

# The Microbial Contamination, Toxicity and Quality of Turned and Unturned Outdoor Floor Malted Sorghum

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## ABSTRACT

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Turned and unturned outdoor floor malted sorghum were studied for their total microbial contamination, nature and extent of contamination by moulds, cytotoxicity (IC<sub>50</sub>) and quality in terms of diastatic power (DP). The presence of aflatoxins, fumonisins, deoxynivalenol and zearalenone were also investigated. Total microbial counts were high (10<sup>7</sup>–10<sup>8</sup> cfu/g) in both turned and unturned samples. All samples showed contamination by different moulds, with the dominant being *Mucor species*, *Rhizopus oryzae*, *Fusarium moniliforme* and *Phoma sorghina* as well as *Aspergillus flavus* and *Alternaria alternata*. The latter four are known for producing mycotoxins. Malt samples had very low cytotoxicity (IC<sub>50</sub> from 62.5 to >1000 kg/kg), though all contained fumonisins, deoxynivalenol and zearalenone at levels of <0.25–2 µg/g, 15–20 and 10–15 µg/kg, respectively. Malt DP was generally lower in turned samples compared to unturned samples probably because the heat conserved in the latter ensured better germination conditions. Overall, turning during germination did not affect the microbial load, mould population and levels of deoxynivalenol and zearalenone in sorghum malt but decreased sorghum malt DP. Thus, alternative methods of controlling the sorghum malt microbial load should be sought.

**Key words:** Cytotoxicity, mycoflora, mycotoxins, sorghum malt.

## INTRODUCTION

In Southern Africa some 200 000 tons of sorghum is malted commercially annually and is mostly used to brew sorghum beer<sup>29</sup>. In South Africa, most sorghum malting is by traditional outdoor floor malting. Floor malting involves germination of the steeped grain outdoors for 4–6 days in layers of about 10 to 30 cm, on a very slightly sloped floor (generally rough concrete) to allow drainage of rain water<sup>28</sup>. The germinating grain is sprayed once or twice daily using a hose. Unlike in barley floor malting, it is general practice in sorghum floor malting that the grain

is not turned. Rather, sorghum grains are allowed to germinate undisturbed, resulting in the stratification of the malt bed<sup>27</sup>. The germination bed temperature and aeration are difficult to control under such conditions. Hence the practice whereby thinner beds are employed in hot weather (for easier dissipation of heat) as against the use of much thicker beds during winter (better preservation of metabolic heat)<sup>27,30</sup>. These germination conditions apparently encourage the proliferation of fungi and lead to high levels (10<sup>5</sup>–10<sup>6</sup> cfu/g) of fungal counts<sup>21,29</sup> in South African sorghum malt. Rabie and Marais<sup>23</sup> have suggested that sorghum malt has the highest fungal load of all South African foods and feeds. While sorghum malt microflora also include bacteria and yeasts, the mould contaminants have been of the greatest concern because of their potential mycotoxigenicity<sup>7,8</sup>.

A five-year survey of industrial sorghum beer brewing in South Africa<sup>30</sup> has shown South African sorghum malt to contain on average 2.18 µg/kg of aflatoxin, with 68% of malts containing ≤1 µg/kg, 20% having 1–3 µg/kg and only 4.5% yielding more than 10 µg/kg. Recently, zearalenone has also been detected in South African sorghum malt grain samples<sup>17</sup>.

Ideally, sorghum malt should be completely free of mycotoxin producing fungi and mycotoxins. The objective of this study was to determine the effect of turning the grain during germination, as is practised in barley floor malting<sup>1</sup> on the microbiological quality of outdoor floor malted sorghum<sup>1</sup>. In barley floor malting the germinating grain is raked, turned and mixed periodically to equalize the temperature and to prevent the roots from matting. Matting leads to “hot spots” where mould growth occurs.

## MATERIALS AND METHODS

### Sorghum grain

Sorghum cultivar NK 283, a condensed tannin-free red hybrid, was obtained from Tiger Brands, Potchefstroom, South Africa.

### Malting

Malting was repeated twice in the winter season with a relatively thin depth of grains (10 cm) to maximally stress the grains. The malting procedure used simulated South African commercial outdoor floor malting conditions. Cleaned sorghum grains (2.5 kg) were weighed into plastic baskets (12 cm depth) to make a 10 cm thick layer. The

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baskets were steeped continuously in 4.0 L of tap water for 8 h at room temperature (20°C). Subsequently grains were germinated outdoors with malt bed temperatures of 18–20 and 14–17°C. Germination was for 7 days with the baskets placed on slightly sloped surfaces (15° angle) to allow drainage of excess water. The surface of the germinating grains was covered with plastic sacking to reduce evaporation and to prevent attack by birds. At intervals (12 h) grains were sprayed with 350 mL of distilled water using an atomizer spray. Temperature could not be controlled because the malting was done outdoors but it was measured three times a day (in the morning, midday and in the evening). To determine the effect of turning, germinating grains in one basket were turned during each watering period, while grains in the other were not turned. At the end of germination, unturned grains were separated into the top, middle and bottom layers (about 3 cm each) according to the different visual estimation of moisture content of the grains (dry top, damp middle and the wet bottom). The grains of the turned sample were randomly separated into the top, middle and bottom layers for comparison. The moisture content of the different layers was determined. The green malt was dried in a forced-draft oven at 50°C for 24 h and milled with a laboratory hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 500 µm opening screen.

### Microbiological analysis

**Microbial population.** Malt samples (10 g) were placed in sterile bags and homogenized in 90 mL 0.1% peptone, 0.85% NaCl, for 30 sec. Tenfold serial dilutions were prepared and appropriate dilutions spread-plated in triplicate onto Plate Count Agar (PCA) for total aerobic counts, Potato Dextrose Agar (PDA) for yeasts and moulds and MRS (de Man Rogosa and Sharp) agar for lactic acid bacteria (LAB) counts. Pour plate technique with the second overlay with agar was performed with the Violet Red Bile agar (VRB) for coliforms. PCA plates were incubated at 35°C, MRS plates at 30°C, VRB plates at 37°C and PDA plates 25°C for 24 to 48 h. All media preparations were according to Pattison *et al.*<sup>18</sup>.

**Mould isolation and identification.** Mould enumeration, isolation and identification were according to Rabie and Lübben<sup>21</sup>. The direct plating method used to quantify the growth of moulds used is more efficient than dilution plating for detecting moulds from sorghum malt samples<sup>22</sup>. Briefly, alcohol-disinfected grains (5) were placed on plates (ten each) of Potato Dextrose Agar (PDA), Malt Salt Agar (MSA), acidified Czapek-Dox Agar and Pentachlorobenzene Agar and incubated at 25°C for 2 to 14 days. The results are reported as a percentage of isolated mould species per sample. Moulds were identified to species level where possible, using morphological identification keys, by light and stereo microscopy, as described in several texts<sup>5,15,19</sup>.

### Diastatic Power (DP)

Diastatic Power (DP) which measures the overall amylase activity, was measured as the parameter to determine the sorghum malt quality because with the Southern African sorghum malt industry, sorghum malt quality is defined in terms of its overall DP<sup>24</sup>. The South African Bu-

reau of Standards method 235<sup>25</sup> was used, except that water was used as the extractant and 5 g of malt used. The volume of the extractant was reduced accordingly. Results are expressed as sorghum diastatic units (SDU)/g dry weight.

### Cytotoxicity assays

**Sample extraction.** Sample extraction was by the multi-mycotoxin screen method of Dutton and Westlake<sup>4</sup>. The extracts (neutral fraction) were weighed and dissolved to 1 mg/mL in complete medium containing ethanol and dimethylsulphoxide (DMSO). Samples were sonicated until they dissolved, then filter-sterilised through a 0.22 µm filter.

**Cells and mycotoxin standards.** Sp2/0 cell line from Balb/C mice was used. Cell maintenance was as described in Hanelt *et al.*<sup>9</sup>, except that Dubelcco's Modified Eagle's Medium (DMEM) was used instead of Modified Eagle's Medium (MEM). Aflatoxin B<sub>1</sub>, deoxynivalenol (DON) and zearalenone (ZEA) standards were from Sigma-Aldrich (catalogue numbers A6636, D0156 and Z2125, respectively).

**MTT-cell culture test.** The cytotoxicity of sorghum grain samples was analysed using the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) cell culture assay which monitors a reduction of yellow tetrazolium (MTT) salt by mitochondrial dehydrogenase enzymes of metabolically active/viable cells to purple formazans<sup>14</sup>. The concentration of samples and mycotoxin standards ranged from 32 to 1000 µg/kg and 0.25 to 500 µg/g, respectively. Assays were performed according to Hanelt *et al.*<sup>9</sup> The absorbance of cultures was measured at 490 nm with an ELISA reader (Multiscan<sup>®</sup> ascent, Lab systems, Finland). Mean extinction values and standard deviations of each sample were compared with those of the corresponding controls and expressed as % cleavage activity in comparison to the cell controls (100%). The cytotoxicity of samples was expressed in terms of their IC<sub>50</sub> value (Inhibitory concentration<sub>50</sub> = concentration resulting in 50% inhibition of the MTT cleavage activity).

### Assay of aflatoxins, fumonisins, deoxynivalenol (DON) and zearalenone (ZEA)

Aflatoxins and fumonisins were assayed by the VICAM Aflatest<sup>™</sup> and Fumonitest<sup>™</sup> (VICAM, Watertown, USA) which is based on affinity chromatography. Aflatoxins were also determined by TLC as described by Trinder<sup>31</sup>. Confirmation of the presence of aflatoxins was carried out by two-dimension TLC. The TLC detection limit is 0.3 µg aflatoxin/kg and the results were expressed as µg/kg of sample. DON and ZEA were determined by a multi-mycotoxin TLC screen method as described by Dutton and Westlake<sup>4</sup>, except that plates were developed and viewed using a method described in Wilbert and Kimmelmeier<sup>34</sup>.

### Statistical analysis

Results were analysed using the SAS package, version 8.2. Analysis of variance (ANOVA) was used to evaluate the data based on a 0.05 level of significance. Differences between means were determined using the least significant difference (LSD) test.

**Table I.** Effect of turning on the bacterial counts (cfu/g) of the unmalted sorghum and the top, middle and bottom layers of sorghum malt when germinated at 14–17°C and 18–20°C.

	APC <sup>1</sup>	LAB <sup>2</sup>	Fungi	Coliforms
Unmalted sorghum	3.2 × 10 <sup>5</sup> a	4.7 × 10 <sup>4</sup> a	2.7 × 10 <sup>4</sup> a	1.8 × 10 <sup>3</sup> a
Sorghum malted at 18–20°C				
Unturned				
Top	6.0 × 10 <sup>7</sup> b	8.9 × 10 <sup>6</sup> b	5.6 × 10 <sup>5</sup> b	4.6 × 10 <sup>4</sup> b
Middle	3.4 × 10 <sup>8</sup> c	3.5 × 10 <sup>7</sup> c	7.1 × 10 <sup>6</sup> c	5.6 × 10 <sup>5</sup> c
Bottom	5.1 × 10 <sup>8</sup> c	5.7 × 10 <sup>7</sup> c	5.9 × 10 <sup>6</sup> c	8.9 × 10 <sup>5</sup> c
Turned				
Top	6.6 × 10 <sup>8</sup> c	5.4 × 10 <sup>7</sup> c	5.3 × 10 <sup>6</sup> c	7.1 × 10 <sup>5</sup> c
Middle	4.9 × 10 <sup>8</sup> c	5.3 × 10 <sup>7</sup> c	5.1 × 10 <sup>6</sup> c	8.9 × 10 <sup>5</sup> c
Bottom	5.7 × 10 <sup>8</sup> c	5.1 × 10 <sup>7</sup> c	5.6 × 10 <sup>6</sup> c	7.1 × 10 <sup>5</sup> c
Sorghum malted at 14–17°C				
Unturned				
Top	1.3 × 10 <sup>7</sup> b	6.3 × 10 <sup>6</sup> b	5.6 × 10 <sup>5</sup> b	1.6 × 10 <sup>4</sup> b
Middle	1.9 × 10 <sup>8</sup> c	3 × 10 <sup>7</sup> c	2.9 × 10 <sup>6</sup> c	3.2 × 10 <sup>5</sup> c
Bottom	2.6 × 10 <sup>8</sup> c	4.6 × 10 <sup>7</sup> c	5.3 × 10 <sup>6</sup> c	7.1 × 10 <sup>5</sup> c
Turned				
Top	2.9 × 10 <sup>8</sup> c	3.6 × 10 <sup>7</sup> c	3.3 × 10 <sup>6</sup> c	5.1 × 10 <sup>5</sup> c
Middle	3.0 × 10 <sup>8</sup> c	3.6 × 10 <sup>7</sup> c	3.7 × 10 <sup>6</sup> c	5.1 × 10 <sup>5</sup> c
Bottom	3.0 × 10 <sup>8</sup> c	4.0 × 10 <sup>7</sup> c	3.8 × 10 <sup>6</sup> c	5.1 × 10 <sup>5</sup> c

<sup>1</sup> Aerobic Plate Count.

<sup>2</sup> Lactic acid Bacteria.

Mean and values with different letters in the same column are significantly different from each other ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### Microbial population

For both germination temperatures the sorghum malt displayed microbial counts as follows:  $1.3 \times 10^7$ – $5.7 \times 10^8$  cfu/g, total aerobic plate count (APC),  $6.3 \times 10^6$ – $5.7 \times 10^8$  cfu/g, LAB count;  $2.7 \times 10^4$ – $7.1 \times 10^6$  cfu/g, fungal count and  $1.6 \times 10^4$ – $8.9 \times 10^5$  cfu/g, coliform count (Table I), all of which were substantially higher ( $p < 0.05$ ) than the microbial load of the unmalted sorghum (APC,  $3.2 \times 10^5$  cfu/g; LAB,  $4.7 \times 10^4$  cfu/g; fungi,  $2.7 \times 10^4$  cfu/g; and coliforms,  $1.8 \times 10^3$  cfu/g). The sorghum malts microbial counts are in the same range with those obtained by Thaoe *et al.*<sup>30</sup>, in which a total aerobic count of  $1.3 \times 10^8$  cfu/g, LAB count of  $1.8 \times 10^7$  cfu/g, yeast count of  $8.7 \times 10^6$  cfu/g, moulds of  $6.3 \times 10^5$  cfu/g and coliforms of  $5.4 \times 10^5$  were obtained from sorghum malts. Lori *et al.*<sup>10</sup> obtained bacterial counts of  $2 \times 10^6$  cfu/mL and  $3.1 \times 10^6$  cfu/mL, in malt samples made from two sorghum varieties. A typical Southern African sorghum malt specification for total bacterial count is  $< 2 \times 10^7$  cfu/g. The aerobic counts of sorghum malt samples in this study exceed the specification and are therefore regarded as unacceptably high.

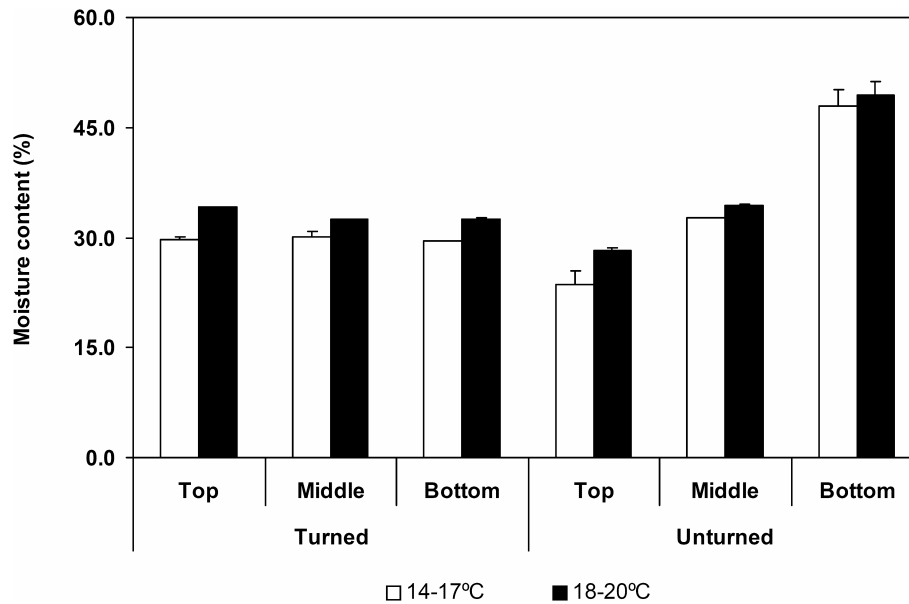
Malt samples germinated at 18–20°C had significantly higher microbial counts ( $p < 0.05$ ) than malt germinated at 14–17°C, probably because the higher malting temperature was more suitable for the growth of the microbial contaminants. Nevertheless, significantly ( $p < 0.05$ ) lower microbial loads occurred within the top layers of unturned samples of both batches in comparison to their middle and bottom layers. This is because much of the water sprayed on the grains during germination passed through the top layer faster than could be absorbed. Some water of the top layer was also lost through evaporation, so that the malt became progressively wetter from the top of the bed to the bottom (Fig. 1). The dry environment of the top layer does

not encourage microbial growth. The rate of water percolation reduced as water passed down through the sorghum grain layers so that some of it was trapped in the middle layer and made it moist and a lot of it was trapped in the bottom layer and made it wet even though the slope allowed for some drainage (Fig. 1). Turning the malt resulted in the moisture being uniformly distributed throughout the malt bed.

### Mould isolation and identification

A wide variety of moulds were isolated from the sorghum grain and malt (Table II). The mould load of malted sorghum far exceeded that of the unmalted grains. *Fusarium moniliforme*, *Mucor* spp., *Phoma sorghina* and *Rhizopus oryzae* were the most abundant, being detected in all analysed sorghum malt samples (100% incidence level). *Alternaria alternata* was also detected at high levels (39–74%), while the incidence of *Fusarium chlamydosporum*, *Aspergillus flavus* and *Eurotium* spp was relatively low (12–35, 5–31 and 3–21%, respectively). *Aspergillus niger*, *Penicillium* spp, *Culvularia* spp, and *Aspergillus fumigatus* occurred at less than 2% (results not shown). Thus the fungal population consisted of field (*Fusarium* spp, *Phoma sorghina* and *Alternaria alternata*) and storage fungi (*Mucor* spp, *Rhizopus* spp and *Eurotium* spp), suggesting that an increase in moisture content during malting<sup>16,33</sup> is quite favourable for fungal growth of different genera. The contamination of sorghum malt by a wide range of fungal species agrees with findings by Rabie and Lübben<sup>21</sup> who earlier demonstrated the contamination of South African sorghum malt samples (65 commercial (floor malting) and 22 industrial (pneumatic malting) by the above fungal species in almost the same order of incidence as was observed in the current study.

The top layer of the unturned samples for both germination temperatures had lower ( $p < 0.05$ ) incidence of some mould spp. (*A. flavus*, *Eurotium* spp, *F. chlamydo-*



**Fig. 1.** Moisture content (%) of the top, middle and bottom layers of the sorghum obtained at temperatures of 18–20 and 14–17°C. The error bars indicate  $\pm$  standard deviation.

*sporum* and *A. alternata*) compared to both the middle and bottom layers. Unlike unturned samples, the mould distribution of turned sorghum malt samples was not influenced ( $p > 0.05$ ) by either grain bed depth (i.e. whether top, middle or bottom layers), or malting temperature. This is probably due to the fact that the mixing effects of turning reduced differences in temperature, moisture content and gas distribution between the three malt bed layers, ensuring that these conditions were nearly identical throughout the malt bed. Presumably, the blending effects of malt turning ensured even distribution of individual mould propagules across the malt bed. The high levels

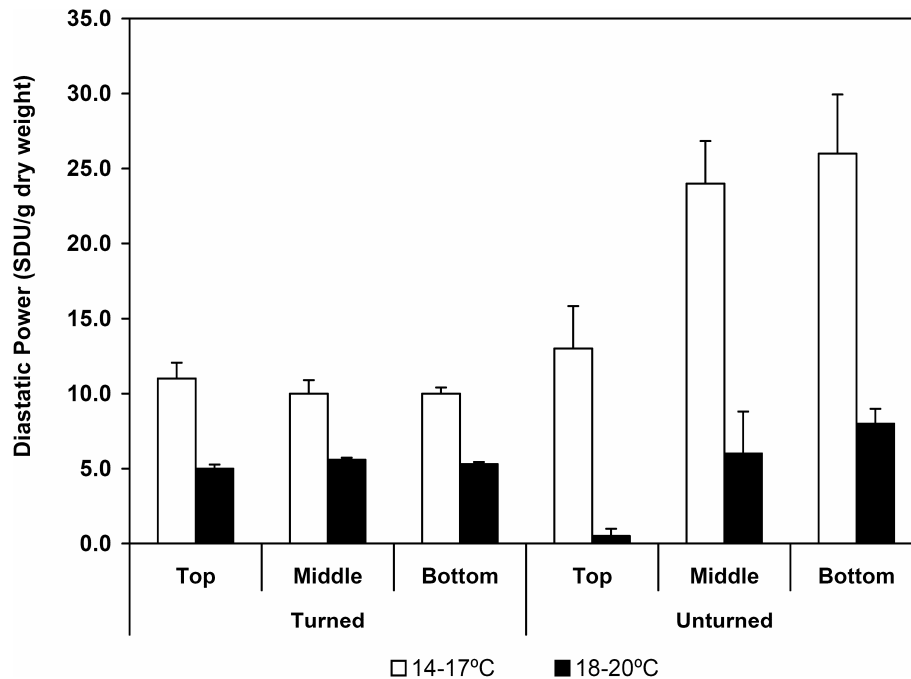
of mould contamination found in this work raise concerns because of the presence of the potentially mycotoxigenic moulds such as *P. sorghina*, *F. moniliforme* and *A. flavus* and suggests the possibility that the malts could be contaminated with unacceptable levels of mycotoxins. Mycotoxins produced by *P. sorghina* are involved in the aetiology of Onyalai disease<sup>20</sup>, while toxins of *F. moniliforme* (fumonisins, fusarin C and moniliformin) are involved in several human and animal ailments<sup>3,12</sup>. Beside potential mycotoxin contamination, high levels of moulds in malt may not be desirable for the simple reason that they could make products unpalatable, resulting in reduced consumer

**Table II.** Incidence of fungal species (% grains infected) of the unmalted sorghum and the top, middle and bottom layers of sorghum malt when germinated at 14–17°C and 18–20°C.

	<i>Aspergillus flavus</i>	<i>Eurotium species</i>	<i>Fusarium chlamydo-sporum</i>	<i>Alternaria alternata</i>	<i>Fusarium monili-forme</i>	<i>Phoma sorghina</i>	<i>Rhizopus oryzae</i>	<i>Mucor species</i>
Unmalted sorghum	0	3 a <sup>1</sup> $\pm$ 1.4 <sup>2</sup>	10 a $\pm$ 3.8	24 a $\pm$ 5.5	86 a $\pm$ 4.3	82 a $\pm$ 9.5	87 a $\pm$ 8.8	87 a $\pm$ 3.5
Sorghum malted at 18–20°C								
Unturned								
Top	9 a $\pm$ 3.0	13 b $\pm$ 3.4	25 c $\pm$ 15.6	73 d $\pm$ 18.3	100 b	100 b	100 b	100 b
Middle	17 b $\pm$ 1.0	15 b $\pm$ 6.0	30 c $\pm$ 16.4	82 e $\pm$ 19.1	100 b	100 b	100 b	100 b
Bottom	31 c $\pm$ 4.8	21 b $\pm$ 2.1	35 c $\pm$ 10.1	83 e $\pm$ 21.9	100 b	100 b	100 b	100 b
Turned								
Top	6 a $\pm$ 1.0	5 a $\pm$ 3.4	22 b $\pm$ 7.1	53 c $\pm$ 8.2	100 b	100 b	100 b	100 b
Middle	5 a $\pm$ 6.6	5 a $\pm$ 5.7	16 b $\pm$ 7.3	56 c $\pm$ 3.8	100 b	100 b	100 b	100 b
Bottom	6 a $\pm$ 4.6	3 a $\pm$ 4.3	18 b $\pm$ 7.2	49 c $\pm$ 2.1	100 b	100 b	100 b	100 b
Sorghum malted at 14–17°C								
Unturned								
Top	10 a $\pm$ 1.0	6 a $\pm$ 3.3	18 b $\pm$ 7.4	34 b $\pm$ 4.2	100 b	100 b	100 b	100 b
Middle	21 b $\pm$ 2.0	7 a $\pm$ 2.1	21 b $\pm$ 2.9	44 c $\pm$ 4.7	100 b	100 b	100 b	100 b
Bottom	22 b $\pm$ 4.0	11 b $\pm$ 3.5	25 c $\pm$ 15.6	65 d $\pm$ 8.7	100 b	100 b	100 b	100 b
Turned								
Top	7 a $\pm$ 7.6	6 a $\pm$ 7.5	15 b $\pm$ 2.0	27 a $\pm$ 3.4	100 b	100 b	100 b	100 b
Middle	6 a $\pm$ 3.8	4 a $\pm$ 1.9	17 b $\pm$ 3.3	35 b $\pm$ 4.5	100 b	100 b	100 b	100 b
Bottom	5 a $\pm$ 7.8	4 a $\pm$ 2.7	12 b $\pm$ 3.0	26 a $\pm$ 3.6	100 b	100 b	100 b	100 b

<sup>1</sup> Mean values with different letters in the same column are significantly different from each other ( $p < 0.05$ ).

<sup>2</sup> Mean  $\pm$  standard deviation.



**Fig. 2.** The effect of turning on the Diastatic Power of the top, middle and bottom layers of the sorghum obtained at temperatures of 18–20°C and 14–17°C. The error bars indicate  $\pm$  standard deviation.

acceptance and therefore loss to the producer. From this perspective the high level of contamination of malts by even presumably innocuous moulds such as those of the genera *Rhizopus* and *Mucor*<sup>21</sup> may not be acceptable.

### Diastatic Power (DP)

The DPs (11–26 SDU/g dry weight) of malt samples germinated at 18–20°C were significantly higher ( $p < 0.05$ ) than that of the malt samples germinated at 14–17°C (0.5–6 SDU/g dry weight) (Fig. 2), probably due to the higher and lower temperatures, respectively to which the grains were subjected during germination<sup>2</sup>. It has been shown that sorghum malts develop DP optimally at temperatures of 24–30°C<sup>4,13</sup>. These results indicate that the low temperature conditions, sometimes used during winter months in South Africa, do not support the production of good quality sorghum malt by outdoor floor malting.

### Cytotoxicity and mycotoxin analyses

The lowest concentration of the DON standard to cause cytotoxic effects in the MTT-bioassay was found to be 1–2  $\mu\text{g/g}$ . ZEA and aflatoxin B<sub>1</sub> did not show any toxicity against the SP 2/0 cells. Unmalted sorghum and the top layer of the unturned, 18–20°C-malted grains showed no toxicity (100% cell growth) even at the highest concentration of 1000 kg examined. Generally, high sorghum malt IC<sub>50</sub> values of 62.5–>1000 kg/kg were obtained which suggest that the malts are relatively non-toxic as those quantities are very large and not normally consumed by humans. However, it is important to note that this assumption pertains only to acute intoxication and does not preclude the possibility of intoxication due to continuous ingestion of sorghum malt over several years (chronic exposure).

Apparently very high levels of aflatoxins were detected in unmalted sorghum (42  $\mu\text{g/kg}$ ) and malted samples (52–160  $\mu\text{g/kg}$ ) using the Vicam Aflatest™ (Table III). These apparent levels are 8 to 32 times higher than the South African legal limit of 5  $\mu\text{g/kg}$ <sup>26</sup> and had never before been obtained in South African sorghum malts. It was therefore necessary to test for aflatoxins using a standard method. TLC was thus performed to confirm the Vicam results. One-dimensional TLC results contrasted with the Vicam Aflatest™ results, indicating that sorghum samples contained about 0.5  $\mu\text{g}$  of aflatoxin B<sub>1</sub>/kg. However, in fact that was a false positive because the two dimension TLC showed that the aflatoxin B<sub>1</sub> suspected spots obtained on the single dimension TLC were not really aflatoxin B<sub>1</sub> spots and that the unmalted sorghum and sorghum malt samples contained less than 0.3  $\mu\text{g}$  of aflatoxin B<sub>1</sub>/kg (minimum detection level). This was confirmed by the detection of a spot of 0.5  $\mu\text{g/kg}$  aflatoxin B<sub>1</sub> spiked in an unmalted sorghum sample. These results agree with findings by Odhav and Naicker<sup>17</sup> who reported the absence of aflatoxin in South African sorghum malt samples. In contrast, Trinder<sup>31</sup> reported the presence of aflatoxins (2–18  $\mu\text{g/kg}$ ) in South African sorghum malt that have been produced by indoor floor malting, so-called industrial malt. We can only hypothesize about the reason why the Vicam test gave false positive aflatoxin results. Perhaps the sorghum polyphenols were bound to the Vicam aflatest antibodies which resulted in a higher aflatoxin count.

Unmalted and turned samples as well as the top layers of unturned 18–20°C-germinated malt contained less than 0.25  $\mu\text{g}$  fumonisin/g (the minimum detection limit). Conversely, middle and bottom layers of the unturned 14–17°C-germinated malt contained 2  $\mu\text{g}$  fumonisin/g (Table III). Fumonisin levels seemed related to malt sample tox-

**Table III.** Concentration of aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>), fumonisins (B<sub>1</sub> and B<sub>2</sub>), deoxynivalenol (DON), zearalenone (ZEA) and the IC<sub>50</sub> levels of the unmalted sorghum and the top, middle and bottom layers of sorghum malt when germinated at 14–17°C and 18–20°C.

	IC <sub>50</sub> <sup>1</sup> (kg/kg)	Concentration				
		Aflatoxins <sup>3</sup> (µg/kg)	Aflatoxins <sup>4</sup> (µg/kg)	Fumonisin <sup>3</sup> (µg/g)	DON <sup>4</sup> (µg/kg)	ZEA <sup>4</sup> (µg/kg)
Unmalted sorghum	>1000 <sup>2</sup>	42	<0.3 <sup>5</sup>	<0.25 <sup>5</sup>	<3 <sup>1</sup>	<3 <sup>1</sup>
Sorghum malted 18–20°C						
Unturned						
Top	>1000 <sup>2</sup>	52	<0.3 <sup>5</sup>	<0.25 <sup>5</sup>	15–20	10–15
Middle	125–250	130	<0.3 <sup>5</sup>	1	15–20	10–15
Bottom	125–250	160	<0.3 <sup>5</sup>	1	15–20	10–15
Turned						
Top	250–500	74	<0.3 <sup>5</sup>	<0.25 <sup>5</sup>	15–20	10–15
Middle	250–500	66	<0.3 <sup>5</sup>	<0.25 <sup>5</sup>	15–20	10–15
Bottom	250–500	69	<0.3 <sup>5</sup>	<0.25 <sup>5</sup>	15–20	10–15
Sorghum malted 14–17°C						
Unturned						
Top	250–500	59	<0.3 <sup>5</sup>	<0.25 <sup>5</sup>	15–20	10–15
Middle	62.5–125	140	<0.3 <sup>5</sup>	2	15–20	10–15
Bottom	62.5–125	140	<0.3 <sup>5</sup>	2	15–20	10–15
Turned						
Top	125–250	53	<0.3 <sup>5</sup>	1	15–20	10–15
Middle	125–250	68	<0.3 <sup>5</sup>	1	15–20	10–15
Bottom	125–250	65	<0.3 <sup>5</sup>	1	15–20	10–15

<sup>1</sup> Inhibitory concentration = concentration resulting in 50% inhibition of the MTT cleavage activity.

<sup>2</sup> Maximum concentration.

<sup>3</sup> Determined using the Vicam kits.

<sup>4</sup> Determined using TLC.

<sup>5</sup> Minimum detection limit.

icity to SP2/0 cells. Both batches of malt contained DON values of 15–20 µg/kg and ZEA values of 10–15 µg/kg.

This study is the first report of the presence of fumonisin and DON in sorghum malt. Levels of ZEA obtained in this study (15–20 µg/kg) are substantially lower than those (387 µg/kg) reported by Rabie and Marais<sup>23</sup> for South African sorghum malt. There are no regulatory levels of DON, ZEA and fumonisins in human foods in South Africa. The United States Food and Drug Administration (FDA) has regulatory levels of DON (500 µg/kg) and no specifications for ZEA<sup>6</sup>. The regulatory levels of fumonisins<sup>32</sup> are 2–3 µg/g and therefore fumonisin levels of 1–2 µg/g were within the limit. The levels of DON, ZEA and fumonisins detected in this work are considered to be too low to be of any concern.

## CONCLUSIONS

The present study shows that malting sorghum using standard commercial type outdoor floor malting conditions under cold temperatures results in malt with unwanted bacteria (coliforms) and fungi. The malt also contained traces of DON, ZEA and fumonisins but showed very low cytotoxicity. Turning of sorghum grains during germination does not affect the microbial populations, the nature and extent of fungal contamination, the level of aflatoxins, DON and ZEA, or the cytotoxicity but it decreases the DP of the sorghum malt. Notwithstanding the low levels of mycotoxins found, it is imperative that effective methods be found to suppress microbiological contamination of outdoor malted sorghum. Addition of dilute alkali (NaOH), lactic acid bacteria or yeast culture during steeping could be other alternatives to reduce the microbial load (especially moulds) of sorghum malt<sup>1,11</sup>.

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