Microbial load and mycotoxins from malted pearl millet and sorghum

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Abstract

Microbial contamination of pearl millet and sorghum grains during the process of malting can compromise the safety of their resulting products. In Namibia, malts of pearl millet and sorghum are used in making *oshikundu*/*ontaku*, *omalovu* (opaque beer) and exclusively sorghum malts for other alcoholic traditional brews. There is limited research on microbial load and mycotoxins from malts of pearl millet intended for making *oshikundu* and possibly *omalovu*. Varieties of pearl millet (*Okashana2, Kantana,* and *Kangara*) and sorghum (*Macia* and Red sorghum) grains were germinated at 30°C for 4 days before drying at 50–55°C for 24 hours. Results showed that malts total microbial load was above the South African recommended limit 6.3 Log cfu/g (2 $\times 10^7$ cfu/g). However, cereal malts showed no contamination by *Salmonella* spp*., Shigella* and coliforms. Regulated mycotoxins aflatoxin, deoxynivalenol, fumonisin and zearalenone were detected from malts irrespective of the cereal. However, detected mycotoxins were below the legal limit set by the European Commission. These suggests that malts may potentially not be of safety concern when it comes to coliforms. Nonetheless, aerobic bacterial load, fungal load and mycotoxins need to be further reduced should the malt be used for *oshikundu, omalovu* and other low alcoholic brews.

Keywords: mycotoxins, aflatoxin, zearalenone, *oshikundu*, *omalovu*,

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1 Introduction

In Africa, cereals are predominantly produced for human consumption (Taylor, 2016a). The top pearl millet (*Pennisetum glaucum (L.) R.Br.*) producers in Africa are Niger, Mali, Burkina Faso, Nigeria and Senegal (Taylor, 2016b). While Nigeria, Ethiopia, Burkina Faso and Sudan (former) are the top producers of sorghum (*Sorghum bicolor (L.) Moench*) (Taylor, 2016a). There are many products from pearl millet and sorghum that are produced and consumed across the continent of Africa. These grains can undergo processes such as malting, fermentation and milling. Generally, cereals are malted for production of weaning food, fermented nonalcoholic and alcoholic beverages such as clear and opaque beers. Malt products from Namibia include *oshikundu/ontaku* (nonalcoholic), *omalovu giilya* (opaque beer) (Embashu and Nantanga, 2019), *otombo, epwaka, okatokele* and *efau* (alcoholic). Pearl millet and sorghum malts are used for brewing *Oshikundu*, however, those of sorghum are commonly used. The malting process is usually not controlled since it takes place in rural household settings. Therefore,

malts can be contaminated with mycotoxins. Mycotoxins of toxicological significance in cereal include aflatoxins, ochratoxin, zearalenone, fumonisin, trichothecenes (T-2 and HT-2 toxins, deoxynivalenol, nivalenol) (Patriarca and Pinto, 2017) tremogenic toxins and ergot alkaloids (Hussein and Brassel, 2001). Different researchers have established a contamination of sorghum malt by aflatoxin, fumonisin, deoxynivalenol, zearalenone (Matomba et al., 2011; Nafuka et al., 2019; Doufour and Melotte, 1992; Lefyedi et al., 2005; Misihairabgwi et al., 2018; Chala at al., 2014). However, limited work is available on pearl millet malts possible contamination by mycotoxins, especially those intended for brewing *oshikundu* and possibly *omalovu*. Misihairabgwi et al. (2018) reported contamination by mycotoxins in malts used for brewing *oshikundu*, but interestingly; none of the regulated mycotoxins were detected in *oshikundu*. This study looked at possible microbial and mycotoxins contamination in malts of pearl millet and sorghum for brewing *oshikundu* and *0malovu*.

2 Material and Methods

2.1 Grains

Pearl millet and sorghum grains of 2015 harvest was obtained from Omahenene Agricultural Research Station (17°26′30″S; 14°47′20″E) of the Ministry of Agriculture, Water and Forestry in Namibia as per method described by Embashu and Nantanga (2019). The varieties of pearl millet grains used in this study are *Okashana 2* (SDMV 93032), *Kantana* (landrace), and *Kangara* (SDMV 92040). For sorghum, it was *Macia* (white, SDS 3220) and a landrace known in Oshiwambo language as *Iilyawala iitiligane* (which literally means "sorghum grains that are red") and herewith referred to as red sorghum.

2.2 Malting

Pearl millet and sorghum grains were malted according to Pelembe et al. (2002) with some modifications by Embashu and Nantanga (2019).

2.2.1 Steeping

Grain samples (1 kg) were washed three times with tap water in a stainless steel bowl to remove floating materials and then steeped in static water at 20–22°C for 2 hours wet and 2 hours air rest for a total of 8 hours. Moisture content was determined at this point for malting loss determination.

2.2.2 Germination

After steeping, 600 g of the grain was transferred into shade cloths and was further wrapped in wet burlap and then incubated at 30°C for 4 days under saturated humidity. The bags with germinating grain were removed from the incubator and steeped in static water (20–22°C) for 10 min and were then returned to the incubator. This was done twice daily (about 8 hrs apart) during the day.

2.2.3 Drying

After the germination period (4 days), grains were removed from the bags and placed in a stainless steel tray. They were then dried in a forced draught oven at 50–55°C for 24 hours. The dry malt was weighed and stored at room temperature until analysis. The moisture content of dry malted grains was measured for malting loss determination. The dry malts were milled using a commercial 2-speed food blender (Waring 7011HS, USA).

2.3 Bacterial and Fungal Enumeration

All analyses were done on nongerminated grains and malts of pearl millet and sorghum. Total plate count, lactic acid bacteria, yeast and mold enumeration was done following work by Lefyedi et al. (2005). MacConkey agar was used instead of Violet Red Bile agar for isolation

of coliforms. Media were prepared as per manufacturer instructions. Colonies were manually counted, those that were 30 counts and above but ≤ 300 were recorded. The colonies were calculated following work by Adekoya et al. (2019) as a mean bacterial load and expressed as the logarithm (base 10) of the colony forming unit per gram of sample (CFU/g).

2.4 Detection of Mycotoxins

All analyses were done on nongerminated grains and malts of pearl millet and sorghum. Enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, USA) were used for quantification of total aflatoxin (AF ELISA kit, Cat no: E-TO-E006, 2017), deoxynivalenol (DON Cat no: E-TO-E003, 2017), fumonisin B1 (FB1 ELISA kit, Cat no: E-TO-E005, 2017), ochratoxin A (OTA ELISA kit, Cat no: E-TO-E001, 2017) and zearalenone (ZEN ELISA kit, Cat no: E-TO-E002, 2017) according to manufacturer instructions. Procedures are summarized as follows: Total flatoxin, fumonisin B1 and ochratoxin A were extracted using 70% methanol, while zearalenone was extracted with 90% methanol and deionised water was used for deoxynivalenol. Extracts were placed on 96x ELISA plates and optical density was measured for each well at 450 nm with a microplate reader. Standard curve was constructed by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis.

2.5 Statistical Analysis

Grains were malted once for each of the varieties of pearl millet and sorghum. Bacterial and fungal isolation on media was repeated once, i.e. it was done twice, with enumeration repeated five times (n = 6). Mycotoxins extraction was done once, with analysis repeated once (n = 2). Data means were compared using a one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test at $p \leq 0.05$ using R software (version 3.5.2, Austria).

3 Results

3.1 Bacterial and Fungal Enumeration

Bacterial, yeast and mold load results from malted pearl millet and sorghum are given in Table 1. Aerobic bacteria, lactic acid bacteria, yeast and mold were not detected in all the nongerminated grains irrespective of the cereal. Also, there was no growth observed from MacConkey agar plates for nongerminated grains and malts irrespective of the cereal. The malted aerobic plate count load was in this order *Kantana* > *Macia* > *Okashana 2* > Red sorghum > *Kangara*. The malted aerobic plate load significantly differed ($p \leq 0.05$) within and between cereals. Highest lactic acid bacterial load was observed from *Okashana 2* malt. Lactic acid bacterial load was not statistically significant ($p > 0.05$) between malts of *Okashana 2, Kangara* and *Macia*. Malts of *Kantana* and *Macia* had the highest yeast and mold load and were statistically the same ($p > 0.05$) same. *Kantana* and *Macia* malts had the highest yeast and mold count and were statistically the same ($p > 0.05$). Overall, malts of *Kantana* followed by malts of *Macia* malt had the highest aerobic plate count, lactic acid bacteria, yeast and molds.

Table 1: Mean bacteria, yeast and molds load in malts of pearl millet and sorghum varieties

Varieties	PCA[1*]	MRS[2*]	PDA[3*]
Pearl millet malt			
Okashana 2	7.7 ±0.1^{c}	7.8 ±0.1^{b}	7.7 ±0.1^{b}
Kantana	8.2 ±0.2^{a}	8.1 ±0.0^{a}	8.0±0.1^{a}
Kangara	7.3 ±0.2^{e}	7.7 ±0.1^{b}	7.6 ±0.1^{b}
Sorghum malt			
Macia (white)	7.9 ±0.1^{b}	7.7 ±0.1^{b}	7.9 ±0.3^{a}

Red Sorghum	7.5 ±0.1[d]	7.6 ±0.2[c]	7.5 ±0.1[b]

[1]Aerobic plate count, [2]Lactic acid Bacteria, [3]Yeast and molds; *Log colonies forming units per gram (Log CFU/g) values are means ± standard deviation; values with the same letter in a column are not significantly different ($p > 0.05$), n= 6.

3.2 Detection of Mycotoxins

Mycotoxins from nongerminated grains and malts of pearl millet and sorghum are shown in Table 2. Aflatoxin was not detected in all nongerminated cereal grains except from red sorghum. Aflatoxin was only detected from malts of *Okashana 2*, *Macia* and Red sorghum. Aflatoxin, ochratoxin A and zearalenone were not detected in all the nongerminated grains varieties of pearl millet. Deoxynivalenol was detected in all cereal, nongerminated and malts. Malting resulted in a significantly decreased deoxynivalenol irrespective of cereal. Malted *Macia* had significantly ($p \leq 0.05$) higher fumonisin B1 than all the other malts. Ochratoxin A was only detected from nongerminated grains of Red sorghum, however following malting, ochratoxin A was not detected in Red sorghum malt. Zearalenone was not detected in cereals' nongerminated grains and their respective malts, except those of malted Red sorghum.

Table 2: Mycotoxins from nongerminated grains and malts of pearl millet and sorghum varieties.

Varieties	Total Aflatoxin*	Deoxynivalenol*	Fumonisin B1*	Ochratoxin A*	Zearalenone*
Pearl millet grains					
Okashana 2	ND	1.13 ±0.32^{d}	0.11 ±0.02^{b}	ND	ND
Kantana	ND	2.13 ±0.33^{c}	0.29 ±0.26^{ab}	ND	ND
Kangara	ND	4.78 ±0.40^{a}	0.17 ±0.16^{b}	ND	ND
Sorghum grains					
Macia (white)	ND	1.33 ±0.18^{d}	0.26 ±0.04^{b}	ND	ND
Red sorghum	0.02 ±0^{a}	2.96 ±0.50^{b}	0.19 ±0.15^{b}	7.53 ±0.42^{a}	ND
Pearl millet malt					
Okashana 2	0.015 ±0^{b}	0.39 ±0.06^{ef}	0.27 ±0.15^{b}	ND	ND
Kantana	ND	0.23 ±0^{f}	0.19 ±0.02^{b}	ND	ND
Kangara	ND	0.28 ±0.09^{ef}	0.09 ±0.02^{b}	ND	ND
Sorghum malt					
Macia (white)	0.01 ±0^{c}	0.49 ±0.04^{ef}	0.54 ±0.05^{a}	ND	ND
Red sorghum	0.02 ±0^{a}	0.88 ±0.31^{de}	0.21 ±0.03^{b}	ND	0.03 ±0^{a}

Note: *part per billion ppb (µg/kg) dry weight. Values are means ±standard deviation; Values with the same letter in a column are not Significantly different ($p > 0.05$); ND- not detected; $n = 2$.

4 Discussion

4.1 Bacterial and Fungal Enumeration

The no growth observed from MacConkey agar plates for nongerminated grains and malts in both cereals, indicates that malts were free from *Salmonella* spp., *Shigella* and coliforms contamination. Malts aerobic plate count load was above the limit set in Southern African sorghum malt specification of 6.3 Log cfu/g or (<2 $\times 10^7$ cfu/g) as noted by Lefyedi et al. (2005). Therefore, this indicates an unacceptable high aerobic plate count load. Lefyedi et al. (2005) also had similar observation of a high aerobic plate count load from sorghum malts. The highest aerobic plate count, lactic acid bacteria, yeast and molds were observed from malts of *Kantana* followed by malts of *Macia*. These can perhaps be linked to *Kantana* and *Macia* malts reducing sugar content of 257.1 ±26.4 and 649.0 ±34.6 mg/g of sample db (Embashu & Nantanga, 2019), respectively. The reducing sugars content in the malt and the absence in nongerminated grains could potentially influence microorganism growth, as they are an easy source of metabolisable sugar. A similar microbial load has been reported from sorghum malts (Lefyedi et al., 2005; Ilori et al., 1991; Thaoge et al., 2003). Lefyedi and Taylor (2006) recommended that steeping sorghum grains in 0.2% (m/v) NaOH reduces bacterial and fungal contamination during sorghum malting. Perhaps, the above procedure can be employed in the reduction of bacteria, yeast and mold loads during malting of grains.

4.2 Detection of Mycotoxins

All mycotoxins analysed were below the regulated legal limits (Commission Regulation No 1881/2006, 2006) except ochratoxin A from nongerminated grains of Red sorghum. Ghali et al. (2008) and Chala et al. (2014) also reported ochratoxin A in sorghum grains above the regulated limits. Aflatoxin, ochratoxin A and zearalenone were not detected in all the nongerminated grains varieties of pearl millet. This is in contrast to Misihairabgwi et al. (2018) and Angula et al. (2024) findings, as they reported ochratoxin in pearl millet meal. Aflatoxin was detected in nongerminated grains of Red sorghum as well as malts of *Okashana 2, Macia* and Red sorghum. This study finding is lower than those reported in sorghum grains, 2.35 ±0.65 µg/kg by Matumba et al. (2011) as well as in malts of pearl millet and sorghum by Misihairabgwi et al. (2018). This study results of deoxynivalenol are lower than those reported in sorghum grains by Ayalew et al. (2006); Chala et al. (2014), sorghum malt by Lefyedi et al. (2005) and finger millet by Chala et al. (2014). The fumonisin B1 observed from nongerminated grains and malts of sorghum is lower than the range of 0.8-123 µg/kg reported by Misihairabgwi et al. (2018) in sorghum malts. Also, lower than the range 200-1400 µg/kg reported by Gamanya (2001) in sorghum grains. Similarly, fumonisin B1 observed from nongerminated pearl millet grains and their respective malts was lower than the range of 0.1-3060 µg/kg reported by Misihairabgwi et al. (2018) in pearl millet malt. The ochratoxin A detected from nongerminated grains of Red sorghum only was above the regulated limits of 5 µg/kg as declared by The European Commission (Commission Regulation N0 1881/2006, 2006). There are reports in contrast to this study's finding that reported detection of zearalenone in sorghum by Lefyedi et al. (2005) and Misihairabgwi et al. (2018) and in pearl millet as reported by Houissa et al. (2019) and Misihairabgwi et al. (2018). There was a low concentration of mycotoxins detected in some malts. However, they were below the legal limits set by the European Commission and those reported by similar studies. The wet and air-rest during steeping step used in this study could perhaps have contributed to washing away of mycotoxins during malting.

5 Conclusions

Over all, malts of pearl millet and sorghum had an unacceptable high aerobic plate count load (total or viable bacteria population). Malts of *Kantana* followed by malts of *Macia* had the

highest aerobic plate count, lactic acid bacteria, yeast and molds. While malts of *Kangara* followed by malts of Red sorghum had the lowest aerobic plate count, lactic acid bacteria, yeast and molds compared to the rest of the malted cereals. However, *Salmonella* spp., *Shigella* and coliforms were not detected from nongerminated grains and their respective malts. This suggest that malted cereals were free of these contaminants. Although coliforms were not detected in the cereals nongerminated grains and malts, a high aerobic plate count load is an indication of a safety concern. Perhaps 0.2% (m/v) NaOH could be used in the reduction of bacterial and fungal contamination during these cereals malting process. Regulated mycotoxins were below the legal limits in all the malts of pearl millet and sorghum varieties, except those of Red sorghum. These cereal malts warrant further investigations on the effect of NaOH use to reduce aerobic bacterial load, yeast and molds count as well as LC-MS/MS quantification of regulated and emerging mycotoxins.

References

Adekoya, I., Obadina, A., Olorunfemi, M., Akande, O., Landschoot, S., De Saeger, S., & Njobeh, P. 2019. Occurrence of bacteria and endotoxins in fermented foods and beverages from Nigeria and South Africa. *International Journal of Food Microbiology*, 305. https://doi.org/10.1016/j.ijfoodmicro.2019.108251.

AF (Total aflatoxin) ELISA kit, Cat no: E-TO-E006, Elabscience Biotechnology Inc, 2017.

Angula, M., Ishola, A., Tjiurutue, M., Sulyok, M., Krska, R., Ezekiel, C. N., & Misihairabgwi, J. 2024. Mycotoxins in stored cereals from rural households in central northern Namibia. *Food Control*, 110532. https://doi.org/10.1016/j.foodcont.2024.110532.

Ayalew, A., Fehrmann, H., Lepschy, J., Beck, R., & Abate, D. 2006. Natural occurrence of mycotoxins in staple cereals from Ethiopia. *Mycopathologia*, 162, 57-63. https://doi.org/10.1007/s11046-006-0027-8

Chala, A., Taye, W., Ayalew, A., Krska, R., Sulyok, M., and Logrieco, A. 2014. Multimycotoxin analysis of sorghum (*Sorghum bicolor L. Moench*) and finger millet (Eleusine coracana L. Garten) from Ethiopia. *Food Control*, 45, 29-35. https://doi.org/10.1016/j.foodcont.2014.04.018

COMMISSION REGULATION (EC) No 1881/2006. 2006. Official Journal of the European Union, 5-24.

DON (Deoxynivalenol) Cat no: E-TO-E003, Elabscience Biotechnology Inc, 2017.

Dufour, J.P., & Melotte, L. 1992. Sorghum malts for the production of lager beer. *American Society of Brewing Chemists*, 50, 110-119.

Embashu, W., & Nantanga, K.K.M. 2019. Malts: Quality and phenolics content of pearl millet and sorghum varieties for brewing non-alcoholic beverages and opaque beers. *Cereal Chemistry* 96 (4), 765-774. https://doi.org/10.1002/cche.10178

FB1 (Fumonisin B1) ELISA kit, Cat no: E-TO-E005, Elabscience Biotechnology Inc, 2017.

Gamanya, S. 2001. Survey of Fusarium moniliforme (F. verticillioides) and production of fumonisin B1 in cereal grains and oilseeds in Zimbabwe. *International Journal of Food Microbiology*, 71, 145-149. https://doi.org/10.1016/s0168-1605(01)00598-0

Ghali, R., Hmaissia-Khlifa, K., Ghorbel, H., Maaroufi, K., & Hedili, A. 2008. Incident of aflatoxins, ochratoxin A and zearalenone in Tunisian foods. *Food Control*, 19, 921-924. http://dx.doi.org/10.1016/j.foodcont.2007.09.003

Houissa et al., 2019. Multimycotoxin LC-MS/MS analysis in pearl millet (*Pennisetum glaucum*) from Tunisia. *Food Control*, 106. https://doi.org/10.1016/j.foodcont.2019.106738

Hussein, H.S., & Brasel, J.M. 2001. Toxicity, metabolism and impact of mycotoxins on humans. *Toxicology*, 167, 101-134. https://doi.org/10.1016/s0300-483x(01)00471-1

Ilori, M.O., Fessehatzion, B., Olajuyigbe, O. A., Babalola, G. O., & Ogundiwin J. O. 1991. Effect of malting and brewing processes on the microorganisms associated with sorghum grains and malt. *Master Brewers Association of the Americas Quarterly* 28, 45- 49.

Lefyedi, M.L., & Taylor, J.R.N. 2006. Effect of dilute alkaline steeping on the microbial contamination, toxicity and diastatic power of sorghum malt. *Journal of the Institute of Brewing*, 112 (2), 108-116. https://doi.org/10.1002/j.2050-0416.2006.tb00240.x

Lefyedi, M.L., Marais, G.J., Dutton, M.F., & Taylor, J.R.N. 2005. The microbial contamination, toxicity and quality of turned and unturned outdoor floor malted sorghum *Journal of The Institute of Brewing*, 111 (2), 190-196. https://doi.org/10.1002/j.2050-0416.2005.tb00665.x

Matumba, L., Monjerezi, M., Khonga, E.B., Lakudzala, D.D. 2011. Aflatoxin in sorghum, sorghum malt and traditional opaque beer in southern Malawi. *Food Control*, 22, 266-268. https://doi.org/10.1016/j.foodcont.2010.07.008

Misihairabgwi, J.M., Ishola, A., Quaye, I., Sulyok, M., & Krska. R. 2018. Diversity and fate of fungal metabolites during the preparation of *oshikundu,* a Namibian traditional fermented beverage. *World Mycotoxin Journal*, 471-481. https://doi.org/10.3920/WMJ2018.2352

Nafuka, S.N., Misihairabgwi, J.M., Bock, R., Ishola, A., Sulyok, A., & Krska, R. 2019. Variation of fungal metabolites in sorghum malts used to prepare Namibia traditional fermented beverages *omalodu* and *otombo*. *Toxins*, 11, 165. https://doi.org/10.3390/toxins11030165

OTA (Ochratoxin A) ELISA kit, Cat no: E-TO-E001, Elabscience Biotechnology Inc, 2017.

Patriarca, A., & Pinto, V.F. 2017. Prevalence of mycotoxins in foods and contamination. *Current opinion in Food Science*, 14, 50-60. http://dx.doi.org/10.1016/j.cofs.2017.01.011

Pelembe, L. A. M., Dewar, J., & Taylor, J. R. N. 2002. Effect of malting conditions on pearl millet malt quality. *Journal of the Institute of Brewing*, *108*, 7–12. https://doi.org/10.1002/j.2050-0416.2002.tb00113.x

Taylor, J. R. N. 2016. Millet pearl: overview. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of food grains, the world of food grains* (2nd ed., pp. 190–198). Oxford: Elsevier.

Taylor, J.R.N. (2016a). Grain production and consumption: Africa. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of food grains, the world of food grains* (2nd ed., pp. 359–366). Oxford: Elsevier.

Thaoge, M.L., Adams, M.R., Sibara, M.M., Watson, T.G., Taylor, J.R.N., & Goyvaerts, E.M. 2003. Production of improved infant porridges from pearl millet using a lactic acid fermentation step and addition of sorghum malt to reduce viscosity of porridges with

high protein, energy and solids (30%) content. *World Journal of Microbiology and Biotechnology,* 19, 305-310.

ZEN (Zearalenone) ELISA kit, Cat no: E-TO-E002, 2017, Elabscience Biotechnology Inc, 2017