbere* pods increases (Yogeesbh & Gowda, 2003), and previous studies have identified
675 fungal species such as *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. as potential
676 contaminants (Mandeel, 2005). However, none of the farmers in the current study reported
677 awareness of mycotoxins, highlighting the need for knowledge and awareness among *berbere*
678 handlers throughout the value chain to prevent their development (Udomkun et al., 2018).

679 Interestingly, none of the farmers reported pest or disease issues causing losses after harvest or
680 during storage of *berbere*. They attributed this to the natural insect deterrent qualities of the
681 red chilli peppers (Lale, 1992; Swamy and Wesley, 2017). It is noteworthy that none of the
682 farmers used any storage protectants or other practices to protect and maintain the quality of
683 their stored *berbere*.

684 Postharvest knowledge for *berbere* is currently predominantly flowing through traditional
685 information pathways, for example, learning from elders or friends, with only 7% of farmer
686 respondents mentioning having learnt about *berbere* postharvest aspects from agricultural
687 officers. Demands for more information about the causes of and methods for reducing
688 postharvest quality deterioration of *berbere* were expressed.

689 **4.3 *Berberere* handling by assemblers, wholesalers and retailers**

690 The assemblers play an important role in the *berbere* value chain serving as intermediaries
691 collecting and purchasing *berbere* from farmers from different villages for resale to
692 wholesalers and retailers. Work by Shumeta (2012) reported from Southwest Ethiopia that
693 individual assemblers collect approximately 50-120 quintals (1 quintal = 100 kg) of *berbere*
694 pre year and resell it to wholesalers across various regions in Ethiopia.

695 Although most assemblers aim to sell their *berbere* on immediately to the next-level
696 purchasers, some do store it for periods ranging from 15 days to 2 years. They may re-dry it
697 during this period using open air sun-drying with the same contamination risks as occur during
698 open-air on ground sun-drying at farmers'-level. If the moisture content is high, even temporary

699 storage of *berbere* can result in quality deterioration and fungal growth and can lead to the
700 production of mycotoxins (Peter et al., 2003). In different regions of Ethiopia, handlers have
701 been observed using floor storage or heap storage for *berbere* pods before packing them into
702 various types of bags (Kuchi et al., 2014). The majority of assemblers remove dirty,
703 discoloured, spoiled, broken pods, and any plant debris, by winnowing outside and manually
704 sorting.

705 Wholesalers play a crucial role in the *berbere* value chain, purchasing *berbere* from farmers
706 and assemblers and supplying it to retailers, local industries, and households (Gobie, 2019;
707 Shumeta, 2012b). The quantities of *berbere* purchased by each wholesaler interviewed in the
708 current study range from 300-4,000 kg per trader per year, which aligns with earlier findings
709 by Shumeta (2012a) where procurement quantities were influenced by population, seasonal
710 variation, and market demand. The wholesalers did not report using any chemical preservatives
711 or other materials to reduce fungal growth problems in their stored *berbere*, and none of them
712 were aware of mycotoxins. They sort and re-dry their produce, discarding any spoiled *berbere*
713 pods, and were aware that during the rainy season increased fungal growth occurs on the
714 *berbere* pods. A lack of formal quality assessment practices exists and the storage facilities are
715 unable to control the temperature and humidity conditions which can potentially impact the
716 quality and safety of *berbere* during this stage of the value chain.

717 Retailers also play a crucial role in the value chain by selling *berbere* directly to customers,
718 local retail shops and industrial processors. They rarely store *berbere* for more than a week.
719 Some retailers explained they had observed fungal growth on *berbere* pods, particularly during
720 the rainy seasons, manifesting as black and white spots on the outside or black dust inside the
721 pods. In cases of high spoilage, one retailer mentioned selling the product at a lower price to
722 consumers, while two other retailers stated that they would return the product to the wholesaler.
723 Retailers reported re-sun-drying the *berbere*, typically on plastic sheets, if the moisture content
724 was too high. Customers are reported to inspect the *berbere*, particularly the pod colour and
725 check for any visible contamination before buying it.

726 **4.4 Domestic and industrial processing**

727 Regarding domestic household processors, the respondents explained that they purchase the
728 *berbere* just after harvest during the peak of its abundance in the markets, when prices are most
729 reasonable (Gobie, 2019). This practice ensures that the *berbere* used for processing is of high
730 quality and freshness, which is crucial for the overall flavour and aroma of the final *berbere*
731 powder.

732 The shelf life of *berbere* powder depends on various factors, including the methods employed
733 in pre-processing, processing, storage conditions, and the antioxidant composition of the
734 *berbere* (Pruthi, 2003). Microbial contamination of the *berbere* and susceptibility to spoilage
735 once ground are issues of concern to domestic processors. Ground *berbere* is more prone to
736 spoilage (Buckenhuskas, 2003). This highlights the need for appropriate handling and storage
737 practices throughout the value chain to maximize the shelf-life and quality and minimize the
738 risk of microbial contamination and subsequent spoilage during the processing and storage of
739 *berbere* powder by processors, food service providers or consumers.

740 The results indicate that small-scale or cottage industries involved in *berbere* production rely
741 on traditional methods and subjective assessments for quality control, with limited use of
742 technical instruments or formal quality management systems. The lack of temperature,
743 moisture or rodent control in storage facilities raises concerns about product integrity and
744 potential spoilage. Implementing appropriate handling practices and storage conditions could
745 help mitigate the risk of microbial growth and maintain the overall quality of the *berbere*.
746 Increasing awareness and implementing mycotoxin control measures would be beneficial to
747 ensure the safety of the *berbere* products.

748 **4.5 Water activity and mycotoxins occurrence**

749 Water activity (a_w) is a critical factor affecting fungal growth and mycotoxin production. The
750 most favourable growth conditions for *Aspergillus flavus*, a fungus associated with aflatoxin
751 production, are within a temperature range of 25-30°C and a_w higher than 0.970 (Chuaysrinule
752 et al., 2020). Respondents in the current study noted that when *berbere* were not properly dry
753 (high moisture content) it encourages fungal growth. The relationship between water activity
754 and moisture content in preserving *berbere* is described by Shirkole & Sutar (2018) and Pruthi
755 (2003). Shirkole & Sutar (2018) report that a water activity of 0.4199 corresponds to a moisture
756 content of 6.93%, while Pruthi (2003) suggests a moisture content of around 10% (a_w 0.59) is
757 optimal for *berbere* pod preservation.

758 The sun-drying method commonly used by *berbere* farmers, whereby pods are spread directly
759 on the ground, can lead to fungal contamination. The correlation between sun-drying duration
760 and aflatoxin formation in red-chillies has been reported previously by Sahar et al., 2017. The
761 rainy season poses additional challenges, as heavy rains and moisture re-absorption can
762 increase the risk of fungal contamination. Moreover, poor weed management is reported to
763 contribute to termites, pest development, pod damage, and *Aspergillus* infection (ICRISAT,
764 2016; Tadesse, 2009).

765 The current study found a lack of knowledge and awareness among farmers and processors
 766 regarding mycotoxins. Training and education are deemed crucial for effective management of
 767 aflatoxin problems (Udomkun et al., 2018), and the adoption of improved agricultural
 768 practices, including effective weed and pest management is crucial to mitigate mycotoxin
 769 contamination in *berbere* (Kimanya & Mlalila, 2020).

770 Storage and postharvest handling practices also contribute to mycotoxin contamination. The
 771 poor storage structures and methods used at the different levels in the *berbere* value chain,
 772 illustrate the lack of mechanisms for controlling environmental conditions. The absence of
 773 moisture content analysis and the inadequate implementation of insect pest and fungal
 774 management strategies were also important factors. Previous studies on red chilli peppers have
 775 highlighted how technologies such as cold storage, radiation, vacuum packing, and hermetic
 776 bags can control fungal growth and maintain quality (Ravi Kiran et al., 2005; Onyenekwe et
 777 al., 1995; Chetti et al., 2014; Abrar et al., 2023). However, none of these technologies are used
 778 in the study location currently and the affordability of several of them may be challenging for
 779 most of the value chain actors.

780 Contamination during milling may also occur due to inadequate hygiene practices. The addition
 781 of other spices, such as dried ginger and garlic, could also potentially introduce fungal toxins
 782 into the *berbere* powder (Hacibekiroğlu & Kolak, 2013; Hammami et al., 2014; Macdonald &
 783 Castle, 1996). An overview of some of previous research finding on red-chilly pepper is
 784 summarised in Table 8.

785 **Table 8.** Overview of research findings on aflatoxin and ochratoxin presence in *berbere*/ dry
 786 red-hot chilli and related products across different countries

Country	No. of samples	% of samples testing positive for		Reference
		<i>Aflatoxin</i>	<i>Ochratoxin</i>	
Spain	21	90 (AFT B1)	67	(Hierro et al., (2008)
India	55	85.5	72.2	Jeswal & Kumar, (2015)
India	25	52.7	40	Jeswal & Kumar, (2016)
India	40	85.45	72.7	Jeswal & Kumar, (2014)
Sri Lanka	86	75	40	Yogendrarajah et al., (2014)
Belgium	35	35	45	Yogendrarajah et al., (2014)
USA	169	64%	-	Singh & Cotty, (2017)

Nigeria	55	93%	-	Singh & Cotty, (2017)
Pakistan	331	48.33	-	Khan et al., (2014)
Pakistan	176	66	-	Shamsuddin, et al., (1995)
Pakistan	312	-	40.4%	Iqbal et al., (2017)
Pakistan	13	100	-	Paterson (2007)
Turkey	25	72	-	Yilmaz (2017)
Turkey	120	62.5%	-	Set & Erkmén, (2014)
Turkey	125	65.7	-	Ankara, (2012)
Nigeria	70	69	-	Ezekiel et al., (2019)
Ethiopia	60	8.33%	-	Fufa & Urga, (1996)
Ethiopia	135	47	-	Tsehaynesh et al., (2021)
Malaysia	80	65	81.25	Jalili & Jinap, (2012)
Italy	30	-	40.0	Prelle et al., (2013)l
Italy	130	-	78.9%	Prelle, et al., (2014)

787

788 **4.6. Conclusions and recommendations**

789 In conclusion, this study revealed that all the *berbere* samples tested positive for aflatoxin and
790 ochratoxin, indicating the widespread presence of these mycotoxins. The findings also
791 highlighted the continued use of traditional agricultural practices in *berbere* cultivation and
792 handling within the Amhara region of Ethiopia. Factors such as rain-fed cultivation, exposure
793 to rain during growth stages, poor weed management, absence of pesticide applications, sun-
794 drying directly on the ground, inadequate storage structures, low-quality packing materials, and
795 the absence of formal quality control methods were identified as contributors to reduced quality
796 and mycotoxin contamination throughout the *berbere* value chain. The packing materials are
797 also poor quality and damaged. No formal method nor analytical devices are used to determine
798 the moisture and any other quality parameter at any stage of the domestic *berbere* value chain.
799 In Ethiopia, the *berbere* pods are converted into a powder and different spices are added. At
800 the industrial *berbere* processor scale, there are no formal quality control systems being
801 implemented. We conclude that poor handling practices in the value chain contribute to the
802 presence of aflatoxins and ochratoxins in all the *berbere* samples tested in this study. At all
803 stages, the training of actors is poor, but they are eager to have their capacity strengthened.

804 Potential recommendations to improve the quality of and reduce the mycotoxin contamination
805 of *berbere*, include:

- 806 • Working with the value chain actors to co-develop, promote and test the use of an
807 integrated pest management system for the *berbere* crop, including:
- 808 • Practising irrigated cultivation for suitable *berbere* varieties
- 809 • Regular weed management using a range of appropriate methods and training
- 810 • Improved drying of *berbere* on plastic sheets, and for some actors artificial dryers to
811 support faster and more uniform drying in hygienic conditions to maintain quality
- 812 • Training of the different value chain actors on the use of good hygiene practices
- 813 • Training of the different value chain actors on sorting, handling and storage, quality
814 management practices, and implementation of quality control systems in *berbere*
815 industries
- 816 • Use of simple moisture determination facilities at all stages of the value chain
- 817 • Use of good packing bags and materials
- 818 • Use of appropriate storage facilities at each value chain step.
- 819 • Supporting greater awareness and knowledge about the spoilage factors and the
820 importance of managing mycotoxins in *berbere* industries
- 821 • Drafting and supporting regulations to enable government agencies to monitor and
822 support the implementation and achievement of standards in *berbere* powder handling
823 and processing in domestic value chains.

824 **Declaration of conflict of interests**

825 The authors declare that they have no known conflict of interest that could have appeared to
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1126 **Supplementary information**

1127 **Supplementary Table 1**

1128 Relationship between Water activity (aw) and Moisture content

Moisture content (%)	6.93	10.81	14.75	18.65	23.05
Water activity (aw)	0.4199	0.5948	0.6873	0.7530	0.7832
	(± 0.007)	(± 0.002)	(± 0.002)	(± 0.002)	(± 0.004)

1129 (Source: Sahar et al., 2015)

1130 **Supplementary Table 2**

1131 Maximum tolerable levels set for aflatoxin and ochratoxin in *berbere* by different regulatory
1132 bodies

S. No	Regulation for	Accepted maximum tolerable levels (µg/kg)	Reference
Ochratoxin in <i>berbere</i>			
1	European Union according to Regulations (EC) No. 1881/2006 and 105/2010.	15	Duarte et al., (2010)
2	WHO/ CODEX/ FAO	20	FAO, WHO, & CODEX, (2018)
3	AFRICAN UNION (AU)	20	FAO/ WHO and CODEX (2018)
Aflatoxin in <i>berbere</i>			
1	WHO/ CODEX/ FAO	20 - 30	FAO (2018)
2	EUROPEAN UNION (EU)	10	FAO/ WHO and CODEX (2018)
3	AFRICAN UNION (AU)	20 - 30	FAO/ WHO and CODEX (2018)

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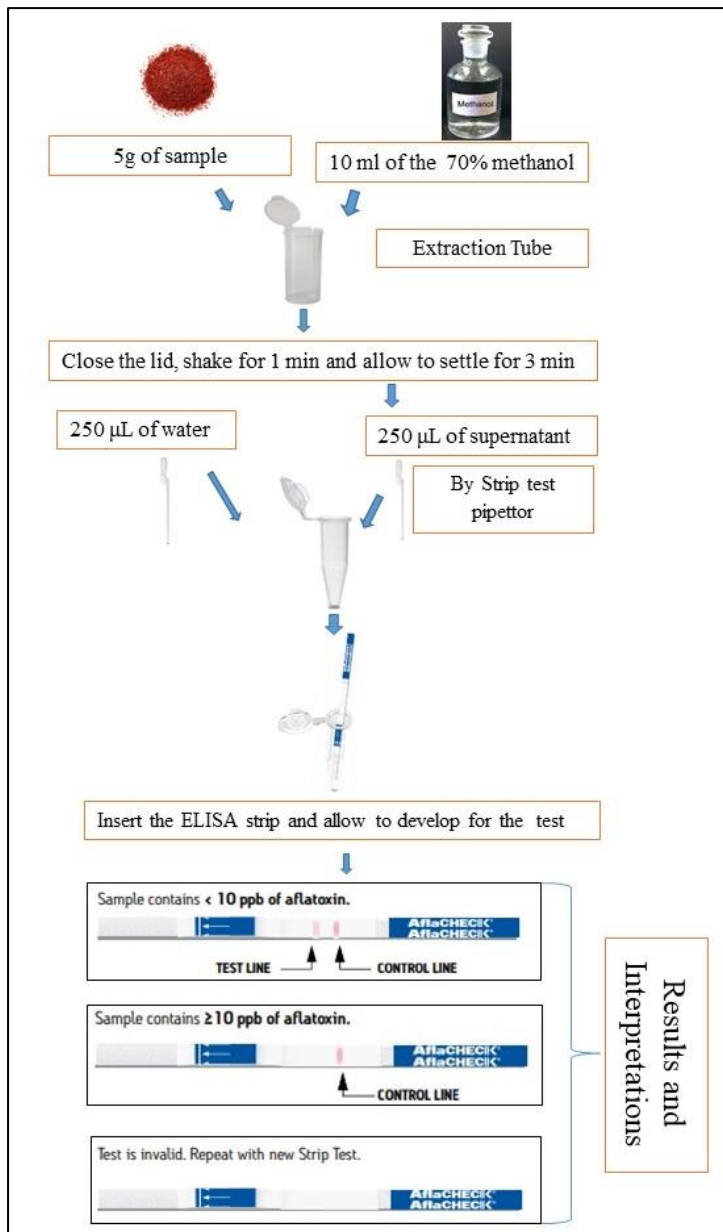
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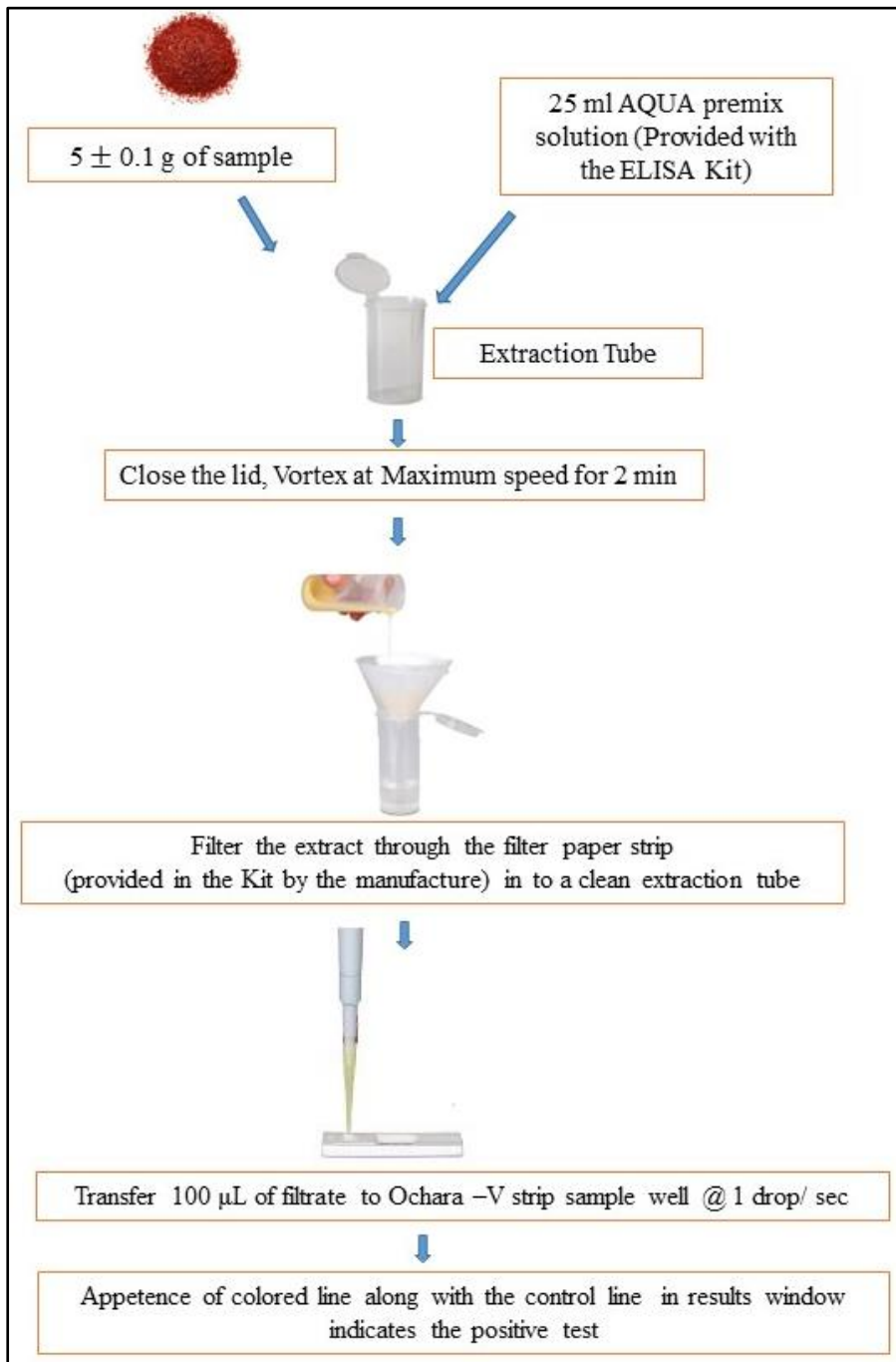
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Supplementary Figure 1: Sample extraction and determination of Aflatoxin by ELISA strip



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1152 **Supplementary Figure 2. Sample extraction and determination of Ochratoxin by ELISA**

1153 **kit method.**