# **OPTIMISATION OF *ONTAKU/OSHIKUNDU*: PEARL MILLET AND SORGHUM MALTS QUALITY AND CONVENIENT PREMIX DEVELOPMENT**

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**Abstract**

*Oshikundu/Ontaku* is a nonalcoholic, acidic, opaque fermented beverage. It is comprised of pearl millet (*Pennisetum glaucum (L.) R. Br*) meal/flour, commonly known as *mahangu*, malts of pearl millet or sorghum (*Sorghum bicolour (L.) Moench*) and/or brans (pearl millet). Brewing of *oshikundu* remains an art in households with no empirical improvement of a controlled fermentation flow process to give a consistent product. Some of the major limitations to the formal commercialisation of this brew are the absence of standardised malting process that gives a consistent malt quality and microbial safety. The lack of standard ingredient ratios (flour/meal: malt: water) of brewing. Also, the sedimentation of adjunct particles at the bottom (dreg), thus creating the difference in viscosity of *oshikundu*. Well-defined fermenting microorganisms are not used, and the preparation method still relays on a laborious time-consuming process. Therefore, this study investigated conditions for malting, reduction of dreg, identification of fermenting microorganisms and formulation of ingredient ratio for an improved preparation process. Grains used in the study were collected from Omahenene Agricultural Research Station (2015 harvest), of the Ministry of Agriculture, Water and Forestry. Pearl millet varieties *Okashana 2* (SDMV 93032), *Kantana* (landrace) and *Kangara* (SDMV 92040), while sorghum varieties are *Macia* (SDS3220) and landrace commonly referred to as red sorghum. Malts of the two cereals were prepared by steeping in static water at 20-22$^{o}$C for 2 hours wet and 2 hours air-rest for a total of 8 hours and germinated at 30$^{o}$C. Malts were dried between 50-55$^{o}$C for 24 hours. Cereals germinative energy was above 90% as recommended for sorghum by the European Brewery Convention. Malting loss was high up to 30% in pearl millet varieties and *Macia*. Crude protein and fibre were found to increase following malting. Reducing sugars were not detected in nongerminated pearl millet grains. Malts reducing sugars were statistically significant ($p \leq 0.05$) between cereals. The malt reducing sugars was as follows *Macia*> Red sorghum>

*Kantana*>*Okashana 2* = *Kangara*. Malting resulted in significantly increased free amino nitrogen (FAN) content. *Kantana* had the highest FAN followed by *Macia* malt. No amylolytic activity was detected in nongerminated grains irrespective of the cereal. Pearl millet was found not to contain condensed tannins. Malts had an unacceptable high aerobic plate count load above 6.3 Log cfu/g or ($2 \times 10^7$ cfu/g) as specified for Southern African sorghum malts. However, results show that the malts were not contaminated by *Salmonella spp., Shigella* and coliforms. Regulated mycotoxins in malts were found to be below the legal limits. Cereal malts are not of safety concern from coliforms and mycotoxins under these malting conditions. *Oshikundu* is likely fermented by lactic acid bacteria (*Lactobacillus. plantarum, L. pentosus, L. acidifarinae, L. paraplantarum, L. spicheri, L. namurensis, L. zymae, L. fermentum, L. brevis, L. delbrueckii subsp bulgaricus, L. buncheri, Leuconostoc gurlium and Pediococcus acidilactici*) and yeast (*Saccharomyces cerevisiae and S. paradoxus*). However, the dynamics of LAB and yeast during fermentation are not known. The use of smaller amounts of dry ingredients and pre-gelatinisation of pearl millet meal in the process of making *oshikundu* significantly decreases total solids. This suggests that the amount of suspended particles in *oshikundu* that tend to settle during storage can also be reduced through this route, in the absence of consumer acceptability test. The study demonstrated a creative formulation of a dry powder premix for brewing *oshikundu*. Preliminary sensory evaluation showed that panellists extremely liked the ease of preparation method, where only water was required to be added to the premix. The study demonstrated that malting pearl millet and sorghum grains under set conditions gave malts of acceptable quality (reducing sugars, free amino nitrogen, alpha and beta amylase activity, phenolic content, radical scavenging activity, mycotoxins and microbial load). Also, a lower amount of pre-gelatinised adjunct can be used to achieve the same yield of *oshikundu* and at the same

time reduce dregs. Also, ingredient ratios were formulated by the use of dry ingredients premix for making *oshikundu* that was easy to prepare.

**List of Publications**

1. Embashu, W., & Nantanga, K.K.M. (2019). Pearl millet grain: A mini-review of its milling, fermentation and brewing of *Ontaku*, a non-alcoholic traditional beverage in Namibia. *Transaction of the Royal Society of South Africa*, 74 (3), 276-282.
2. 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.
3. Embashu, W., Simeon, M.P, Haimbondi, N.T, Ekandjo, A.K., Cheikhyoussef, A., & Kahaka, G. (2018). Identification of lactic acid bacteria and yeast from *Oshikundu* using 16S and 26S rDNA gene sequencing. *International Science and Technology Journal of Namibia*, 11, 3-14.
4. Hepute, N.R., Embashu, W., Cheikhyoussef, A., Nantanga, K.K.M. (2016). Effect of pre-cooking pearl millet (*Pennisetum glaucum*) flour on the reduction of dregs in *Oshikundu*. *International Science and Technology Journal of Namibia* 8, 91-97.

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**List of Abbreviations and/or Acronyms**

| | |
|---|---|
| BLAST | Basic local alignment search tool |
| CFU | Colony forming unit |
| DNA | Deoxyribose nucleic acid |
| DP | Diastatic power |
| ELISA | Enzyme-linked immunosorbent assay |
| FAN | Free amino nitrogen |
| GC | Gas chromatography |
| HUT | Home use test |
| LAB | Lactic acid bacteria |
| LC-MS | Liquid chromatography-mass spectrometry |
| PCR | Polymerase chain reaction |
| rDNA | Ribosomal Deoxyribose nucleic acid |
| TLC | Thin layer chromatography |

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## Dedication

I would like to dedicate this work to my mother Mrs Ally Embashu for all the love, support and guidance that helped mould me into who I am today. This is also dedicated to Ambili Iileka, who passed on shortly before examination of the thesis.

## Declaration

I, Werner Embashu, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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……………………………… Date …………………….

# Chapter 1

## Introduction

## 1.1. Background of the study

Cereal products from millet, sorghum and maize are the staple food in Africa (Taylor, 2016a). Malting, fermentation and milling are applied mostly in traditional settings (Taylor, 2016b; Waniska, Rooney & McDonough, 2016; Adebiyi, Obadina, Adebo & Kayitesi, 2018). An example of a cereal based product from Namibia is *oshikundu/ontaku* (Embashu, Iileka & Nantanga, 2019). It is a nonalcoholic beverage that is brewed almost daily. *Oshikundu* is brewed with pearl millet (*mahangu*) meal, bran (pearl millet) and malt meal of sorghum or pearl millet (Mallet & Du Plessis, 2001; Embashu, Cheikhyoussef, Kahaka & Lendelvo, 2013; Taylor, 2016a). Similar products from sorghum and/or millet have been reported in Africa (Taylor & Emmambux 2008) such as *togwa* (Tanzania), made from maize meal and finger millet (Hellstr¨om, V´azques-Ju´arez, Svanberg & Andlid, 2010) and *kunun-zaki* (Nigeria) made using pearl millet and white fonio (Akoma, Jiya, Akunmka & Mshila, 2006). The brewing and consumption of *Oshikundu* is mostly done in rural households (north and northern east), but a few do brew in urban areas around the country. The brewing of *Oshikundu* remains a household art with no standard malting process. The malt safety of mycotoxins is of concern as reported by Misihairabgwi et al., (2018). This study looks at the standardisation of malting process and formulation of dry ingredients to easy preparation method of brewing *Oshikundu*.

## 1.2. Statement of problem

*Oshikundu* is a popular beverage commonly found in the north and north-east of Namibia in at least 50% of households. It is a daily drink in many rural households, not would you like a cup of coffee, tea or juice? It is the first food/beverage presented upon minutes of greeting and it is often the common weaning food in rural areas. Primary school going children would carry it to schools in bottles for breakfast and perhaps lunch. Despite these, it is not commercially available. It is only vended by women at informal markets and construction sites in towns. The brewing of *Oshikundu* remains an art in households with no empirical

improvement of a controlled brewing flow process to give a consistent brew. Some of the major limitations to the formal commercialisation of this drink are the absence of standardised malting conditions, ingredients ratio for a consistent quality. There are studies that have established malting conditions that gave acceptable amount of free amino nitrogen and α- and β-amylase, for opaque beer brewing with sorghum (Okafor & Aniche, 1980; Palmer, Etokakpan & Igyor, 1989; Dewar & Taylor, 1999; Lyumugabe, Gros, Nzungize, Bajyana & Thonart, 2012). Similary Pelembe, Dewar & Taylor (2004) has reported malting conditions for quality pearl millet malt. There are studies on pearl millet and sorghum but limited to the nutritional composition and brewing flow process of *Oshikundu* (Embashu et al., 2013; Embashu, 2014). There is no literature found on the conditions of malting and the quality of pearl millet or sorghum malt varieties for brewing *Oshikundu*. Malting of pearl millet or sorghum for *Oshikundu* brewing takes place in traditional settings. There is generally no temperature, moisture control or air rest during malting.

The role of phenolic compounds potential long-term health promoting properties in millets and sorghum and their foods has been given (Dykes & Rooney, 2006; Taylor & Duodu, 2017). The health promoting properties have been reviewed to be antidiabetic, anti-inflammatory, anticancer and antimicrobial (Taylor & Duodu, 2015). Also, phenolics in vitro radical scavenging properties in millets and sorghum have been established (Dykes & Rooney, 2006; Chandrasekara & Shahidi, 2010). There was no literature found on proximate content, phenolic contents and their possible antioxidant activities of pearl millet and sorghum varieties used in brewing *Oshikundu*. In addition, health properties of fermentation, Short-chain fatty acids, pre and pro biotic effect if any or not know in *oshikundu*. Mycotoxins are a concern in cereal as food, since recognised to harm human health (Bhat, Rai & Karim, 2010; Udomkun et al., 2017). Sorghum malt microbial quality and mycotoxins for brewing opaque beer has been reported by Lefyedi (2005). Mycotoxins contamination in pearl millet

and sorghum malts for brewing *Oshikundu* and opaque beer from open markets has been reported (Misihairabgwi, Ishola, Quaye, Sulyok & Krska, 2018). There in no literature found on *Oshikundu* pearl millet and sorghum malt microbial quality and possible contamination with mycotoxins.

Ingredients amount/ratios (malt: meal: water) that are used for *oshikundu* brewing are subjective by the makers measuring technique, which is usually by use of hands (dry ingredients). This creates possible variations within the household and between households. Furthermore, with no straining, filtration or other appropriate separation steps and with limited gelatinisation of starch in the flour/meal used, *Oshikundu* is an unstable suspension that has a large amount of floating particles (flour/meal). Particles collect and sediment at the bottom as a denser layer of dregs (*ehete*) due to gravity. Consequently, *Oshikundu* has a differential consistency or viscosity. The sediment dregs in *Oshikundu* are largely composed of partially and un-gelatinised starch, which is used as chicken and small livestock feed. However, this is a concentrated form of energy (starch) that goes to animals as opposed to humans. Lactic acid bacteria (LAB) and yeast has been reported in millet and sorghum fermented products (Blandino, Al-Aseeri, Pandiella, Cantero & Webb, 2003; Franz et al., 2014). Lactic acid bacteria have been reported in *Oshikundu* by Embashu (2014) but not possible yeast. The microorganisms in *Oshikundu* are not known, especially those potentially responsible for fermentation such as lactic acid bacteria (LAB) and yeast. The preparation method of mixing dry ingredients to water is laborious. It requires acquired skills to know at which stage to add ingredients. All of the above gaps may possibly contribute to the inconsistency of the final product. Therefore, this study aims at investigating the appropriate conditions of malting process, reduce dregs and formulate an easy preparation method of dry ingredients for *Oshikundu* fermentation.

## 1.3. Objectives

The general objective of the study is to standardize malting conditions, reduce dregs formulate an easy preparation method of dry ingredients for *Oshikundu* fermentation.

The specific objectives of the study are:

1. To determine the effect of malting on pearl millet and sorghum quality (free amino nitrogen, reducing sugar, alpha & beta amylase and diastatic power), proximate content, phenolic content and their antioxidant activities.

2. To determine the effect of malting on malts safety of pearl millet and sorghum: total microbial quality and mycotoxins.

3. To identify LAB and yeast from *Oshikundu*.

4. To determine the effect of pre-cooking on dreg reduction in making *Oshikundu*.

5. To formulate an innovative ready to use dry ingredients of fermenting microbes for *Oshikundu* brewing.

## 1.4. Hypothesis

Ho: Malting has no significant difference on malt quality, proximate content, phenolics and antioxidant activities between pearl millet and sorghum.

Ho: Malting has no significant effect on malt safety: total microbial quality and mycotoxins.

Ho: There are no lactic acid bacteria or yeast in *Oshikundu*.

Ho: Pre-cooking has no significant effect on dreg reduction in making *Oshikundu*.

Ho: The dry ingredients of fermenting microbes will not result into an innovative ready to use for *Oshikundu* brewing.

### 1.5. Significance of the study

Standardise the malting conditions of pearl millet and sorghum for *Oshikundu* brewing will be new contributions to knowledge. This will contribute to ensuring consistency in malt treatment and possibly the quality of the final product. The total microbial load and mycotoxins give a degree of assurance on the malts safety when employing the standardized malting process. Identification of LAB and yeast would add to the knowledge of other traditional fermented beverages in Africa. The study contributes to the scanty information of possible LAB and yeast that may be responsible for *Oshikundu* fermentation. Information on potential fermenting microorganisms can be used as a template in starter culture development, especially in prospective scaling up of *Oshikundu* production. During pearl millet meal pre-cooking, starch undergoes partially gelatinization. Consequently, a lower amount of dry ingredients can be used while producing the same yield. At some time, this will reduce the inconsistency viscosity as well as the reduction in the amount of dreg that sediment at the bottom of *Oshikundu*. Formulations of a dry ingredients premix for making *Oshikundu* can easy the preparation method.

### 1.6. Limitation of the study

The study is limited to mycotoxins quantification as it was not repeated. Also, mycotoxins were only quantified in the malts but not from *Oshikundu*. Also, the identification of microorganism was limited to LAB and yeast only. The number of consumer panellist evaluating the ease of preparation using the formulated dry ingredients powder premix was limited. Also, a control product was not used in the sensory evaluation to compare to the new product.

### 1.7. Delimitation

Mycotoxins quantification was done only one independent extraction and analysis were repeated once (n =2). Only 10 panellists were used to evaluate the ease of preparation using the formulated dry ingredients powder premix.

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# Chapter 2

## Literature Review

Section of the literature review is published in the Transaction of the Royal Society of South Africa titled, Pearl millet grain: A mini-review of its milling, fermentation and brewing of *Ontaku*, a non-alcoholic traditional beverage in Namibia

## 2.1. Introduction

### *2.1.1. Pearl millet*

Grasses collectively known as millet are variable small seeded plants (Rooney, 1996). Cultivated millet species are classified as major and minor (Taylor, 2017). Major includes white fonio (*Digitaria exilis (Kippist) Stapt*), black fonio (*Digitaria iburus Stapt*), finger millet (*Eleucine coracana L. Gaertn*), foxtail millet (*Setaria italic (L.*) *P beauv.* subsp. *Italic*), proso millet (*Panicum miliaceum L.* subsp. *miliaceum*), pearl millet (*Pennisetum glaucum (L.) R.Br.*) and teff (*Eragrostis tef (Zuccagni) Trotter*). While those of barnyard millet (*Echinochloa frumentacea*), Japanese barnyard millet (*Echinochloa esculenta (A. braun) H. Scholz*), kodo millet (*Paspalum scrobiculatum*) and little millet (*Panicum sumatrense*) are minor. Pearl millet is the most produced among the millet species and known by different vernacular languages (Taylor, 2016a). These include *bajra* (India), *gero* (Nigeria, Hausa language), *hegni* (Niger, Djerma language), *sanyo* (Mali), *dukhon* (Sudan, Arabic) and *mahangu* (Namibia, Oshiwambo language). Pearl millet (*Pennisetum glaucum (L.) R. Br*) is one of the cereal grains with the potential to become a global food crop (Taylor, 2016a). It is cultivated in arid and semiarid regions in Asia and Africa. This ability gives it a competitive advantage in the era of climate change. India in Asia and Nigeria, Niger and Mali in West Africa are some of the major producers of pearl millet. In southern Africa, pearl millet is domestically produced in countries such as Zimbabwe, Mozambique and Namibia (Taylor, 2016a). It is regarded as one of the most hypoglycemic cereals (Annor, Tyl, Marcone, Ragaee and Marti, 2017), which has potential in the management of Type 2 diabetes. In Namibia, it is a staple food to over 60% of the human population.

Pearl millet grain is usually processed by malting, fermentation and milling. The grain can also be boiled as a whole or following decortication and then consumed. One of pearl millet uses in Namibia is in the brewing of a drink known as *Ontaku or Oshikundu* (Figure 1) in

Oshiwambo languages. It is a non-alcoholic, fermented and acidic beverage comprising of pearl millet (*Pennisetum glaucum (L.) R. Br.*) meal (Taylor, 2016a), sorghum (*Sorghum bicolor*) or pearl millet malt, water and/or pearl millet bran (Mallet & Du Plessis, 2001; Embashu, Cheikhyoussef, Kahaka & Lendelvo, 2013; Hepute, Embashu, Cheikhyoussef & Nantanga, 2016; Taylor, 2016b;). It is brewed almost daily for immediate consumption in more than half of the Namibian households (Hepute et al., 2016), primarily as a source of energy and hydration. This is because, under the current processing methods, it has a short shelf life of about 10-14 hours at ambient conditions.

The cultural importance of *Ontaku* in the majority of Namibian households cannot be overemphasised. For instance, unlike in many societies where one would be commonly offered a cup of coffee or tea, *Ontaku* is the first food/beverage offered to visitors in most households in Namibia. Furthermore, *Ontaku* is also used as a weaning food and is often frequently fed to children, the sick, lactating mothers and the elderly. Although *Ontaku* is currently not produced for formal commercial markets, it is sold to low income earners at the informal markets and labourers at construction sites in most towns in Namibia. Essentially, *Ontaku* is important to the food security of Namibia. With modernisation, via rapid urbanisation in most developing nations (Watson, Ngesa, Onyang'O, Alnwick & Tomkins, 1996), the indigenous traditional techniques of food processing can be easily lost and die together with the current rural elders if not documented. Contrast this with developed nations where fermented foods are becoming fashionable in Western diets, where there is an emphasis on artisanal food processing. One reason for this is the health-promoting potential of fermented foods. Scientific publications have even appeared lately suggesting the inclusion of fermented foods in national dietary recommendations (Chiltin, Burton & Reid, 2016; Bell, Ferrão & Fernnandes, 2017). Currently, there is limited documentation (Embashu et al., 2013; Hepute et al., 2016) on the brewing processes and characteristics of *Ontaku*, a popular

fermented beverage in Namibia. To document the traditional techniques involved in the processing of pearl millet in Namibia, this contribution collates and provides a mini-review on the available pertinent information on the malting, fermentation and milling processes involved in the production of the ingredients used in the brewing of *Ontaku*. It also discusses the flow processes commonly followed by brewers of this non-alcoholic African beverage. Furthermore, brief basic science underlying the key processing steps has been included. The information presented in this contribution should aid future research in the field of functional cereal foods.

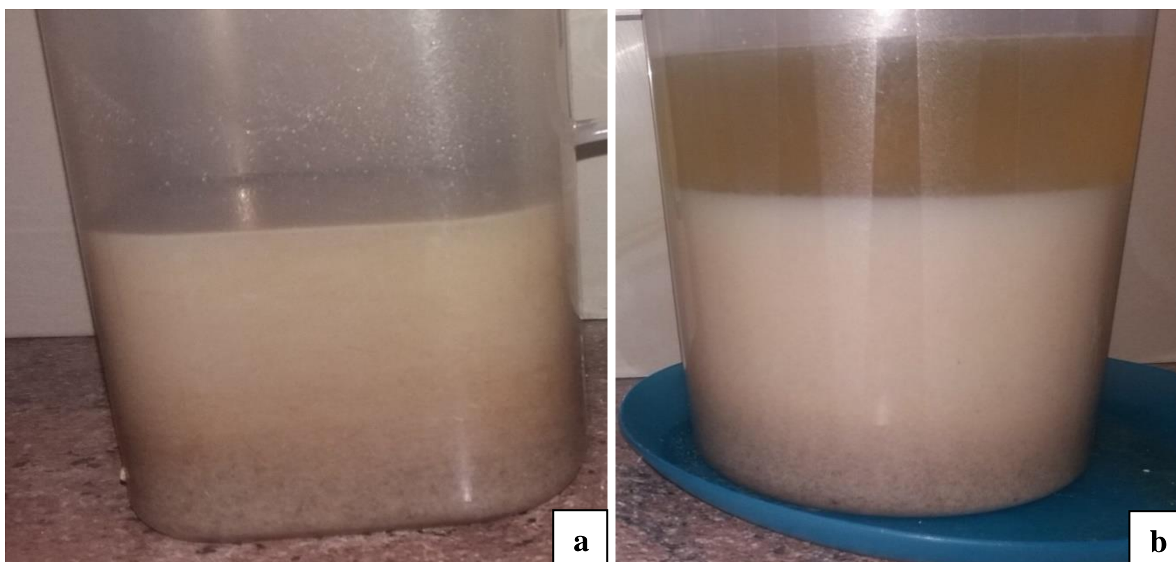



**Figure 1.** a) Fresh *Ontaku* and b) Expired *Ontaku*.

*2.1.2. Grain morphology and chemical composition*

Pearl millet 1000 kernel weighs between 2.5-15g (Taylor, 2016a; Serna-Saldivar & Espinosa-Ramirez, 2019). Pearl millet kernel is made up of three major anatomical structures (Figure 2), pericarp (7.2-10.6%), germ (15.5-17.4) and endosperm (73.9-76.2%) (Abdelrahman, Hoseney & Varriano-Marston, 1984). Chemical and proximate compositions of pearl millet are shown in Table 1. The germ contains the highest protein, fat and ash compared to pericarp and endosperm. Amylose content and gelatinization temperature of pearl millet are similar to that of sorghum.

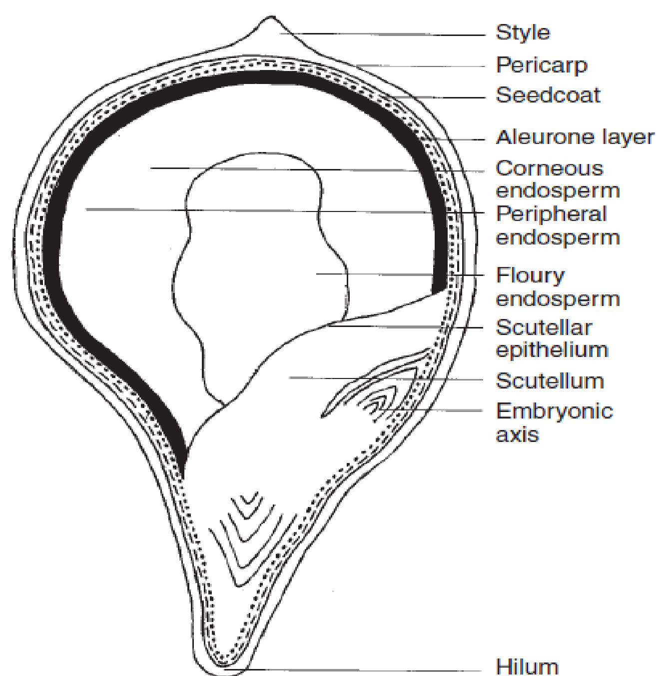



**Figure 2:** Diagrammatic longitudinal section through a pearl millet grain (From Taylor, 2016a).

**Table 1:** Proximate composition, structural features and gelatinization properties of pearl millet kernel.

| Composition | Whole grain | Pericarp (%) | Germ (%) | Endosperm (%) |
|---|---|---|---|---|
| Protein | | 17.1 [a] | 24.5 [a] | 10.9 [a] |
| Fat | | 5.0 [a] | 32.2 [a] | 0.5 [a] |
| Ash | | 3.2 [a] | 7.2 [a] | 0.3 [a] |
| Soluble sugars (g/100 g) | 1.4-2.8 [b] | | | |
| Starch (g/100 g db) | 63.1-78.5 [a,b] | | | |
| Amylose (%) | 17.0-32.5 [a,b] | | | |
| Amylopectin (%) | 78.9-83.0 [c] | | | |
| Starch granules (µm) | 3.0-14.0 [a] | | | |
| Protein body size (µm) | 0.6-1.5 [a] | | | |
| Gelatinization temperature ($^{o}$C) | 61-73 [a,b] | | | |

[a]Serna-Saldivar & Espinosa-Ramirez, 2019, [b]Taylor, 2016a, [c] Serna-Saldivar & Rooney, 1995

### 2.2. Nutritional composition

The nutrient composition of the pearl millet grain can be affected by among others the soil, environmental condition and variety of grain. The nutrients are distributed variably in the different anatomical parts (pericarp, endosperm and germ) of the grain (Serna-Saldivar & Espinosa-Ramírez, 2019). The pericarp (8.4%) of pearl millet contains mostly the insoluble fibers, proteins, minerals and phenolic compounds. For pearl millet, like sorghum, the pearl millet pericarp may contain starch granules. The endosperm that is 75.1% of the grain anatomy comprises primarily of starch and proteins (prolamins). It also contains B-vitamins, phytic acid and phenolic compounds. Pearl millet has the largest germ of all the cereals, about 16.6%. This is where the oil, proteins (albumins and globulins), soluble sugars, minerals and vitamins are found. Removal of some of these anatomical parts during the milling of the grain adversely affects the original nutrient content of the grain.

The content of some nutrients present in the variously milled pearl millet meals is given in Table 2. The energy content of whole pearl millet grain is between 1646 and 1691 g/100 g dry weight (Taylor, 2004; Taylor, 2016a; Taylor, 2016b). These amounts decrease during milling. For instance, the energy content was reported to be 1381 g/100 g dry weight in pearl millet meal obtained from service millers (Mallet & Du Plessis, 2001). Some components such as fat that contribute to the overall energy content of the grain are usually removed during milling for shelf-life extension and phenolics are removed for palatability (Taylor, 2016a). The protein content of the various pearl millet meals ranges from 8.6 to 19.4 g/100 g dry weight.

The ranges of carbohydrate, dietary fibre, fat, and ash contents (g/100 g dry weight) are as follow, respectively, 63-78.5, 8-11.7, 1.5-5.8 and 0.76-3.6. During the milling process, decortication reduces the amount of nutrients such as the fat, mineral (ash) and fibre contents in the resulting meal or flour. These nutrients are often removed as part of bran (pericarp and

germ) during decortication (Taylor, 2004; Barrion, 2008). This loss of outer layers of the grain "concentrates" the amount of starch. Thus, the starch content is higher 71 g/100 g dry weight in service milled pearl millet meal than in the raw grain (59.8 g/100 g dry weight) (Barrion, 2008). Steeping of the grain can also possibly cause a reduction of some nutrients through the leaching of water-soluble proteins and phenolics, minerals and vitamins into the steeping water.

It is noteworthy that whole unprocessed pearl millet grain contains compounds that adversely affect mineral bioavailability, those implicated in goitre and those with disagreeable effects on the sensory quality of some pearl millet products (Taylor, 2016a). Phytate present in pearl millet represents the reserve of phosphorus like in other cereal grains but it binds minerals such as Cu (II), Fe (II) and Zn (II) rendering them biounavailable. C-glycosyl flavones in pearl millet such as vitexin, glucosyl vitexin and glucosyl orientin are not only responsible for the gray/brown colour of pearl millet grain, but they are also implicated in the goitre incidents reported in Sudan. This should, however, be noted together with the fact that the communities where the incidents were reported to have a very limited diet that might simply be deficient of iodine (Taylor, 2016a). The C-glycosyl flavones have also been attributed to the mousy or mouse-dropping-like flavour associated with damp, improperly dried pearl millet flour. The phytate and the C-glycosyl flavones are concentrated in the outer layers of the grain. They therefore significantly reduced together with the minerals by decortication. Malting and fermentation free the minerals through enzymatic hydrolysis of phytate.

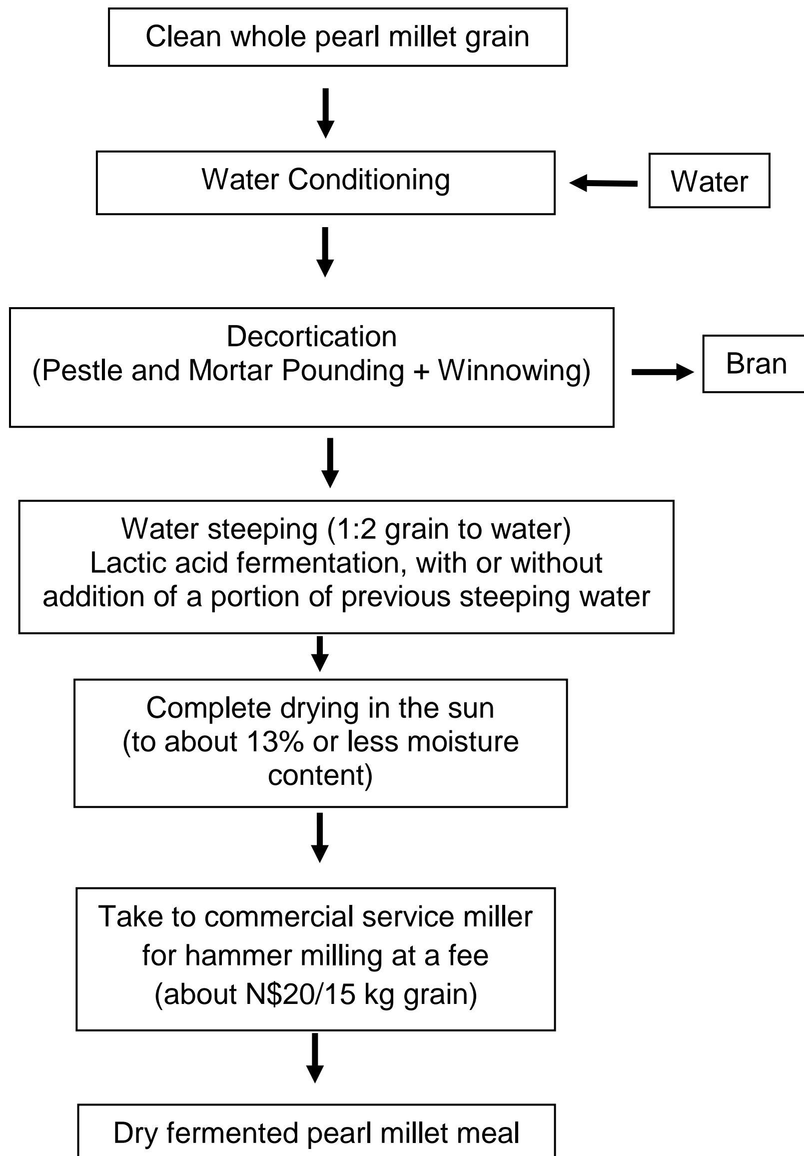
**Table 2:** Comparison of some nutrients of pearl millet meals obtained through different ways of milling and from different millers.

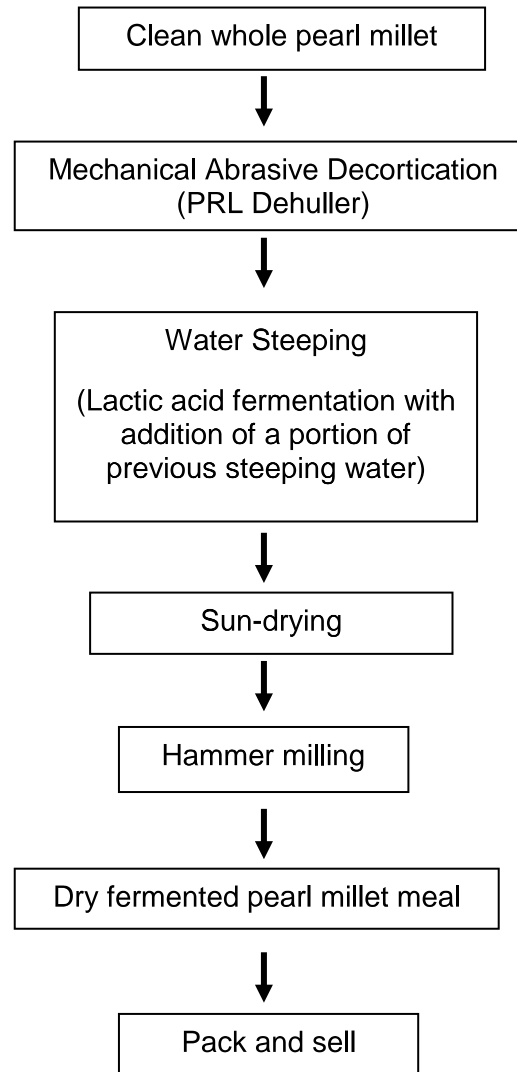
| Nutrient on dry basis | Whole pearl millet grain[a] | Commercial milling Service pearl millet meal[b] | Commercial milling Service pearl millet meal[c] | Industrial milled pearl millet meal[b] |
|---|---|---|---|---|
| Food energy (kJ/100 g) | 1646 – 1691 | | 1381 | |
| Protein (g per 100 g) | 8.6 - 19.4 | 13.2 | 12.4 | 13.4 |
| Carbohydrate (g per 100 g) | 63.1 - 78.5 | 70.8 | 72.8 | 69.8 |
| Dietary fibre (g per 100 g) | 8.0 - 9.0 | 11.4 | 7.5 | 11.7 |
| Fat (g per 100 g) | 1.5 - 6.8 | 3.8 | 5.8 | 4.1 |
| Ash (g per 100 g) | 1.6 - 3.6 | 0.76 | 1.5 | 1.12 |
| Total polyphenols (mg per 100 g) | | 80 | | 140 |
| Phosphorus (mg per 100 g) | 373 | | 226 | |
| Calcium (mg per 100 g) | 41 | | 188 | |
| Magnesium (mg per 100 g) | 125 | | 73.4 | |
| Potassium (mg per 100 g) | 460 | | 204 | |
| Zinc (mg per 100 g) | 2.4 | | | |
| Copper (mg per 100 g) | 0.5 | | | |
| Iron (mg per 100 g) | 10.8 | | | |
| Niacin (mg per 100 g) | 2.9 | | 1.32 | |
| Riboflavin (mg per 100 g) | 0.2 | | 0.064 | |
| Thiamin (mg per 100 g) | 0.3 | | 0.25 | |

[a] Taylor, 2016a, [b] Barrion, 2008, [c] Mallet & Du Plessis, 2001

### 2.3. Milling of pearl millet in Namibia

The ingredients used in the making of *Ontaku* are water, pearl millet meal and malted sorghum meal or malted pearl millet meal. Pearl millet meal (locally known as *uusila/oufila* (Mallet & Du Plessis, 2001; Taylor, 2016b) *woMahangu* in Oshiwambo, a local language in Namibia) can be obtained from traditional domestic milling (Taylor, 2004) (Figure 3a) or from service millers (Figure 3b) or from industrial milling (Figure 3c) (Barrion, 2008). The industrial milling process includes conditioning of the grains with water, abrasive decortication and roller milling of the decorticated grain. The traditional and service milling processes are generally similar. They both usually (but not always) include a decortication, fermentation, partial drying of fermented grain and size-reduction and then solar- drying of the flour steps. Service milling refers to milling services that reduce the size of the grain using a hammer mill, whereas traditional milling uses laborious wooden pestle and mortar and then sieving (Taylor, 2004; Barrion, 2008). Customers can buy pearl millet meal from service millers and/or can deliver their own grain (decorticated and fermented or whole grains fermented by steeping) for milling at a fee.


a) Household pre-milling preparation
Clean whole pearl millet grain
Water Conditioning
Water
Decortication
(Pestle and Mortar Pounding + Winnowing)
Bran
Water steeping (1:2 grain to water)
Lactic acid fermentation, with or without
addition of a portion of previous steeping water
Complete drying in the sun
(to about 13% or less moisture
content)
Take to commercial service miller
for hammer milling at a fee
(about N$20/15 kg grain)
Dry fermented pearl millet meal


b) Commercial Service Milling
Clean whole pearl millet
Mechanical Abrasive Decortication
(PRL Dehuller)
Water Steeping
(Lactic acid fermentation with
addition of a portion of
previous steeping water)
Sun-drying
Hammer milling
Dry fermented pearl millet meal
Pack and sell

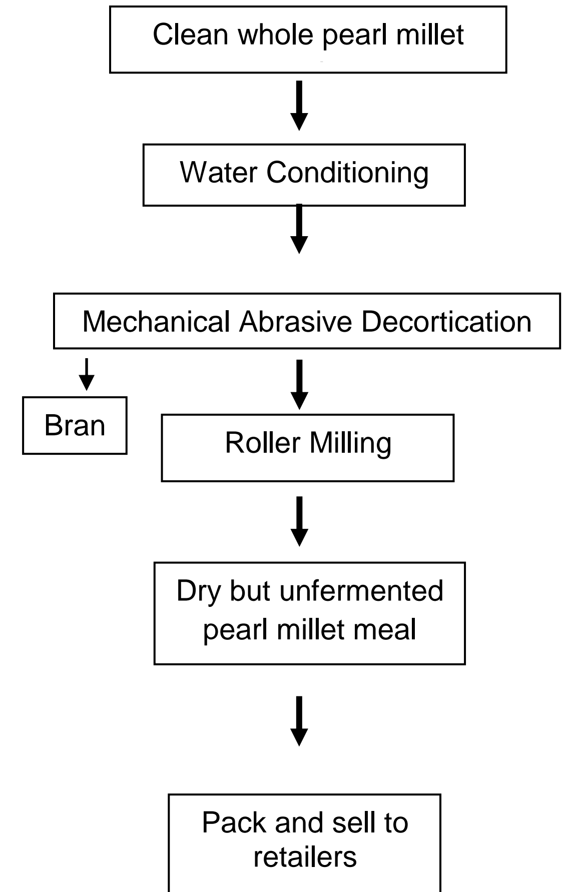

c) Industrial Milling
Clean whole pearl millet
Water Conditioning
Mechanical Abrasive Decortication
Bran
Roller Milling
Dry but unfermented
pearl millet meal
Pack and sell to
retailers

**Figure 3.** General flow processes of traditional pre-milling processing and milling by commercial hammer milling service providers and industrial milling (Taylor, 2004; Barrion, 2008).

Pearl millet meal used in the brewing of *Ontaku* can either be fermented or unfermented. This often depends on the brewer's and consumers' preference and/or on the availability of the ingredients. The first step in the traditional and the service milling processes is decortication (Taylor, 2004; Barrion, 2008). Using the pestle and mortar or a mechanical dehuller, between 10-30% of the grain may be removed during this step. The pericarp and germ and thus their chemical components such as oil, fiber and phenolics are lost into the bran. The removal of the germ during decortication reduces the oil content in the resulting flour. The oil, which is rich in unsaturated fatty acids, is prone to rancidity via oxidation. Its removal therefore positively contributes to the shelf stability of the meal. The decorticated grain can then be directly milled into a meal or it can be subjected to fermentation before milling.

Different techniques of fermentation can be applied during traditional milling at household level or applied by service millers (Figures 4a and 4b) to produce fermented meals. One of the techniques involves steeping of the grain in water (1:2 parts, respectively) and the other involves the use of *oomuma/eendjeke*, which are grain remnants from previously fermented grain (starter culture) when milled using the pestle and mortar (Shimbwadala, Nantanga & Shikongo-Nambabi, 2018). The duration of steeping of cleaned pearl millet grain in water is variable. It can be allowed for several hours or days. This depends among other factors on the ambient temperature, availability of money for service millers or of labour to pound using the wooden pestle and mortar, need of the meal and/or desired whitening of grain. The steeping step brings about lactic acid fermentation, which brightens (Taylor, 2004) the grain and subsequently yields a less dark meal. Lactic acid fermentation can occur spontaneously but often backslopping technique is employed. This involves the use of a previous portion of

steeping water, which contains a relatively high load fermenting microorganisms, mostly lactic acid bacteria (Shimbwadala et al., 2018), to inoculate the new steep water. Alternatively, the new steeping process may be carried out in the same container as the previous steeping but intelligently washed to leave portions of previous dry or semi-wet deposits in it. These deposits possibly carry a lactic acid "starter culture".

In the other technique, fermentation of pearl millet is sped up by the use of *eendjeke/oomuma*. Like backslopping (Taylor, 2004; Barrion, 2008), *eendjeke* also contains live microorganisms. They act as a source of "starter culture". The isolation, identification and characterisation of the microorganisms in *eendjeke* are not clearly documented. The practice of using *eendjeke* in the fermentation of pearl millet meal is currently limited to households.

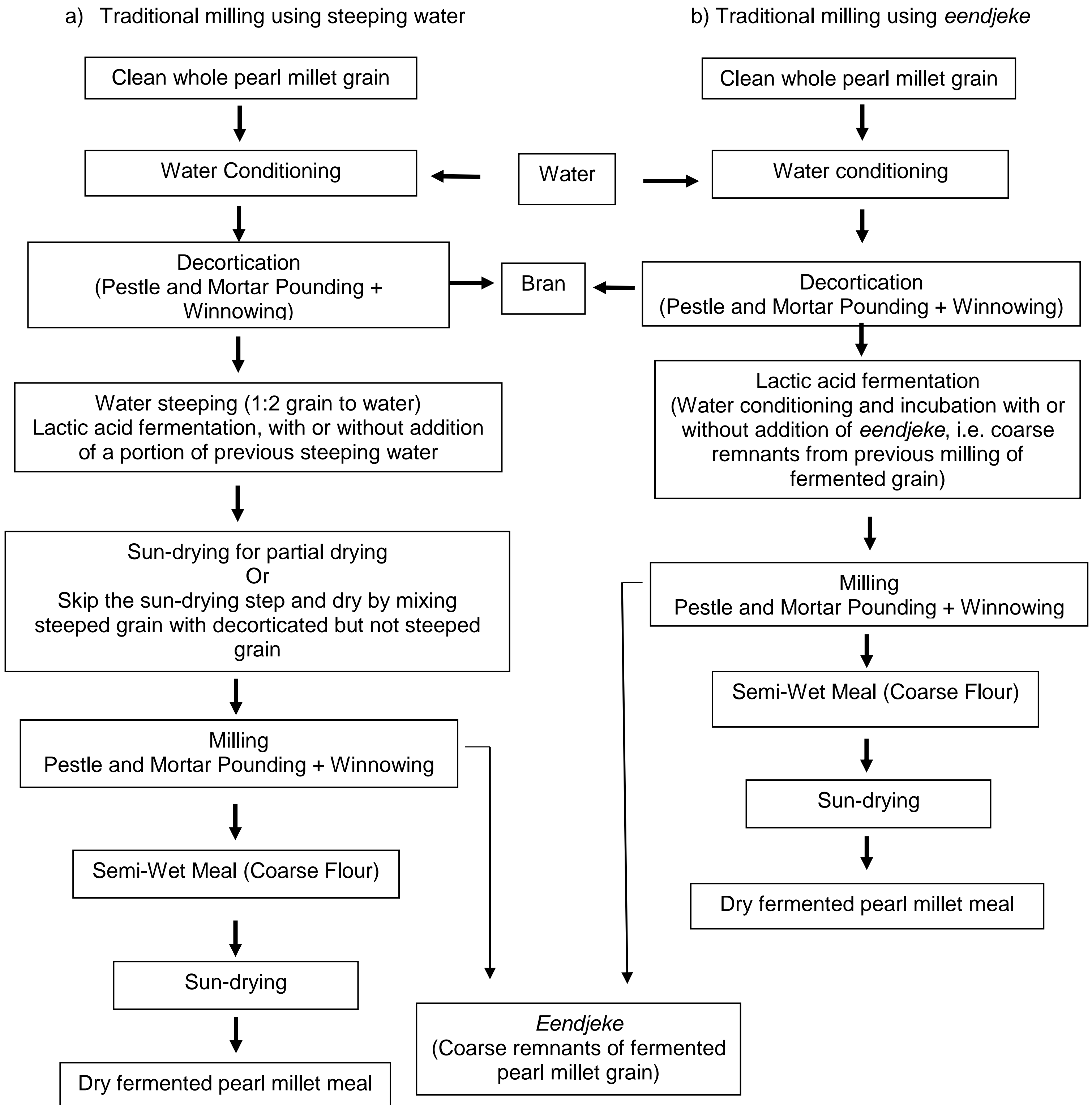



**Figure 4.** Common processing steps involved in the production of traditional fermented pearl millet meal. These are currently carried out domestically only.

### 2.4. Sorghum

Sorghum (*Sorghum bicolor (L.) Moench*) belongs to the grass family and its domestication started in East Africa (Cruickshank, 2016). The top sorghum producing countries are The United States, Nigeria, India, Mexico, Sudan, Argentina, Ethiopia, Australia, China (Mainland), Brazil, Burkina Faso, Niger, Mali and Cameroon (Cruickshank, 2016). Sorghum 1000 kernel weighs between 23-55g (Taylor & Duodu, 2017; Serna-Saldivar & Espinosa-Ramirez, 2019). The sorghum anatomical structure (Figure 5) consists of pericarp, endosperm and germ which vary but in most cases, it is 6%, 84% and 10% respectively (Waniska, Rooney & McDonough, 2016). Proximate composition, structural properties and chemical composition of sorghum are shown in Table 3. The range of protein, fat, fibre and ash content (g/100 g dry weight) are as follows, respectively 7.3-16.8, 0.5-6.2, 1.1-7.1 and 0.4-7.3.

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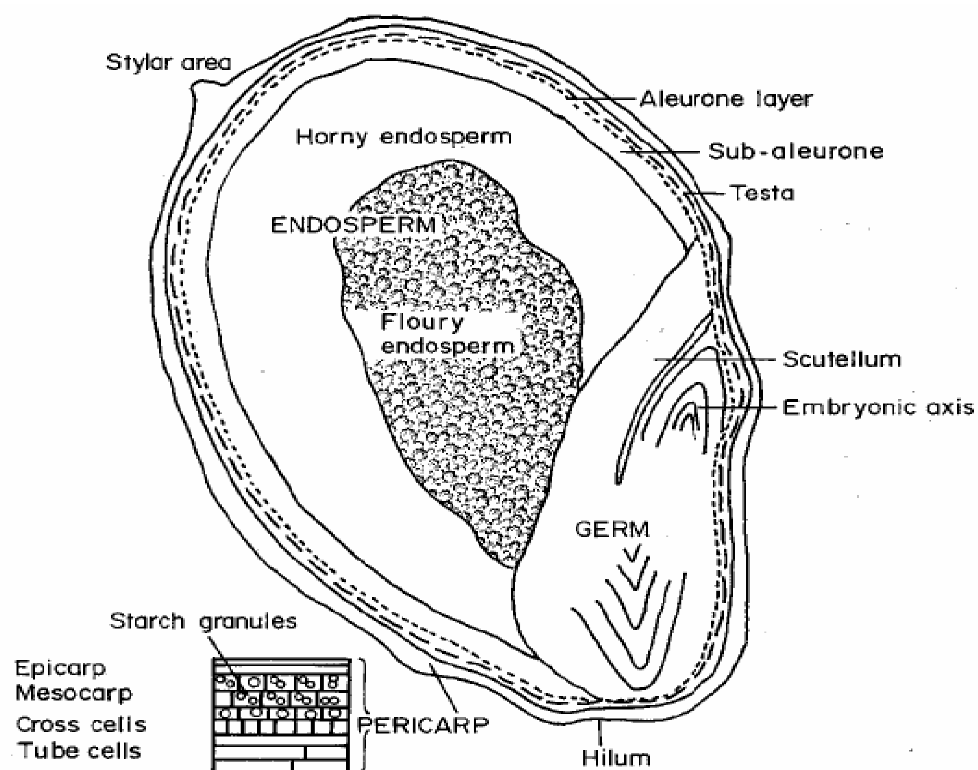


**Figure 5:** Diagrammatic section through a sorghum kernel (From Rooney & Miller, 1982).

**Table 3:** Proximate composition, structural features and gelatinization properties of Sorghum kernel.

| Composition | Whole grain | Pericarp (%) | Germ (%) | Endosperm (%) |
|---|---|---|---|---|
| Protein (g/100 g db) | 11 (7.3-16.8) [a,b] | 6.0 (5.2-7.6) [a] | 18.4 (17.8-19.2) [a] | 10.5 (8.7-13.0) [a] |
| Fat (g/100 g db) | 3.2 (0.5-6.2) [a,b] | 4.9 (3.7-6.0) [a] | 28.1 (26.9-30.6) [a] | 0.6 (0.4-0.8) [a] |
| Ash (g/100 g db) | 1.8 (1.1-7.1) [a,b] | 3.4 (2.0-3.8) [a] | 9.2 (3.9-10.4) [a] | 0.6 (0.3-0.7) [a] |
| Crude fibre (g/100 g db) | 2.7 (0.4-7.3) [a,b] | | | |
| Starch (g/100 g db) | 70.8 (55.6-75.2) [a] | 3.5 [a] | 13.4 [a] | 82.5 (81.3-83.0) [a] |
| Amylose (%) | 20-30 [a,b] | | | |
| Amylopectin (%) | 70-80 [b] | | | |
| Starch granules (µm) | 2-35 [a,c] | | | |
| Protein body size (µm) | 0.4-2 [a,c] | | | |
| Gelatinization temperature ($^{o}$C) | 68-91 [a,d] | | | |

[a]Serna-Saldivar & Espinosa-Ramirez, 2019, [b]Waniska, Rooney & McDonough, 2016, [c]Taylor & Duodu, 2017, [d]Awika, 2017

## 2.5. Lactic acid bacteria and Yeast

Steinkraus (2002) define fermented food (also beverages) as "food substrates that are invaded or overgrown by edible microorganism whose enzymes, particularly amylase, protease, lipases hydrolyse the polysaccharides, protein and lipids to nontoxic products with flavours, aromas and texture pleasant and attractive to the human consumer." Lactic acid bacteria (LAB) and yeast fermentation are usually common. According to Taylor (2016b), LAB is gram positive, catalase negative, nonspore-forming spheres and rod shaped. LAB genera are *Carnobacterium, Enterococcus, Lactococcus, Lactobacillus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Vagococcus* and *Weissella*. These LAB convert fermentable sugars to lactic acid (Steinkraus, 2002). Yeast is a single cell organism. Yeast converts fermentable sugars into ethanol and carbon dioxide. LAB are known to be responsible for many fermented African traditional products such as *injera* (Ethiopia), *ting* (Southern Africa), *ogi* (Benin, Guinea and Nigeria), *togwa* (Tanzania), *amahewu/magoe* (South Africa), *mukonyo* (Zambia), *Oshikundu* (Namibia) (Taylor, 2016b). Some of sorghum and millet based African alcoholic products that are yeast fermented are sorghum beer (South Africa) (Dewar & Taylor, 1999), *merissa* (Sudan), *pito* (Nigeria, Ghana) (Blandino, Al-Aseeri, Pandiella, Cantero & Webb, 2003), *omalovu* (Namibia) (Embashu, Iileka & Nantanga, 2019).

## 2.6. Phenols

Phenolic compound basic structure composes of benzene ring with one or more hydroxyl groups (Dykes & Rooney, 2007). Phenolics are categorised based on the basic carbon skeleton (Giada, 2013). The C6 (simple phenol, benzoquinone), C6- C1 (phenolic acids), C6-C2 (acetophenones, phenylacetic acids), C6- C3 (hydroxycinnamic acids, phenylpropenes, coumarins, chromones), C6- C4 (Naphthoquinones), C6- C1-C6 (xanthones), C6- C2-C6 (stilbenes, anthraquinones), C6- C3-C6 (flavonoids), (C6- C3)2 (lignans and neolignans),

(C6- C3)2 (lignins) and (C6- C3-C6)n (condensed tannins). Phenolics of major significance from pearl millet and sorghum have been reviewed (Dykes & Rooney, 2007). Phenolic acids that are hydroxybenzoic acid derived protocatechuic, p-hydroxybenzoic, gentistic, vanillin and syringic acid are both reported in pearl millet and sorghum grains. While those of gallic and salicylic acid only in sorghum grains. The hydroxycinnamic acid derived ferulic, caffeic, o-coumaric, m-coumaric, p-coumaric, cinnamic and sinapic acid are reported in both cereals. Sorghum compared to pearl millet grains has flavonoids from classes such as anthocyanins, flavones, flavanones, dihydroflavonols, flavan-4-ols and flavanols. While pearl millet has flavones: glucosylorientin, glucosyvitexin, vitexin. Condensed tannins are reported in sorghum (Awika, McDonought & Rooney, 2005; Dykes & Rooney, 2007), finger millets (Siwela, Taylor, de Milliano & Duodu, 2007) but not in pearl millet (Dykes & Rooney, 2006; Taylor & Duodu, 2015; Embashu & Nantanga, 2019).

### 2.7. Mycotoxins

Mould from genera *Aspergillus, Penicillium, Alternaria* and *Fusarium* (Stark, 2010; Patriarca & Pinto, 2017) are considered as contaminants from a food point of view in cereals, oilseeds and spices (Bhat, Rai & Karim, 2010). Grains are contaminated either in the field (*Alternaria, Fusarium*) or during storage (*Aspergillus, Penicillium*) (Patriarca & Pinto, 2017). Contamination of grains by mould is associated with factors such as moisture, available water and temperature (Patriarca & Pinto, 2017; Neme & Mohammed, 2017). Moulds produce mycotoxins as secondary metabolites (Bilgrami & Choudhary, 1998). Mycotoxins of toxicological significance in cereal include aflatoxins, ochratoxin, zearalenone, fumonisin, trichothecenes (T-2 and HT-2 toxins, deoxynivalenol, nivalenol) (Patriarca & Pinto, 2017) tremogenic toxins and ergot alkaloids (Hussein & Brassel, 2001). These toxins have been recognised to affect human and animal health (Bhat at al., 2010: Udomkun et al., 2017). Therefore, prevention strategies and contamination detection methods should be robust.

Detection of mycotoxins contaminants generally would follow the following steps (Pereira, Fernandes & Cunha, 2014). The process starts with sampling, homogenization, extraction, clean up, sample concentration, ends with separation and detection. The analytical methods used in the detection of mycotoxins contamination vary. These can be from simple, cheap, sensitive, rapid and able to simultaneous identify and quantify (Pereira et al., 2014: Anfossi, Giovannoli & Baggiani, 2016). The analytical methods include enzyme-linked immunosorbent assay (ELISA), thin layer chromatography (TLC), gas chromatography (GC) and liquid chromatography mass spectrometry (LC-MS).

*2.7.1. Aflatoxins*

Aflatoxins are difuranocoumarin derivatives synthesized by polyketide pathway (Cousin, Riley & Pestka, 2005; Patriarca & Pinto, 2017). Aflatoxins are produced by *Aspergillus* genera (Pascari, Ramos, Marín & Sanchís, 2018), from species of *A. flavus* and *A. parasiticus* (Patriarca & Pinto, 2017). Among the different types of aflatoxins, AFB1, AFB2, AFG1 and AFG2 are the most toxic and carcinogenic (Adebo, Njobeh, Gbashi, Nwinyi & Mavumengwana, 2015). The AFB1, AFB2 are produced by *A. flavus* while AFG1, AFG2 and also AFB1, AFB2 by *A. parasiticus* (Bhat et al., 2010). The optimum conditions for aflatoxin production are 30-33$^{o}$C and 0.99 aw (water activity) (Milani, 2013). Aflatoxins contamination have been reported in grains of sorghum (Ayalew, Fehrmann, Lepschy, Beck & Abate, 2006; Matumba, Monjerezi, Khonga & Lakudzala, 2011: Vismer, Shephard, Rheeder, van der Westhuizen & Bandyopadhyay, 2015; Misihairabgwi, Ishola, Quaye, Sulyok & Krska, 2018) and pearl millet (Vismer et al., 2015; Misihairabgwi et al., 2018). Likewise, aflatoxins also reported in sorghum malts (Trider, 1988; Matumba et al., 2011; Misihairabgwi et al., 2018; Nafuka, Misihairabgwi, Bock & Ishola, 2019). There are limited reports on possible aflatoxins contamination in malts of pearl millet. The European Commission has set legal limits of aflatoxins (Commission Regulation N0 1881/2006, 2006).

Aflatoxin AFB1 is set to be 2 µg/kg and total aflatoxins 4 µg/kg for all cereal and their product derivatives for human consumption. Meanwhile those of processed cereal-based food and baby foods for infants and young children to be 0.10 µg/kg. Some studies reported aflatoxins to be below the European Commission legal limits in grains of sorghum (Matumba et al., 2011) and pearl millet (Misihairabgwi et al., 2018). However, some found aflatoxins to be above the legal limits in pearl millet (Houissa et al., 2019), sorghum grains (Ghali et al., 2008) and malt (Nkwe, Taylor & Siame, 2005).

### *2.7.2. Ochratoxin*

Ochratoxin is difuranocoumarin derivatives synthesized by polyketide pathway (Cousin et al, 2005). Ochratoxin A and B are produced by *Aspergillus* (*A. ochraceus, A. carbonarius, A. alliaceus, A. auricomus, A. glaucus, A. melleus, A. niger*) and *Penicillium* (*P. verrucosum*) species (Milani, 2013; Reddy et al., 2010). The conditions that favour ochratoxin production are temperature (25-37$^{0}$C) and humidity (0.94- 0.98 aw) (Lasram et al., 2016: Lahoua et al., 2017).

The European Commission has set legal limits of ochratoxin A (Commission Regulation N0 1881/2006, 2006). The unprocessed cereals to be 5 µg/kg. That of all products derived from unprocessed cereal products and cereals intended for direct human consumption to be 3 µg/kg. At the same time, 0.50 µg/kg is set for processed cereal-based food and baby foods for infants and young children. Ochratoxin contamination in sorghum has been extensively reported (Ayalew et al., 2006; Ghali et al., 2008; Zaied et al., 2009; Makun et al., 2013; Chala et al., 2014). Also, reported in sorghum malts (Misihairabgwi et al., 2018). Contamination in pearl millet grains has also been reported (Sangare-Tigori et al., 2006; Makun et al., 2013; Misihairabgwi et al., 2018; Houissa et al., 2019).

*2.7.3. Zearalenone*

Zearalenone is produced from polyketide pathway (Cousin, Riley & Pestka, 2005). It is a phytoestrogenic compound (Diekman & Green, 1992), 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resor-cylic acid µ-lactone (Hussein & Brasel, 2001: Cousin et al., 2005). Zearalenone is a metabolite of *Fusarium* species (*F. culmorum, F. graminearum, F. sporotrichioides*) (Hussein & Brasel, 2001). The European Commission has set legal limits of zearalenone (Commission Regulation N0 1881/2006, 2006). Unprocessed cereal is set to be 100 µg/kg. Cereal intended for direct human consumption, cereal flour, bran as end product marketed for direct human consumption and germ to be 75 µg/kg. Meanwhile, processed cereal-based foods and baby foods for infants and young children to be 20 µg/kg. Zearalenone contamination has been reported in sorghum grains (Lefyedi et al., 2005; Ayalew et al., 2006; Ghali et al., 2008; Chala et al., 2014) and similarly in malts (Nkwe et al., 2005; Lefyedi et al., 2005; Misihairabgwi et al., 2018). There are limited reports of contamination in finger millets (Chala et al., 2014) and pearl millet (Misihairabgwi et al., 2018).

*2.7.4. Fumonisin*

Fumonisin is synthesised through polyketide pathway (Cousin et al., 2005). Fumonisin is produced by *Fusarium* species (*F. proliferatum, F. verticillioides*) (Fletcher & Blaney, 2016; Lulamba, Staffor & Njobeh, 2019), but also reported in *Aspergillus niger* (Udomkun et al., 2017). Fumonisin metabolites include B1, B2, B3 and B4 (Lulamba et al., 2019), with FB1 being commonly found (Marroquín-Cardona, Johnson, Phillips & Hayes, 2014). The optimum conditions for the production of FB1 (15-30$^{o}$C, humidity 0.93 aw) and *Fusarium* (15-30$^{o}$C, humidity 0.99 aw) have been noted (Lulamba et al., 2019). The European Commission has set legal limits (Commission Regulation N0 1881/2006, 2006). The sum of fumonisin B1 and B2 in unprocessed maize is set to be 2000 µg/k. Maize flour, meal, grits, germ and refined maize oil to be 1000 µg/kg. That of processed maize-based foods and baby

foods for infants and young children to be 200 µg/kg. Contamination with fumonisin has been reported in sorghum grains (Gamanya, 2001; Lefydi et al., 2005; Ayalew et al., 2006; Chala et al., 2014; Vismer et al., 2015). Also, in pearl millet grains (Vismer et al., 2015; Houissa et al., 2019). Malt of sorghum has been reported to be contaminated by fumonisin (Dufour, Melotte, 1992; Lefyedi et al., 2005; Misihairabgwi et al., 2018: Nafuka et al., 2019) and pearl millet (Misihairabgwi et al., 2018).

*2.7.5. Trichothecenes*

Trichothecenes are compounds containing sesquiterpene rings characterized by a 12, 13-ep-oxy-trichothec-9-ene nucleus, with different constituents on position 3, 4, 7, 8 and 15 of the molecule (Hussein & Brasel, 2001). These include T-2 toxin, HT-2 toxin, neosolaniol, scirpentriol, nivalenol and deoxynivalenol (Fletcher & Blaney, 2016). Deoxynivalenol is a trichothecene produced by *Fusarium* species (*F. culmorum, F. graminearum, F. crookwellense*) (Hussein & Brasel, 2001; Fletcher & Blaney, 2016). The optimum conditions for production the production of deoxynivalenol are $25^0$C after 40 days of incubation and $0.995a_w$ (Lulamba et al., 2019). The European Commision has set legal limits of deoxynivalenol in unprocessed cereal other than durum wheat, oats and maize to be 1250 µg/kg. In addition, cereal intended for direct human consumption, cereal flour, bran as end product marketed for direct human consumption to be 750 µg/kg (Commission Regulation N0 1881/2006, 2006). Contamination in sorghum grains by deoxynivalenol have been reported (Gamanya, 2001; Lefydi et al., 2005; Ayalew et al., 2006; Chala et al., 2014). Similarly, contamination has been stated in the malts of sorghum (Lefyedi et al., 2005). Contamination has been also reported in finger millets grains (Chala at al., 2014) and there is limited data on pearl millet grains and malts.

## 2.8. Malting

Malting is a limited germination of cereal grains or occasionally seeds of pulses under controlled conditions (Briggs, 1998) for a specified period. Malting is divided into three steps steeping, germination and drying or kilning (Briggs, Hough, Stevens and Young, 1981). Malting of cereal grains is done following pneumatic malting (industrial malting) and floor malting (commercial malting) (Novellie and De Schaepdrijver, 1986; Briggs, Boulton, Brookes and Stevens, 2004). Pneumatic and floor malting steeping process is common and differed during germination and dying (Taylor, Dewar and Joustra, 2005). Generally, cereals are malted for production of weaning food, fermented nonalcoholic and alcoholic beverages such as clear and opaque beer.

### *2.8.1. Steeping*

Steeping involves the submerging of grains in water until an acceptable moisture level is attained (Owuama, 1999). During the steeping step, germination is initiated, endosperm structure is modified and as a result malt of desired quality is produced (Lyumugabe, Gros, Nzungize, Bajyana & Thonart, 2012). Therefore, steeping is considered a critical step during malting (French & McRuer, 1990; Dewar, Taylor & Berjak, 1997a). Steeping primary objectives is hydration of dry grain, initiate metabolism, cleaning by washing and removing floaters (Briggs, Hough, Stevens & Young, 1981; Briggs, 1998). The uptake of water by grain during steeping is influenced by factors such as moisture, temperature (Briggs, 1998), cultivar, protein content, structure, size (Pelembe, 2001) and oxygen (French & McRuer, 1990). Dewar et al., (1997a) demonstrated that air rest period during the steeping step resulted in optimum sorghum malt quality (diastatic power, free amino nitrogen, hot water extracts). During the air rest period, carbon dioxide and ethanol are removed (Pelembe, 2001). Steeping duration of 14 to 40 hours, water temperature between 25-30$^{o}$C and air rest period have been reported to produce optimum quality sorghum malt (Dewar, Taylor &

Berjak, 1997a). While steeping duration that of finger millet and pearl millet to be between 6-16 hours (Gomez, Obilana, Marti, Madzvamuse & Moyo, 1997; Muoria & Bechtel, 1998). Moreover, pearl millet steeping water temperature to be between 30-35$^{o}$C for pearl millet (Dahiya, Yadav, Yadav & Yadav, 2018). Pearl millet and finger millet steeping duration are lower compared to those of sorghum nonetheless, steeping water temperatures are higher than of finger and pearl millets.

*2.8.2. Germination*

Germination involves the outgrowth of plumule and radicle of seedling (Owuama, 1999). Hydrolytic enzymes are developed during the stage of germination in the grain such as α-amylase, β-amylase, α-glucosidase, lipases, peroxidases, carboxypeptidases and proteinases (endopeptidases) (Palmer, 1989; Owuama, 1999). During seed germination, stored protein in the endosperm is hydrolysed by proteinases and peptidases to peptides and amino acids that are used for grains growth (Payne & Walker-Smith, 1987). The α-amylase hydrolyses α-1, 4 glucosidic bond of starch to produce dextrins (short chains of glucose molecules), maltotriose, maltose and glucose randomly (Briggs, 1998; Pelembe, 2001). Furthermore, β-amylase hydrolyse penultimate α-1, 4 glycosidic bonds at the non-reducing end of the starch molecule releasing maltose (Taylor & Robbins, 1993). Sorghum germination conditions for optimum malt quality are reported as the temperature between 25-30$^{o}$C and a period of 3-6 days (Morral, Boyd, Taylor & Van der Walt, 1986; Dewar, Taylor & Berjak, 1997b). Germination conditions of sorghum are similar to that of pearl millet suggested by Pelembe, Dewar and Taylor (2002) with a temperature between 25-30$^{o}$C and germination time 3-5 days were found to be optimum, although they suggested a higher temperature 30-35$^{o}$C for a shorter period of 1-3 days.

*2.8.3. Drying/Kilning*

Drying/kilning is the last stage of malting (Lefyedi, 2006). Drying terminates growth of the green malt, this lowers moisture content, water activity and as a result, a shelf-stable product with active enzymes is produced (Novellie & De Schaepdrijver, 1986). The drying temperature of sorghum (Novellie, 1962; Dewar, Taylor & Berjak, 1997b) and pearl millet (Pelembe et al., 2002) diastatic green malt are both up to 50°C for 24 hours.

## 2.9. Malting and malt quality of pearl millet or sorghum for use in the brewing of *Ontaku*

Malt quality is central to the brewing of beers and other beverages. In Namibia, pearl millet and sorghum can be malted for use to brew *Ontaku* (Mallet & Du Plessis, 2001; Taylor, 2004; Embashu et al., 2013; Taylor, 2016a; Hepute et al., 2016; Taylor, 2016b) or an opaque beer (*omalovu*) (Embashu et al., 2019). The traditional malting process and its set-up in Namibia are not controlled, standardised or well documented. Nevertheless, this traditional malting process follows the common malting steps of steeping, germination and drying (Taylor, 2016a). The duration of steeping of pearl millet or sorghum grains in Namibia ranges from 1 to 3 days (Embashu et al., 2019). There is generally no temperature control or air rests.

During steeping, the water diffuses into the grain. The grain swells and biochemical changes and germination are induced. The steeped grain is drained and then allowed to germinate for 7 to 14 days with some intermittent watering. The amount and frequency of watering of the germinating grain are mostly subjectively applied. During germination, the seedling grows (Taylor, 2016a). The various endogenous enzymes are activated and mobilised from the germ into the endosperm to modify the grain structure. The most important enzymes in the malting process are the amylases, proteases and peptidases (Taylor & Duodu, 2010). The α- and β-amylases hydrolyse starch, the major component of the grain, into dextrins and fermentable sugars such as maltotriose and maltose. The degree of activity of these two enzymes in the

malt is referred to as diastatic power (DP). DP estimates the ability of the malt to solubilise starch during brewing.

The activities of proteases and peptidases cumulatively convert proteins into amino acids. The amino acids are estimated as free amino nitrogen (FAN) content in malts and are utilised by the fermenting microorganisms during brewing (Taylor & Duodu, 2010). The malted grain is then subjected to size-reduction by semi-wet milling, primarily by women using wooden pestle and mortar. The milled malt is then sun dried for up to 3 hours depending on the season, at ambient temperature, usually below 50°C to conserve the activity of the mobilised enzymes. This also produces shelf stable malt because of its low moisture content.

There is no literature found on the conditions of malting and on the quality of pearl millet or sorghum malt for use in the brewing of *Ontaku*. However, the malting conditions which give an acceptable amount of free amino nitrogen and α- and β-amylase, for opaque beer brewing, have been reported and reviewed for sorghum (Okafor & Aniche, 1980; Palmer, Etokakpan & Igyor, 1989; Dewar & Taylor, 1999; Lyumugabe, Gros, Nzungize, Bajyana & Thonart, 2012) and pearl millet (Pelembe, Dewar & Taylor, 2004) to be generally between 25-30°C and germination time between 1-3 days. The quality of traditionally produced malt currently used domestically in Namibia needs to be studied and then relate aspects such as its diastatic power, free amino nitrogen and related parameters to the quality of *Ontaku*. Safety aspects of the malt such as the occurrence of harmful bacteria and mycotoxins also need to be investigated. Recently, aflatoxin B1 was detected in pearl millet meals and sorghum malts (Misihairabgwi, et al., 2018). Sorghum malt had levels above the European Union regulatory limit of 5 parts per billion. Fumonisin B1 in pearl millet meals and in sorghum malts. The amount of fumonisin B1 was above the European Union regulatory limit of 2,000 parts per billion in pearl millet meal. Interestingly, none of the regulated mycotoxins were detected in

*ontaku*. Information on the quality and safety of the malt can be useful in the processes that can lead towards the commercialization of malt for use in the brewing of *Ontaku*. Recently, there is a dry premix of ingredients (pearl millet meal containing sorghum malt) produced industrially and is available in the supermarkets in Namibia for use in the brewing of *Ontaku*.

### 2.10. Flow process for brewing *Ontaku*

The traditional brewing methods of *ontaku* have been described (Taylor, 2004; Embashu et al., 2013; Hepute et al, 2016). The general flow process used in the making of *Ontaku* is shown in Figure 6. Pearl millet meal is mixed with boiling water in a ratio of 1:2.5. Pearl millet bran may be used as part of the ingredients (Embashu et al., 2013). This paste is stirred until it is relatively homogeneous without lumps. The boiling water partially gelatinises the starch granules of the meal. The paste is then cooled to temperatures below 40$^{o}$C. Thereafter, pearl millet or sorghum malt, ranging from a quarter to half the amount of the meal is then added and thoroughly mixed. The addition of malt introduces among other soluble sugars, enzymes such as amylases and fermenting microorganisms. The enzymes can act on starch molecules to thin the paste. After some time of cooling, the paste is diluted with tap water of about 2 to 3 times the amount of the boiling water that was added. The amount of water added is subject to the consistency desired by the brewer. It is noteworthy that there are no mashing and straining steps in the brewing of *Ontaku* that are typical in the brewing of beers. The diluted paste is usually directly allowed to spontaneously ferment at ambient conditions for at least 3 hours before consumption. Backslopping using a previous portion of *Ontaku* is also commonly employed. The duration of fermentation is usually overnight but commonly ranges from 3 to 6 hours. Taylor, (2016a) suggests that it is the lactic acid bacteria (LAB) that are mostly responsible for the fermentation of soluble sugars in the brewing of *Ontaku.* However, some levels (see succeeding text) of alcohol and acidity develop over fermentation

time. This suggests that there are different populations of fermenting microorganisms other than LABs.

An alternative but also traditional brewing method of *Ontaku* exists. In this method, the dry ingredients (malt and pearl millet meal) are mixed first before the addition of water. Importantly, the water added has to be lukewarm and not boiling water. The exact temperature of the lukewarm water is not determined but is simply described as such based on the experience of the brewers. The addition of lukewarm water to the meal containing the malt is hypothesised to be aimed at not inactivating the enzymes present in the malt. The rest of the processing steps are the same as those described in the first method.

*Ontaku* is greenish brown with a buttery sour taste (Taylor, 2004). It is an effervescent beverage with up to 98% water content on wet basis. It is generally watery but can have a consistency similar to that of yoghurt. It has a pH of less than 4 and lactic acid content of up to 0.6%. It should be noted that not all *Ontaku* beverages are non-alcoholic. The alcohol content that appears over time has been recorded up to 1.6%. For weaning, the freshest and non-alcoholic *Ontaku* is commonly used. Upon spoilage (expiration), *Ontaku* generally separates into two layers (Figure 1). A more watery layer sits on top of dense solids and the effervescence dissipates. There is little literature (Taylor, 2004; Hepute et al., 2016) found on the nutrient composition and characteristics of *Ontaku*.

The protein, ash and insoluble fibre contents of the watery part (excluding *Ontaku* dregs) of ontaku were 0.013-0.018%, 0.07-0.8% and 0.25-0.31%, respectively, on wet basis (Embashu, 2014; Misihairabgwi & Cheikhyoussef, 2017). The energy content was in the range of 0.38-0.58 kJ/mL. While studies on the nutrient composition and safety of the whole *Ontaku* need to be conducted, it suffices to note that *Ontaku* might not be nutrient dense especially for use as a sole weaning food.

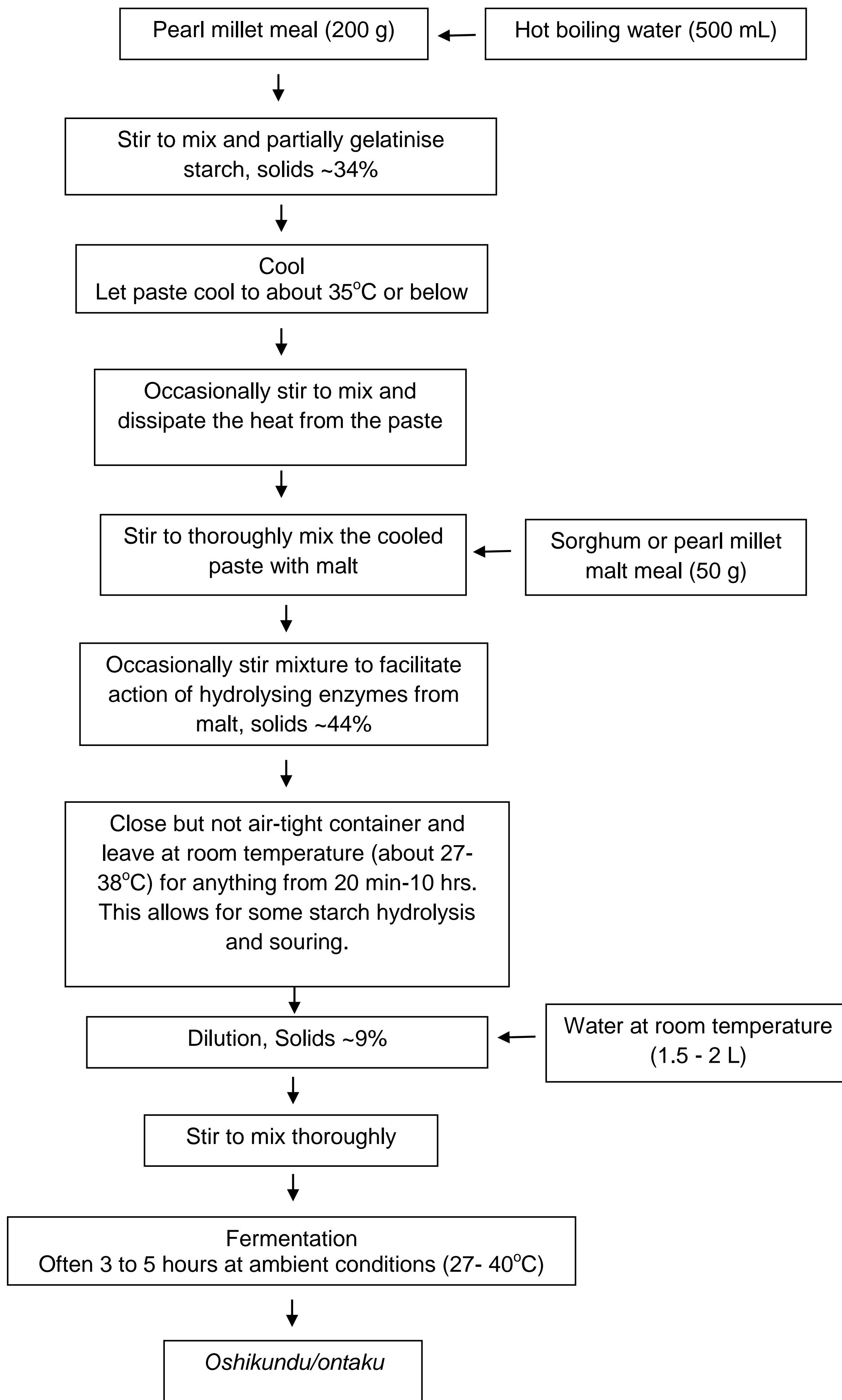


**Figure 6.** *Ontaku* brewing flow process (Taylor, 2004; Hepute et al., 2016).

One of the possible major limitations to the commercialisation of this beverage is the absence of standardised flow processes to produce it such that it is safe and has consistent quality and improved shelf life. For instance, the ingredient ratio, mixing regime and processing conditions such as temperature and time at which *Ontaku* is brewed are not standardised. Furthermore, with no straining, filtration or other appropriate separation steps and with limited gelatinisation of starch in the flours/meal used, *Ontaku* is an unstable suspension that has a large amount of floating particles (flour/meal). It also contains live populations of microbes. Within minutes these particles collect and sediment at the bottom as a denser layer of dregs (*ehete*) (Figure 1) due to gravity. Consequently, *Ontaku* has a differential consistency or viscosity. This also results in the differential preferences of these layers or their mixture by consumers.

Currently, the processing conditions for brewing of *Ontaku* are as diverse as the brewers. For improved brewing of this beverage, further research needs to be conducted. For example, the quality and amount of ingredients, temperature of water added, the consistency of *Ontaku* paste (pearl millet), time of dilution, duration of conversion by the malt are some of the parameters that are not controlled and needs investigation The extent of gelatinisation and conversion and extent of dilution during the brewing process of *Ontaku* also warrant assessment.

The microflora responsible for the fermentation and its potential health benefits also needs to be explored. The various malting and fermentation of pearl millet grain could result in the delivery of populations of different microorganisms involved in the brewing of *Ontaku*. Moreover, the use of unfermented whole grain, fermented whole grain or fermented decorticated grain meals presents different substrates for fermentation. This offers opportunities for investigations into possible prebiotics, probiotics and snybiotics which may

arise during different stages of brewing and in *ontaku*. Clinical studies on the health effects of consuming *Ontaku* in 60% of the Namibian human population may give insight on the functional benefits of this beverage. Understanding the metabolites in *Ontaku*, coupled with *ontaku* being a cereal product without complications of celiac issues presents alternative avenues for the development of new healthful products in the market of functional foods of cereal origin. However, for the improved safety and quality of *Ontaku*, there is a need for further investigations on the quality and safety of the ingredients, processing conditions, sensory quality, flavour compounds profile and safety aspects (e.g. methanol level) of *Ontaku*.

## 2.11. Conclusion

*Oshikundu/Ontaku* is a very popular fermented drink in Namibia. It is fermented using pearl millet. It is acidic and has live cultures of fermenting microflora. It has relatively low or no alcohol content. It is currently indispensable to household food security and daily hunger nourishment to the majority of the Namibian people. Improved processing of pearl millet grain and of *Ontaku* brewing process can potentially enhance the utilisation and diversification of high quality fermented products of pearl millet grain. Development of starter cultures for the fermentation steps in the milling of the grain and in the fermentation of *Ontaku* can result in improved control of the products quality and safety. Pearl millet has the potential of becoming a global staple food crop in many regions that are affected by the effects of climate change. This is because it is well adapted and flourishes in hot and arid regions where most other cereal crops can hardly grow and produce grains.

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# Chapter 3

## Malts: Quality and phenolic content of pearl millet and sorghum varieties for brewing nonalcoholic beverages and opaque beers

Chapter 3 addresses objective 1.

To determine the effect of malting on pearl millet and sorghum quality (free amino nitrogen, reducing sugar, alpha & beta amylase and diastatic power), proximate content, phenolic content and their antioxidant activities.

Chapter is published in the Journal of Cereal Chemistry.

## Abstract

The use of malted sorghum and pearl millet in the production of traditional foods and beverages is ubiquitous in Africa and India. However, there is limited industrial production and little data on the phenolic content and quality of pearl millet and sorghum malts of different varieties. Therefore, this study investigated the proximate content, malt quality, and phenolics of pearl millet (*Okashana 2, Kantana,* and *Kangara)* and sorghum (*Macia* and a landrace referred to as Red sorghum) varieties. Malting increased the protein in all the varieties, except for *Kangara*. Germinative energies were > 97% for all varieties, except for Red sorghum. Malt quality (reducing sugars, free amino nitrogen, and β-amylase activity) was highest for *Macia* followed by *Kantana*. All pearl millet varieties and *Macia* had no condensed tannins. The total phenolic content and radical scavenging capacity decreased after malting for all the varieties. *Macia* and *Kantana* can be candidates for industrial malting for brewing nonalcoholic beverages and opaque beers. *Kantana* and Red sorghum had higher amounts of phenolic compounds and can potentially be vectors of delivering phenolics into human diets. This study investigated the phenolic content and quality of malts of different pearl millet and sorghum varieties, which can potentially be used to brew particularly low-alcohol beverages.



Keywords: brewing, malt quality, pearl millet, phenolics, sorghum, tannins

## 3.1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R.Br.) and sorghum (*Sorghum bicolor* (L.) Moench) are tropical cereal grains that are grown in many parts of the world such as India, the United States, Brazil, China, Australia, and Africa (Taylor, 2016a; Taylor & Duodu, 2017). Traditionally, fermentation, malting, and milling are applied to these grains. Various traditional foods are produced which include unleavened breads, porridges, boiled grain and non-alcoholic gruels and opaque beers (Taylor, 2016b; Waniska, Rooney & McDonough, 2016). Pearl millet and sorghum malts are made into various products including *Bushera* (Uganda), *Fura* (Nigeria), *Injera* (Ethiopia, Eritrea), *Ting* (Botswana, South Africa), *Oshikundu, Omalovu* (Namibia), *Togwa* (Tanzania), *Kisra* (Arabian Gulf, Sudan, Iraq), *Dakuwa* (Nigeria), and weaning food (India) (Adebiyi, Obadina, Adebo, & Kayitesi, 2018; Blandino, Al-Aseeri, Pandiella, Cauntero, & Webb, 2003; Dias-Martins, Pessanha, Pacheco,Rodrigues, & Carvalho, 2018; Embashu, Iileka, & Nantanga, 2019). The malting process of sorghum for brewing of beer is relatively reported and standardized at commercial level (Owuama, 1999; Ratnavathi & Chavan, 2016). The brewing of beer using sorghum malt exists in a number of countries such as Nigeria, Zambia, and South Africa (INTSORMIL, 2008; Kutyauripo, Parawira, Tinofa, Kudita, & Ndengu, 2009). Furthermore, un-malted sorghum is also used as adjunct to brew a lager-type beer (Eagle lager) in Tanzania, Uganda, Zambia, and Zimbabwe (INTSORMIL, 2008). Similarly, a lager-type beer (Eagle lager) is brewed commercially using un-malted pearl millet flour (*Mahangu*) in Namibia. While there is limited, if any, commercial breweries that use pearl millet malts for brewing of beer, pearl millet malt is traditionally produced and used to brew alcoholic and nonalcoholic beverages at household levels in many countries in sub-Saharan Africa (Adebiyi et al., 2018;Embashu et al., 2019; Hepute, Embashu, Cheikhyoussef, & Nantanga, 2016). The quality of malts produced using traditional processes is generally unknown, and the traditional malting

processing steps and conditions are uncontrolled and not standardized. There are relatively few studies that investigated the effects of malting conditions on the malt quality of pearl millet (Nzelibe & Nwasike, 1995; Pelembe, Dewar, & Taylor, 2002). Pearl millet and sorghum grains have higher total phenolic compounds and antioxidant activities than wheat, barley, and rye (Ragaee, Abdel-Aal, & Noaman, 2006). Phenolic compounds may confer protection against oxidative stress, cardiovascular diseases and can have anticancer properties (Awika & Rooney, 2004; Taylor & Duodu, 2017). Pearl millet and sorghum products are presumably some of the common vectors that deliver phenolic compounds in human diet in developing nations. As stated, there is scant information on the malt quality of pearl millet varieties. Moreover, there is a need to understand the quality and characterize the phenolic content of malts of pearl millet and sorghum varieties for use in the production of nonalcoholic and low-alcohol opaque beverages. Therefore, this study investigated the effect of malting on the quality of malt and phenolic content of malted pearl millet and sorghum varieties for use in the production of value-added products such as beverages.

## 3.2. Materials and methods

### *3.2.1. Grains*

Peal millet and sorghum grains of 2015 harvest were obtained from Omahenene Agricultural Research Station (17°26′30″S; 14°47′20″E) of the Ministry of Agriculture, Water and Forestry in Namibia. The varieties of pearl millet grains used in this study were *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”). The sorghum landrace is herein referred to as Red sorghum variety. The grains were grown in a sandy-loam soil under irrigation of 16 mm per hour every 3 days on average till fruiting stage (Megameno Amutenya, Agricultural Research Officer, Omahenene Research Station, *personal*

*communication*), except when it rained. The cultivation field was fertilized with 300 kg per hectare of NPK (basal fertilization) during planting. At about 3 leaves stage and also before flowering, 200 kg per hectare of urea was applied. At the booting stage, superphosphate was also applied at 200 kg per hectare. The grains were obtained within one month of harvest from Omahenene Research Station where they were stored in shaded silos at ambient temperatures (apparently 20–30°C). The grains were then kept at 7–10°C in a cooled room and were malted within one month of storage in this cooled room.

#### *3.2.2. Malting*

Pearl millet and sorghum grains were malted according to Pelembe et al., (2002) with some modifications.

##### *3.2.2.1. 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 hr wet and 2 hr airrest for a total of 8 hr. Moisture content was determined at this point for malting loss determination.

##### *3.2.2.2. Germination*

After steeping, 600 g of the grain was transferred into shade cloth 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 hr apart) during the day.

##### *3.2.2.3. 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 hr. 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).

*3.2.3. Determination of weight (1000) kernels*

The 1,000 kernel weight was determined using method described by Serna-Saldivar (2012). This was repeated five independent times ($n$ = 6) per variety.

*3.2.4. Determination of germinative energy*

Germinative energy, that is, the ability of the grain to germinate fully and with vigor, which is essential to normal malting was determined following method used by Ebbah, Laryea, Barimah & Djameh (2015). This was repeated five independent times ($n$ = 6) per variety. To a 90 mm petri dishes, placed 100 kernels of three pearl millets and two sorghum varieties with two layers of filter paper (Whatman No.1) wetted with 4 mL of water. The petri dishes with pearl millet and sorghum kernels were placed in an incubator at 28$^o$ C. The germinated kernels were counted and removed at 24, 48 and 72 hours. The germination energy was calculated using the following equation.

Germination energy (%) = $(n_{24} + n_{48} + n_{72})/3$

Where: $n_{24}$, $n_{48}$, $n_{72}$ – numbers of germinated kernels at 24, 48 and 72 hours.

*3.2.5. Proximate analysis and malt quality*

The proximate content and reducing sugars, free amino nitrogen, and activities of amylases were analysed in both the nongerminated grains and the malts except malting loss determined on loss difference in nongerminated grains and germinated grains.

*3.2.5.1. Determination of proximate content*

Crude fiber (method 4.6.01) and crude fat (method 4.5.01) were determined according to AOAC (1995a, 1995b) methods, crude protein was determined using combustion nitrogen analysis (CHN628 Carbon/protein/Nitrogen Analyzer) ($N \times 6.25$), and moisture was

determined using a moisture analyzer (Sartorius MA35). The proximate content was determined on both the nongerminated grains and on the malts.

*3.2.5.2. Malting loss*

Shoots and roots were included in malting loss determination according to formula below:

$$\text{Malting loss (\%)} = \frac{[[SGW-(SGW\times(SGM/100))]-[DMGW-(DMGW\times(DMGM/100))]]\times 100}{[SGW-(SGW\times(SGM/100))]}$$

Where SGW—steeped grains weight, SGM—steeped grains moisture (%), DMGW—dry malt grains weight, and DMGM—dry malt grains moisture (%).

*3.2.5.3. Determination of reducing sugars*

Pearl millet and sorghum varieties reducing sugars were determined using the method by Nelson-Somogyi (1944). Complete method in appendex A.

*3.2.5.4. Determination of free amino nitrogen*

The FAN was determined by the European Brewery Convention Ninhydrin assay, method 8.8.1 (EBC, 1987) using glycine as a reference amino acid. Complete method in appendex B.

*3.2.5.5. Determination of α- and β- amylase activities*

The betamyl-3 and ceralpha methods by Megazyme International (2015) were used to determine the α- and β-amylase activities.

*3.2.6. Phenolics and antioxidant activity*

*3.2.6.1. Detection of tannin by bleach test*

The chlorox bleach test for qualitative determination of tannins in sorghum grain was used as described by Taylor & Taylor (2008). It was done on the nongerminated grains of sorghum and pearl millet varieties. If the grain turns black over the entire surface upon the bleach test, this indicates a positive test (presence of a pigmented testa, i.e., tannin grain). On the other hand, a completely white or brown grain over part of the surface indicates a negative test (absence of pigment testa, i.e., nontannin grain). It is noteworthy that no standard check

sample was used to compare with the results observed for pearl millet and sorghum varieties sample in this study.

*3.2.6.2. Extraction*

For the extraction of phenolic compounds, 0.5 g of nongerminated grains and malts was weighed into a 50-ml centrifuge tube. This was extracted using 5 ml of 1% HCl (v/v) in methanol. The content was mixed and left to stand for 15 min with gentle shaking. The tubes were then placed in a sonicator (Elmasonic S10H) at 25°C for 10 min before centrifugation (Eppendorf 5810R, Germany) at 3220 *g* for 3 min. The supernatant was decanted into a separate centrifuge tube, and the extraction was repeated once. The supernatants were stored at −4°C until analysis.

*3.2.6.3. Determination of total phenolic content*

Total phenolic content was determined using the Folin–Ciocalteu method as described in Ainsworth & Gillespie (2007). The estimation of phenolic content was expressed as milligrams of gallic acid equivalent per gram of sample on dry basis.

*3.2.6.4. Determination of flavonoid content*

The aluminum chloride method as described by Chang, Yang, Wen & Chern (2002) was used to determine the flavonoid content, which was expressed as milligrams of quercetin equivalent per gram of sample on dry basis.

*3.2.6.5. Determination of condensed tannins*

Condensed tannins were determined using vanillin–HCl method as described by Gaytan-Martinez et al., (2017). The condensed tannin content was expressed as milligrams of catechin equivalent per gram of sample on dry basis.

*3.2.6.6. DPPH free radical scavenging activity*

The capacity of extracts to scavenge free radicals (2,2-diphenyl-1-picrylhydrazyl, DPPH) was determined according to the method by Gaytan-Martinez et al., (2017). The free radical

scavenging ability (Sompong, Siebenhandl-Ehna, Linseberger-Martina, & Berghofer, 2011) was calculated using the following equation:

$$\text{Scavenging ability (\%)} = \frac{A_{\text{control}} - A_{\text{sample/AA}}}{A_{\text{control}}} \times 100$$

where $A$ = absorbance at 515 nm; $A$control = DPPH solution without extract or standard (ascorbic acid); $A$sample = DPPH solution with extract; $A$AA = DPPH solution with ascorbic acid.

*3.2.7. Statistical analysis*

The grains were malted once for each of the varieties of sorghum and pearl millet. The proximate analyses were repeated five times ($n$ = 6). The malt quality and phenolic extractions were performed two independent times. The analyses were then conducted in triplicates for each of the two independent extractions. The effect of malting on malt quality and phenolics of pearl millet and sorghum varieties was determined using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test at $p \leq 0.05$ using R software (version 3.5.2, Austria).

## 3.3. Results and discussions

*3.3.1. Kernel weight, germinative energy, and proximate content*

Kernel weight and germinative energy of nongerminated pearl millet and sorghum grains varieties are shown in Table 4. Nongerminated sorghum grain varieties had the highest ($p \leq 0.05$) kernel weight compared to all pearl millet varieties. Among pearl millet varieties, *Kangara* had a relatively higher ($p \leq 0.05$) kernel weight than the other two studied varieties. The pearl millet kernel weight of *Okashana 2* and *Kantana* was in the range of 0.008–0.015 g/kernel reported by Abdelrahman, Hoseney and Varriano-Marston (1984) and Serna-Saldivar and Espinosa-Ramirez (2019). However, for *Kangara* the kernel weight was slightly

higher than the same reported range. This suggests that *Kangara* kernel is relatively larger and/or denser than *Okashana 2* and *Kantana*. The kernel weight of sorghum varieties was within the reported range of 0.023–0.035 g/kernel (Serna-Saldivar & Espinosa-Ramirez, 2019; Serna-Saldival & Rooney, 1995).

The germinative energy was statistically the same ($p > 0.05$) among the varieties of pearl millet and *Macia* grains, except that of Red sorghum which was significantly lower ($p \leq 0.05$), but they were all higher than the recommended minimum germinative energy of 90% for sorghum by the European Brewery Convention (EBC, 1998). The germinative energy (>97%) for pearl millet and *Macia* observed in this study is the same as that reported by Pelembe et al., (2002) for pearl millet. Red sorghum germinative energy was in the range of 94%–95% reported by Morrall, Boyd, Taylor, and Walt (1986) and Ebbah et al., (2015) for sorghum. However, other studies have found higher (96%–99%) germinative energy (Bekele, Bultosa, & Belete, 2012) for Red sorghum. Crude fat in nongerminated grains differed significantly ($p \leq 0.05$) between the varieties irrespective of the cereal (Table 4). Crude fat content in nongerminated grains was in this order *Kangara* > *Okashana 2* > *Kantana* > Red sorghum > *Macia*. Pearl millet varieties had significantly ($p \leq 0.05$) higher crude fat content than sorghum varieties. Pearl millet (*Okashana 2*, *Kantana*) and sorghum (*Macia*, Red sorghum) varieties had fat contents that are within the respective ranges reported by Serna-Saldivar and Espinosa-Ramirez (2019). However, the crude fat content of *Kangara* was higher than the fat content range reported by Serna-Saldivar and Espinosa-Ramirez (2019). Malting decreased crude fat content in all the cereals except *Macia*. The decrease was more pronounced in pearl millet than in sorghum. *Kangara* had the most decrease in fat content. Adebiyi, Obadina, Adebo, and Kayitesi (2017) also observed a decrease in fat content of pearl millet following malting. The decrease in fat content is attributed to hydrolysis and utilization of fat as energy source for biochemical reactions during germination (Nkhata,

Ayua, Kamau & Shingiro, 2018). Crude protein contents of nongerminated grains of *Kantana* and *Macia* were statistically the same ($p > 0.05$) but higher than those of the other grains, whereas *Okashana 2* and Red sorghum had the lowest crude protein contents. These findings were within the reported range of 6.9%–20.9% db (Serna-Saldivar & Espinosa-Ramirez, 2019; Taylor, 2017) for pearl millet and of 7.3%−15.6% db (Awika, 2017; Serna-Saldivar & Espinosa-Ramirez, 2019) for sorghum. Unlike the fat content, the protein content increased after malting in the following order *Kantana* > Macia > Red sorghum > *Okashana 2*. Malting, however, did not affect the protein content of *Kangara*. Adebiyi et al., (2017) also noted an increase in pearl millet protein content following malting. The increase in protein content may be due to loss of dry weight some carbohydrate and fats are utilized during respiration but also some amino acids are synthesized during germination (Nkhata, Ayua & Kamau, Shingiro, 2018). Crude fiber content of nongerminated grains differed significantly ($p \leq 0.05$), except those of *Kangara* and Red sorghum. The highest crude fiber content was observed in Red sorghum and *Kangara* nongerminated grains. Nongerminated *Kantana* grains had the lowest fiber content. These results were within the range of 1.7%–7.3% db for pearl millet and of 1.2%–6.6% db for sorghum reported by Serna-Saldivar and Espinosa-Ramirez (2019). Malting resulted in increased fiber contents of *Okashana 2*, *Kantana,* and *Macia*, whereas those of *Kangara* and Red sorghum remained statistically ($p > 0.05$) unchanged. Adebiyi et al., (2017) also observed an increase in crude fiber content in pearl millet after malting.

**Table 4:** Kernel weight, crude fat, protein, fiber and germinative energy of pearl millet and sorghum grains and malt varieties

| Varieties | Kernel weight (g/kernel) | Germinative energy (%) | Crude fat (%, db) | Crude protein (%, db) | Crude fiber (%, db) |
|---|---|---|---|---|---|
| **Pearl millet grains** | | | | | |
| *Okashana 2* | 0.010 ±0.00$^{c}$ | 99.66 ±0.57$^{a}$ | 6.40 ±0.42$^{b}$ | 7.70 ±0.42$^{f}$ | 2.50 ±0.00$^{e}$ |
| *Kantana* | 0.010 ±0.00$^{c}$ | 99.33 ±1.15$^{a}$ | 5.30 ±0.28$^{c}$ | 13.80 ±0.84$^{b}$ | 2.05 ±0.07$^{f}$ |
| *Kangara* | 0.016 ±0.00$^{b}$ | 99.66 ±0.57$^{a}$ | 8.10 ±0.40$^{a}$ | 11.25 ±0.77$^{c}$ | 3.45 ±0.07$^{c}$ |
| **Sorghum grains** | | | | | |
| *Macia* (white) | 0.030 ±0.00$^{a}$ | 97.66 ±2.08$^{a}$ | 2.40 ±0.14$^{fg}$ | 11.95 ±0.63$^{c}$ | 3.05 ±0.07$^{d}$ |
| Red sorghum | 0.030 ±0.00$^{a}$ | 93.00 ±4.35$^{b}$ | 3.60 ±0.28$^{e}$ | 8.10 ±0.56$^{ef}$ | 3.80 ±0.28$^{bc}$ |
| **Pearl millet malt** | | | | | |
| *Okashana 2* | N/A | N/A | 4.55 ±0.21$^{d}$ | 9.15 ±0.07$^{de}$ | 4.70 ±0.14$^{a}$ |
| *Kantana* | N/A | N/A | 4.30 ±0.14$^{d}$ | 17.95 ±0.35$^{a}$ | 3.80 ±0.28$^{bc}$ |
| *Kangara* | N/A | N/A | 4.25 ±0.07$^{d}$ | 11.85 ±0.07$^{c}$ | 3.45 ±0.21$^{c}$ |
| **Sorghum malt** | | | | | |
| *Macia* (white) | N/A | N/A | 2.05 ±0.42$^{g}$ | 14.75 ±0.07$^{b}$ | 4.05 ±0.21$^{b}$ |
| Red sorghum | N/A | N/A | 2.65±0.07$^{f}$ | 9.75 ±0.07$^{d}$ | 3.70 ±0.14$^{bc}$ |

Note: Values are means ±standard deviation; Values with the same letter in a column are not significantly different ($p > 0.05$); db, dry basis; N/A- not applicable; $n = 6$.

*3.3.2. Malting loss, reducing sugars, free amino nitrogen, and amylases activities*

The malting loss, reducing sugars, free amino nitrogen (FAN), and amylases activities results are given in Table 5. There was no statistically significant difference ($p > 0.05$) in the malting loss between the varieties except for Red sorghum, which had the lowest. Nevertheless, *Kantana* had a relatively high malting loss of 30% although it was not statistically significant from the other varieties, except for the Red sorghum. These pearl millet malting losses were higher than those (<10%) reported by Pelembe et al., (2002) but were lower than 35% reported by Badau, Nkama, and Jideani (2006). For sorghum, the malting losses were less than or similar to those reported by Badau et al., (2006) and Iwuoha and Aina (1997). Reducing sugars were not detected in all the nongerminated pearl millet grains. The sorghum nongerminated varieties had small amounts of reducing sugars, which were statistically the same ($p > 0.05$). These findings are indirectly corroborated by the absence of diastatic power reported in pearl millet (Pelembe et al., 2002) and sorghum (Novellie, 1962; Novellie & De Schaepdrijver, 1986). Following malting, reducing sugars were detected in all the sorghum and pearl millet grains. *Macia* had the highest reducing sugars content followed by Red sorghum, then by *Kantana, Okashana 2,* and *Kangara*. The relative order of amounts of reducing sugars in all pearl millet and sorghum malts was positively linked to the respective levels of β-amylase activity (Table 5), except for Red sorghum. This is because it is β-amylase rather than α-amylase which primarily produces fermentable sugars such as maltose (Briggs, 1998; Taylor, 2016a). The α-amylase activity was however much higher than that of β-amylase in malts (Table 5). No amylolytic activity was detected in nongerminated grains irrespective of the cereal (Table 5). This is similar to the findings whereby no α-amylase activity (Pelembe et al., 2002) and only negligible or no β-amylase activity (Pelembe et al., 2002; Taylor & Robbins, 1993) were noted in nongerminated pearl millet and sorghum grains. For malts, the significant ($p \leq 0.05$) order was *Macia* > Kantana = Red sorghum > Kangara = *Okashana 2*. The α-amylase activities in all the malts were higher than 17–27

PMDU/g dry weight malt reported by Pelembe et al., (2002), except for *Okashana 2*, which was within the range. The FAN was detected in both the nongerminated grains and the malts for all the cereals (Table 5). Malting resulted in a significantly increased FAN content. Malted *Kantana* had significantly ($p \leq 0.05$) higher FAN contents than all the other malts. The FAN content of *Macia* and *Kantana* malts was higher, and those of *Okashana 2*, *Kangara,* and Red sorghum were lower than the FAN of 163 mg/100 g db reported by Pelembe et al., (2002) for pearl millet malted under similar conditions.

**Table 5:** Malting loss, reducing sugars, free amino nitrogen and amylase activities of pearl millet and sorghum varieties.

| Varieties | Malting loss (%) | Reducing sugars (mg/g of sample, db) | FAN (mg/100 g of sample, db) | α- Amylase (PMSDU/g of sample, db) | β-Amylase (PMSDU/g of sample, db) |
|---|---|---|---|---|---|
| **Pearl millet grains** | | | | | |
| *Okashana* 2 | 23.6 ±2.1[a] | ND | 4.0 ±2.2[i] | ND | ND |
| *Kantana* | 30.4 ±0.4[a] | ND | 12.6 ±2.3[h] | ND | ND |
| *Kangara* | 24.3 ±1.7[a] | ND | 22.2 ±1.1[g] | ND | ND |
| **Sorghum grains** | | | | | |
| *Macia* (white) | 28.6 ±6.3[a] | 29.6 ±2.7[e] | 17.2 ±2.2[gh] | ND | ND |
| Red Sorghum | 11.0 ±0.7[b] | 30.5 ±2.6[e] | 31.9 ±1.2[f] | ND | ND |
| **Pearl millet malt** | | | | | |
| *Okashana* 2 | N/A | 225.9 ±8.8[d] | 120.5 ±4.3[d] | 24.6 ±1.4[c] | 2.4 ±0.4[b] |
| *Kantana* | N/A | 257.1 ±26.4[c] | 174.9 ±1.2[a] | 57.5 ±2.2[b] | 2.7±0.1[b] |
| *Kangara* | N/A | 206.0 ±26.7[d] | 132.5 ±6.6[c] | 35.9 ±20.8[c] | 1.4 ±0.7[c] |
| **Sorghum malt** | | | | | |
| *Macia* (white) | N/A | 649.0 ±34.6[a] | 167.2 ±1.9[b] | 200.6 ±17.1[a] | 8.3 ±0.2[a] |
| Red Sorghum | N/A | 285.6 ±11.2[b] | 111.8 ±9.2[e] | 55.3 ±1.9[b] | 0.4 ±0.1[d] |

Note: PMSDU = pearl millet or Sorghum diastatic unit; Values are mean ±standard deviation; Values with the same letter in a column are not significantly different ($p > 0.05$); ND- Not detected, db- dry basis, N/A- not applicable; $n = 6$.

### *3.3.3. Phenolics and antioxidant activity*

#### 3.3.3.1. *Tannins by bleach test*

The bleach test was negative for all the pearl millet varieties (*Kantana, Kangara*, *Okashana* 2) and for *Macia* (Figure 7). This suggests that these grains do not have pigmented testa; thus, they do not contain tannins. According to Taylor and Taylor (2008), tannin-containing sorghum grains turn black over the entire surface when subjected to the bleach test, thereby indicating a positive test (presences of pigmented testa) tannin grains. However, the Red sorghum grains after the bleach test in this study turned partially black on the surface (Figure 7). This suggests the possibility of a presence of a pigmented testa. These results were corroborated by the quantitative data of condensed tannins reported in Section 3.3.3.2.

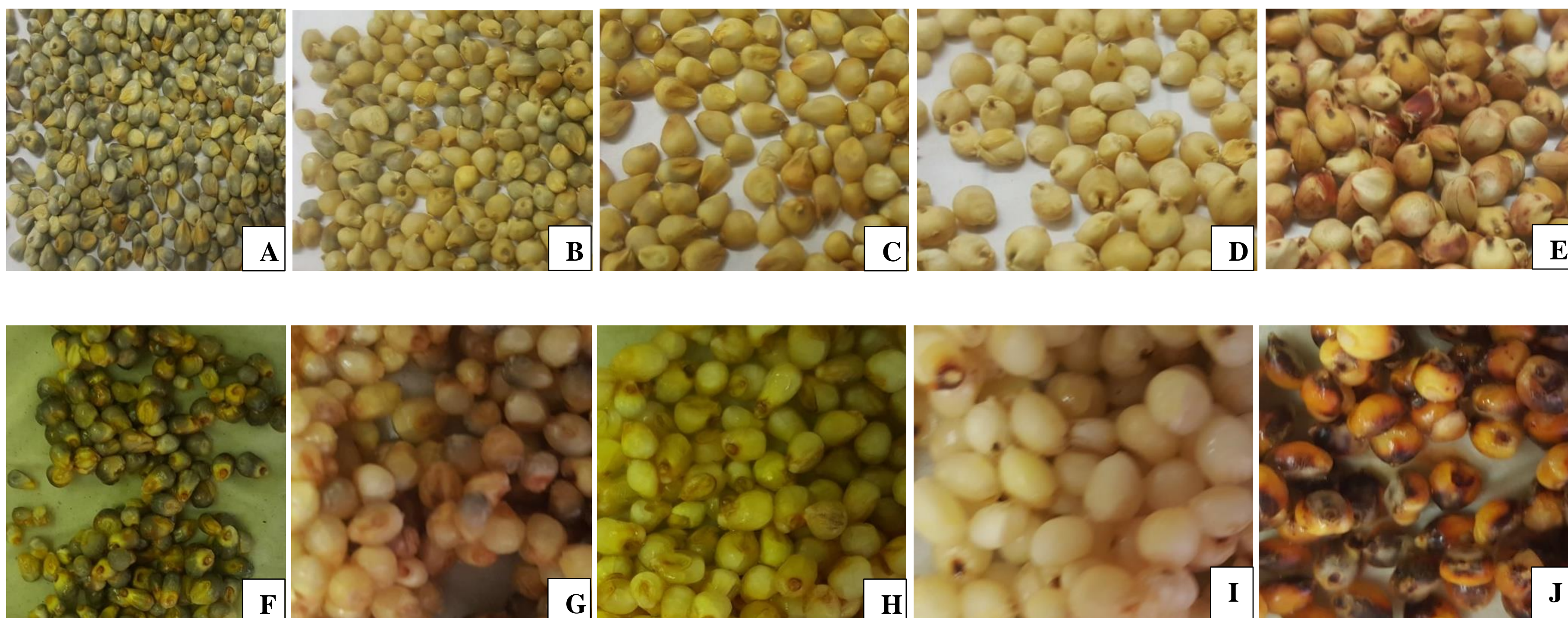


**Figure 7:** Grains before bleach test (left to right) pearl millet A) *Okashana 2*, B) *Kantana*, C) Kangara, D) *Macia* (white) sorghum, E) Red sorghum and after bleach test (left to right) pearl millet F) *Okashana* 2, G) *Kantana*, H) *Kangara*, I) *Macia* (white) sorghum, J) Red sorghum.

*3.3.3.2. Total phenolic (TPC), flavonoid (FC), condensed tannnis (CT) content, and antioxidant activity*

The total phenolic, flavonoid, condensed tannin contents, and antioxidant activity of pearl millet and sorghum varieties are given in Table 6. The TPC of nongerminated grains was as follows: *Kantana* > *Kangara* = *Okashana 2* = Red sorghum > *Macia*. The TPC of all nongerminated pearl millet and sorghum grains was lower than 70.34 mg GAE/g reported by Adebiyi et al., (2017) in nongerminated pearl millet, but was higher than the TPC of 1.387 ±0.013 and 4.128 ±0.009 mg GAE/g for pearl millet and sorghum, respectively reported by Ragaee et al., (2006). These differences in the TPC can be attributed to the use of different extracting solvents and/or extraction conditions, which can yield different levels of phenolics (Dykes & Rooney, 2006; Salar, Purewa, & Bhatti, 2016). Malting significantly ($p \leq 0.05$) decreased TPC for all the grains. A decrease in TPC was also noted by Sehgal and Kawatra (1998) in pearl millet and in sorghum (Iwuoha & Aina, 1997) after malting. However, Adebiyi et al., (2017) found an increase in pearl millet TPC after malting. The FC of nongerminated grains differed significantly ($p \leq 0.05$), except those of *Kangara* and Red sorghum. Malting significantly ($p \leq 0.05$) decreased the FC for all the grains, except in *Macia*, which did not change ($p > 0.05$). Khoddami, Mohammadrezaei, and Roberts (2017) also observed a decrease in FC in sorghum after malting. The decrease in TPC after malting in pearl millet and sorghum can be attributed to the leaching of phenols during steeping and germination or phenolics entering the endosperm together with imbibed water during steeping and germination, and then, polyphenols may bind with proteins and other macromolecules and become less extractable (Taylor & Duodu, 2015). TPC decrease could also be due to the activity of enzymes such as polyphenol oxidase (PPO) during germination whereby polyphenols, the substrate of PPO, might be used up. There was no CT detected in all the pearl millet varieties before and after malting. This is in agreement with McDonought and Rooney (1989) who also detected no CT in pearl millet and with the negative bleach test

(Section 3.8.3.1). However, Lestienne, Besancon, Caporiccio, Lullien-Pellerin, and Treche (2005) found low levels of CT in nongerminated pearl millet. There was also no CT detected in nongerminated *Macia*, but relatively high CT content was found in nongerminated Red sorghum. Based on this, nongerminated *Macia* can be regarded as a tannin-free sorghum, whereas the nongerminated Red sorghum can be regarded as tannin sorghum because its CT values were within the range (10.0–68.0 mg CE/g db), which is regarded as tannin sorghum as suggested by Awika and Rooney (2004). These findings corroborate the Bleach test results (Section 3.3.1). Low levels of CT were observed in *Macia* and those of Red sorghum significantly ($p \leq 0.05$) decreased after malting. The CT contents of Red sorghum and *Macia* malts determined in this study were higher than 20.54 ±0.97 and 0.89 ±0.01 mg CE/sample in Red and white sorghum, respectively, reported by Gaytan- Martinez et al., (2017). The radical scavenging capacities of the nongerminated grains were statistically higher ($p \leq 0.05$) than those of the malts for all the cereals. Following malting, the radical scavenging capacities significantly decreased ($p \leq 0.05$) as follows: *Okashana 2* > *Kantana* > *Kangara* > *Macia*> Red sorghum. The decrease in radical scavenging capacities could be due to the observed decrease in phenolic after malting. Taylor and Duodu (2015) have reviewed some of the reasons why phenolic decrease after malting. Leaching of phenolic compounds during steeping and germination. Phenolic compounds enter the endosperm together with imbibed water during steeping and germination, endosperm and polyphenols may bid with proteins and other macromolecules and become less extractable. Therefore, the above may decrease the activity of phenolic compound scavenging capacities. The capacities for nongerminated and the malts were significantly higher ($p \leq 0.05$) than that of 6.22 mg/ml ascorbic acid, except for Red sorghum.

**Table 6:** Total phenolics content (TPC), flavonoids content (FC), condensed tannins (CT) content and antioxidant activity of pearl millet, sorghum non-germinated grains and malted grains varieties.

| Varieties | TPC (mg GAE/g of sample db) | FC (mg QE/g of sample db) | CT (mg CE/g of sample db) | Scavenging capacity (%) |
|---|---|---|---|---|
| **Pearl millet grain** | | | | |
| *Okashana* 2 | 34.09 ±1.86$^{b}$ | 6.72 ±0.02$^{d}$ | ND | 98$^{a}$ |
| *Kantana* | 38.48 ±0.29$^{a}$ | 7.98 ±0.11$^{a}$ | ND | 97$^{b}$ |
| *Kangara* | 35.07 ±0.54$^{b}$ | 7.66 ±0.18$^{bc}$ | ND | 97$^{b}$ |
| **Sorghum grain** | | | | |
| *Macia* (white) | 28.98 ±3.80$^{c}$ | 5.45 ±0.21$^{g}$ | ND | 97$^{b}$ |
| Red Sorghum | 34.40 ±0.87$^{b}$ | 7.69 ±0.19$^{b}$ | 52.90 ±2.09$^{a}$ | 83$^{c}$ |
| **Pearl millet malt** | | | | |
| *Okashana* 2 | 24.81 ±0.47$^{d}$ | 6.17 ±0.03$^{e}$ | ND | 96$^{c}$ |
| *Kantana* | 28.97 ±3.34$^{c}$ | 7.50 ±0.16$^{c}$ | ND | 95$^{d}$ |
| *Kangara* | 26.14 ±2.78$^{cd}$ | 5.64 ±0.89$^{f}$ | ND | 94$^{e}$ |
| **Sorghum malt** | | | | |
| *Macia* (white) | 24.72 ±0.87$^{d}$ | 5.62 ±0.05$^{fg}$ | 4.38 ±0.29$^{c}$ | 89$^{f}$ |
| Red Sorghum | 29.13 ±2.69$^{c}$ | 6.21 ±0.07$^{e}$ | 23.67 ±2.55$^{b}$ | 76$^{h}$ |
| Vitamin C$^{*}$ | | | | 84$^{g}$ |

Note: GAE- gallic acid equivalent; QE- quercetin equivalent; CE- catechin equivalent; Values are mean ± standard deviation; Values with the same letter in a column are not significantly different ($p$>0.05); db- dry basis; ND- Not detected; *Vitamin C 6.22 mg/mL; $n$ = 6.

### 3.4. Conclusions

All the pearl millet varieties and *Macia* had higher germinative energy values than Red sorghum. Malted pearl millet varieties had the same but significantly higher crude fat contents than the sorghum varieties. *Kantana* malt had the highest protein content while *Okashana 2* and Red sorghum had the lowest. *Okashana 2* malt had the highest fiber content than all the other malts. For sorghum varieties, *Macia* had the highest malting qualities (FAN, reducing sugars, α- and β-amylases activities), whereas for pearl millet, *Kantana* had the highest malting qualities. Therefore, *Macia* and *Kantana* can be candidates for industrial malting for brewing non-alcoholic and low alcoholic beverages such as *Oshikundu* and *Omalovu* (opaque beer). For the delivery of phenolics, *Kantana* and Red sorghum had higher amounts of phenolic compounds and can potentially be vectors of delivering phenolics into human diets.

## 3.5. References

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## Chapter 4

**Mycotoxins analysis of pearl millet (*Pennisetum glaucum (L.) R. Br*) and sorghum (*Sorghum bicolour (L.) Moench*) malts from Namibia**

Chapter 4 addresses objective 2.

To determine the effect of malting on malts safety of pearl millet and sorghum: total microbial quality and mycotoxins.

**Abstract**

Pearl millet and sorghum form part of daily foods in Africa. Preparation of these cereal foods involves malting, fermentation and milling. The traditional malting process of pearl millet and sorghum grains is mostly intended for making weaning food, nonalcoholic beverages and opaque beer. During malting, pearl millet and sorghum just like other cereals get contaminated by mycotoxins. Food intended for human consumption must be safe when it comes to mycotoxins. Malts for *Oshikundu* brewing are not excluded. Pearl millet (*Okashana2, Kantana,* and *Kangara*) and sorghum (*Macia* and Red sorghum) grains were malted for brewing *Oshikundu*. 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 in malts irrespective of the cereal. However, detected mycotoxins were below the legal limit set by the European Commission. This suggests that these malts may not be of safety concern when it comes to coliforms and mycotoxins although bacteria and fungi load is high. Therefore, these pearl millet and sorghum malt can be rendered safe from mycotoxins as well as coliforms and thus can be used in *Oshikundu* fermentation.

Keywords: mycotoxins, malt, pearl millet, sorghum, *Oshikundu*

## 4.1. Introduction

Cereals are produced mainly for human consumption, especially in Africa (Taylor, 2016a). The top pearl millet (*Pennisetum glaucum (L.) R.Br.*) producers in Africa are Niger, Mali, Burkina Faso, Nigeria and Senegal (Taylor, 2016b). Meanwhile, Nigeria, Ethiopia, Burkina Faso and Sudan (former) are the top producers of sorghum (*Sorghum bicolor (L.) Moench*) (Taylor, 2016a). Various products from pearl millet and sorghum exist. They are processed through malting, fermentation and milling. African traditional products from pearl millet and sorghum malt are common. Even though malt uses is possibly limited to nonalcoholic, alcoholic beverages and opaque beers brewing. Malt products from Namibia include *oshikundu/ontaku* (nonalcoholic), *omalovu giilya* (opaque beer) (Embashu & 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 household settings. Therefore, malts can be contaminated with mycotoxins. Mycotoxins of toxicological significance in cereal includes aflatoxins, ochratoxin, zearalenone, fumonisin, trichothecenes (T-2 and HT-2 toxins, deoxynivalenol, nivalenol) (Patriarca & Pinto, 2017) tremogenic toxins and ergot alkaloids (Hussein & Brassel, 2001). Different researchers have adequately established contamination of sorghum malt with aflatoxin, fumonisin, deoxynivalenol, zearalenone (Matomba, Monjerezi, Khonga & Lakudzala, 2011; Nafuka, Misihairabgwi, Bock, Ishola, Sulyok & Krska 2019; Doufour & Melotte, 1992; Lefyedi, Marais, Dutton & Taylor, 2005; Misihairabgwi, Ishola, Quaye, Sulyok & Krska, 2018; Chala, Taye, Ayalew, Krska, Sulyok & Logrieco, 2014). However, limited work is available on pearl millet malts possible contamination by mycotoxins, especially those intended for brewing *Oshikundu*. Misihairabgwi, Ishola, Quaye, Sulyok, and Krska (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 contamination in malts of pearl millet and sorghum for *Oshikundu* fermentation.

## 4.2. Material and methods

### *4.2.1. 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. The varieties of pearl millet grains used in this study were *Okashana 2* (SDMV 93032), *Kantana* (landrace), and *Kangara* (SDMV 92040). For sorghum, it was *Macia* (white, SDS 3220) and a landrace is known in Oshiwambo language as '*Iilyawala iitiligane'* (which literally means "sorghum grains that are red"). The sorghum landrace is herein referred to as Red sorghum variety. The grains were grown in a sandy-loam soil under irrigation of 16 mm per hour every 3 days on average till the fruiting stage (Megameno Amutenya, Agricultural Research Officer, Omahenene Research Station, personal communication), except when it rained. The cultivation field was fertilized with 300 kg per hectare of NPK (basal fertilization) during planting. At about 3 leaves stage and also before flowering, 200 kg per hectare of urea was applied. At the booting stage, superphosphate was also applied at 200 kg per hectare. The grains were obtained within one month of harvest from Omahenene Research Station where they were stored in shaded silos at ambient temperatures (apparently 20–30°C). The grains were then kept at 7–10°C in a cooled room and were malted within one month of storage in this cooled room (chapter 3).

### *4.2.2. Malting*

Pearl millet and sorghum grains were malted according to Pelembe, Dewar and Taylor (2002) with some modifications by Embashu and Nantanga, 2019 as indicated in chapter 3.

#### *4.2.2.1. 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.

#### *4.2.2.2. 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.

#### *4.2.2.3. 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).

### *4.2.3. Bacterial and fungal enumeration*

All analysis was done on nongerminated grains and malts of pearl millet and sorghum or otherwise stated. Total plate count, lactic acid bacteria, yeast and mould enumeration was done following work by Lefyedi, Marais, Dutton and Taylor, (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).

*4.2.4. Detection of mycotoxins*

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. Procedure is 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.

*4.2.5. 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, 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).

## 4.3. Results and discussions

*4.3.1. Bacterial and fungal enumeration*

Malts of pearl millet and sorghum bacterial, yeast and mould load are given in Table 7. Cereals nongerminated grains had 30 or less colony count, no growth on PCA, MRS and PDA plates. MacConkey agar had no growth observed for nongerminated grains and malts in both cereals. This would potentially indicate the safety of the malt from *Salmonella* spp., *Shigella* and coliforms contamination. The aerobic plate count load was in this order *Kangara*

$>$ Red Sorghum $>$ *Okashana 2* $>$ *Macia* $>$ *Kantana*. Further, the aerobic plate load significantly differed ($p \leq 0.05$) between the cereals. Malts aerobic plate count load in the two cereals were above the limit of Southern African sorghum malt specification of 6.3 Log cfu/g or ($2 \times 10^7$ cfu/g) as noted by Lefyedi, Marais, Dutton and Taylor (2005). Therefore, this indicates an unacceptable high aerobic plate count load. Although coliforms were not detected in cereal nongerminated grains and malts, the aerobic plate count load should be taken as a safety concern. Lactic acid bacteria load was not statistically significant ($p > 0.05$) between malts of *Okashana 2, Kangara* and *Macia*. There was no statistical difference ($p \geq 0.05$) between the yeast and mould load of *Kantana* and *Macia* malts. While malts of *Okashana 2, Kangara* and Red Sorghum yeast and mould load was the statistically the same ($p > 0.05$) same. The highest aerobic plate count, lactic acid bacteria, yeast and moulds were observed from *Kantana* malts followed by *Macia*. The above can perhaps be linked to *Kantana* and *Macia* malts reducing sugar content in chapter 3 (Table 5) of 257.1 ±26.4 and 649.0 ±34.6 mg/g of sample db, respectively. The reducing sugars content in the malt and the absence in nongerminated grains could potentially influence microorganism growth, thus observations. A similar microbial load has been reported in sorghum malts (Lefyedi et al., 2005; Ilori, Fessehatzion, Olajuyigbe, Babalola & Ogundiwin, 1991; Thaoge, Adams, Sibara, Watson, Taylor & Goyvaerts, 2003). Lefyedi and Taylor (2006) showed and 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 mould load during malting of both cereals.

**Table 7:** Mean bacteria, yeast and mould 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 moulds; *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.

*4.3.2. Detection of mycotoxins*

Mycotoxins from nongerminated grains and malts of pearl millet and sorghum are shown in Table 8. 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, Hmaissia-Khlifa, Ghorbel, Maaroufi and Hedili (2008) and Chala, Taye, Ayalew, Krska, Sulyok and Logrieco (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, Ishola, Quaye, Sulyok and Krska (2018) who 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. However, aflatoxin was lower than those reported in sorghum grains 2.35 ±0.65 µg/kg (Matumba, Monjerezi, Khonga & Lakudzala, 2011) as well as in malts of pearl millet and sorghum (Misihairabgwi et al., 2018). *Macia* malt had the highest ($p \leq 0.05$) deoxynivalenol contamination. There was no statistically significant difference ($p > 0.05$) in deoxynivalenol from the nongerminated grains between the cereals and Red sorghum malt. Furthermore, there was no statistically significant difference ($p > 0.05$) in deoxynivalenol within the malts of pearl millet varieties. The detected values are lower than those reported in sorghum grains (Ayalew, Fehrmann, Lepschy, Beck & Abate,

2006; Chala, Taye, Ayalew, Krska, Sulyok & Logrieco, 2014), sorghum malt (Lefyedi et al., 2005) and finger millet (Chala, Taye, Ayalew, Krska, Sulyok & Logrieco, 2014). Following malting, deoxynivalenol decreased however, there was no statistically significant difference ($p > 0.05$) between the varieties and cereals. *Macia* malt had the highest ($p \leq 0.05$) fumonisin B1. There was no statistically significant difference ($p > 0.05$) in fumonisin B1 contamination from the nongerminated sorghum and those of pearl millet varieties. Nongerminated grains and malts of sorghum fumonisin B1 were 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. Nongerminated pearl millet grains and their respective malts fumonisin B1 was lower than the range of 0.1-3060 µg/kg reported by Misihairabgwi et al., (2018) in pearl millet. Ochratoxin A was only detected from nongerminated grains of Red sorghum. These were however above the regulated limits of 5 µg/kg as declared by The European Commission (Commission Regulation N0 1881/2006, 2006). Following malting, ochratoxin A was not detected in Red sorghum malt. Zearalenone was not detected in cereals nongerminated grains and their respective malts, except in Red sorghum malt. However, there are reports of zearalenone in sorghum (Lefyedi et al., 2005; Misihairabgwi et al., 2018) and pearl millet (Houissa et al., 2019; Misihairabgwi et al., 2018). Overall, there was no statistically significant difference ($p > 0.05$) within the varieties and between cereals for those that were contaminated with mycotoxins. There is a low concentration of mycotoxins that were detected in some cereals malt. However, they are below the legal limits set by the European Commission and those reported by similar studies. The steeping step of wet and air-rest in this study could perhaps contribute to the above observation that is not employed by similar studies. Misihairabgwi et al., (2018) did not detect regulated mycotoxins in *Oshikundu* fermentation using contaminated malts. However, non-regulated metabolites were detected.

**Table 8:** 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 | $1.13 \pm 0.32^{ab}$ | $0.11 \pm 0.02^{b}$ | ND | ND |
| Kantana | ND | $2.13 \pm 0.33^{ab}$ | $0.29 \pm 0.26^{ab}$ | ND | ND |
| Kangara | ND | $4.78 \pm 0.40^{ab}$ | $0.17 \pm 0.16^{b}$ | ND | ND |
| **Sorghum grains** | | | | | |
| Macia (white) | ND | $1.33 \pm 0.18^{ab}$ | $0.26 \pm 0.04^{b}$ | ND | ND |
| Red sorghum | $0.02 \pm 0^{a}$ | $2.96 \pm 0.50^{ab}$ | $0.19 \pm 0.15^{b}$ | $7.53 \pm 0.42^{a}$ | ND |
| **Pearl millet malt** | | | | | |
| Okashana 2 | $0.015 \pm 0^{b}$ | $0.39 \pm 0.06^{b}$ | $0.27 \pm 0.15^{b}$ | ND | ND |
| Kantana | ND | $0.23 \pm 0^{b}$ | $0.19 \pm 0.02^{b}$ | ND | ND |
| Kangara | ND | $0.28 \pm 0.09^{b}$ | $0.09 \pm 0.02^{b}$ | ND | ND |
| **Sorghum malt** | | | | | |
| Macia (white) | $0.01 \pm 0^{c}$ | $12.49 \pm 16.99^{a}$ | $0.54 \pm 0.05^{a}$ | ND | ND |
| Red sorghum | $0.02 \pm 0^{a}$ | $0.88 \pm 0.31^{ab}$ | $0.21 \pm 0.03^{b}$ | ND | $0.03 \pm 0^{a}$ |

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; $n = 2$.

### 4.4. Conclusions

Malts of pearl millet and sorghum had an unacceptable high microbial load. However, *Salmonella* spp., *Shigella* and coliforms were not detected from nongerminated grains and their malts and this suggests that they were possibly 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. Therefore, the cereal malts are not of concern when it comes to regulated mycotoxins. None the less, this does not guarantee the safety of *Oshikundu* on coliforms and mycotoxins since there is scanty literature on this. Safety can only be ensured when all processes such as treatment, handling of ingredients, fermentation flow process as well as the final product *Oshikundu* are investigated and set. These cereal malts are potentially safe from mycotoxins but with a high bacteria and fungi load. Since possible pathogenic microbes were not detected, it can be concluded that the malt can be used for brewing *Oshikundu*.

### 4.5. References

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## Chapter 5

## Identification of lactic acid and yeast from *Oshikundu* using 16S and 26S rDNA gene sequencing

Chapter 5 addresses objective 3.

To identify lactic acid bacteria (LAB) and yeast from *Oshikundu.*

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**Abstract**

*Oshikundu* is brewed from fermentation of pearl millet (*Pennisetum glaucum* (L.) R. Br) meal commonly known as *mahangu*, sorghum (*Sorghum bicolor* (L.) Moench)/pearl millet malt, water and/or bran (from pearl millet). Consumption of this brew is daily in many households mostly in the Northern Namibia. *Oshikundu* samples were collected from north central and central region of Namibia. Isolation of lactic acid bacteria (LAB) was carried out on deMan Rogosa Sharpe (MRS), M17 and Rogosa agars, however malt extract and potato dextrose agars were used for yeast. DNA from isolates was amplified using standard polymerase chain reaction (PCR) and PCR products were sequenced thereafter. Sequence was multiple aligned through Basic Local Alignment Search Tool (BLAST) and compared to the known DNA sequences in databases found in National Center for Biotechnology Information (NCBI)/GenBank database for LAB and yeast identification from *Oshikundu*. Identified LAB were *Lactobacillus plantarum, L. pentosus, L. fermentum, L. acidifarinae, L. spicheri, L. namurensis, L. zymae, L. brevis, Leuconostoc gurlium, L. delbrueckiee* subs., *bulgaricus, L. buncheri* and *Pediococcus acidilactici*. However, for yeast were *Saccharomyces spp*., and *Pichia kudriavzevii*. From the results, it appears that the dominant LAB is mostly heterofermentative and yeast likely to produce ethanol in *Oshikundu*. The study contributes to the scanty information of possible LAB and yeast that may be responsible for *Oshikundu* fermentation.

Keywords: *Oshikundu, mahangu* (pearl millet), lactic acid bacteria, yeast

## 5.1. Introduction

There is an increasing demand for healthy food, driven by population growth and rising affluence globally. This demand will impose severe challenges on the agro-processing sector, agricultural and social systems (Mace et al., 2013). African dietary ethos consist of fermented and non-fermented foods and beverages that are from sorghum, millet, maize, cassava, wild legume seeds, tubers, meat and milk (Tamang & Samuel, 2010). Most foods and beverages from sorghum and millet in Africa have undergone a lactic acid fermentation or a malting process or both during production, an important characteristic of these products (Belton and Taylor, 2004). Fermentation being one of the oldest, economical forms of food preservation and production (Blandino et al., 2003; Chelule et al., 2010) is of importance as a technology which is affordable and also combating foodborne diseases (Franz et al., 2014). Recently, cereals gained a lot of attention as raw material for non-alcoholic and functional beverages production. Due to their high fiber, whole or multi-grain content cereal-based beverages have become a greater part of the new better-for-you foods and beverages with desirable benefits (Dongmo et al., 2016). The most desirable benefits resulting from cereals are their high nutritional value and bioactive compound content (Adil et al., 2012). Lactic acid bacteria (LAB) and yeast in fermented food and beverages across Africa from sorghum and millet such as *ogi, doro, injera, uji, togwa, busa, bushera, amgba, bel-saalga, bogbe, d`egu`e, kenkey, kisra, kamu-zaki, hulumur, mwenge, kisra, kaffir beer* have been reviewed (Blandino et al., 2003; Franz et al., 2014; Adebiyi et al., 2016). Lactic acid fermented malt-based beverages are non-alcoholic, with low pH value (3.5-4.5) and produced by the fermentation of cereals, cereal substrates or blends by LAB strains. Available lactic acid fermented cereal beverages include yogurt-like cereal functional beverages and traditional cereal fermented beverages (Corbo et al., 2014). Some of these lactic acid fermented malt-based beverages serve as food and thirst quencher such as *oshikundu* from Namibia (Embashu, 2014).

*Oshikundu* is made from fermentation of pearl millet (*Pennisetum glaucum* (L.) R. Br) meal (*uusila/oufila*) commonly known as *mahangu* in Namibia, sorghum (*Sorghum bicolor* (L.) Moench)/pearl millet malt, water and/or bran (from pearl millet). This brew is made in many households across the country and found its way to the informal market. The social aspects and processing methods were reported by Embashu et al., (2013). The brewing of *Oshikundu* takes place at home on a small scale, with recipe passed on from generation to generation (Embashu et al., 2013). *Oshikundu* is perceived as a non-alcoholic and possibly a lactic acid bacteria fermented beverage, thus it is consumed by everyone in a household. However, Embashu (2014) documented alcohol below 2% *v/v* in *Oshikundu* and gentle bubble during fermentation which would suggest possible yeast fermentation as well. Although pearl millet is the main staple and commercial crops for the majority of people in Northern Namibia, there is no sufficient documentation on their nutritional, fermentation process and microbiota associated with their fermented products. The current study aims at isolating and identifying the LAB and yeast associated with *Oshikundu* fermentation by 16S and 26S rDNA gene sequencing and multiple alignments through Basic Local Alignment Search Tool (BLAST).

## 5.2. Materials and methods

### *5.2.1. Sampling*

*Oshikundu* samples (500 *mL*) were collected in 500 *mL* sterile plastic bottles from the Northern-Central (Omusati, Oshana, Oshikoto) and Central (Khomas) region in Namibia, transported in a portable fridge and stored below $4^{o}C$ until further microbiological analysis.

*5.2.2. Isolation of lactic acid bacteria and yeast*

Lactic acid bacterial isolation was done following work by Tanguler and Erten (2012), and V¨akev¨ainen et al. (2018) with few modifications, no supplement or antibiotic was used on the isolation medium. *Oshikundu* samples were diluted ($10^{-1}$ to $10^{-6}$) in peptone water buffered (Biolab, Merck, Germany) and mixed thoroughly using a vortex. The 100 $\mu L$ aliquots of these mixtures were inoculated on deMan Rogosa Sharpe (MRS) (De Man et al., 1960), M17 (Therzaghi & Sandine, 1975) and Rogosa agar and incubated aerobically at $37^0$ $C$ for 24 hours. After the first incubation period, colonies were further purified by successive streaking on MRS (Biolab, South Africa), M17 (Merck, Germany) and Rogosa agar (Biolab, South Africa) respectively. White/milky in colour colonies were then subjected to enzymatic test (catalase) before DNA extraction. Catalase test was done by dissolving colonies from each selective media in a drop of 3% hydrogen peroxide placed on a clean slide (Harrigan, 1998) and only isolates that gave a negative reaction to catalase test were extracted for DNA. Meanwhile, yeast isolation was done following work by Liu et al., (2017) with modifications, no supplement or antibiotic were used on the isolation medium and malt extract and potato dextrose gar were used instead of yeast extract peptone dextrose. Using serial dilution ($10^{-1}$ to $10^{-1}$) samples in sterile peptone water buffered (Biolab, Merck, Germany) and mixed thoroughly using a vortex. To 100 $\mu L$ aliquots of the mixture was inoculated on malt extract agar (MEA) and potato dextrose agar (PDA) before aerobic incubation at $25^oC$ for 48 hours. Colonies were further successive streak on respective agar before DNA isolation.

*5.2.3. Lactic acid bacteria and yeast DNA extraction*

DNA extraction from yeast (11 isolates) and bacteria colonies (28 isolates) was done following the standard phenol/chloroform method (Neumann et al., 1992) improved by Cheng and Jiang (2006). Bacteria and yeast, colonies were transferred into a clean 1.5 $mL$ micro centrifuge tubes. The colonies were suspended in 200 $\mu L$ TE buffer (10$mM$ Tris/HCl, 1$mM$ EDTA, pH 8.0), and 100 $\mu L$ Tris-saturated phenol (pH 8.0) was added to all sample

tubes before vortex mixing for 60 seconds to lyse cells. For yeast samples, the suspension was sonicated for 10 minutes at $5^{o}C$ after the addition of tris-saturated phenol. The samples were subsequently centrifuged at 4000 *rpm* for 5 min at $4^{o}C$ to separate the aqueous phase from the organic phase. The 160 $\mu L$ upper aqueous phase was transferred to a clean 1.5 *mL* micro centrifuge tube. To this 40 $\mu L$ TE buffer was added to make 200 $\mu L$, mixed with 100 $\mu L$ chloroform and centrifuged for 5 min at 4000 *rpm* at $4^{o}C$. Lysate was purified by chloroform extraction until a white interface was no longer present. The 160 $\mu L$ upper aqueous phase was transferred to a clean 1.5 *ml* micro centrifuge tube and 40 $\mu L$ TE was added. Chloroform 100 $\mu L$ volumes was added to the tube, mixed well and centrifuged for 5 min at 4000 *rpm* at $4^{o}C$. A volume of 150 $\mu L$ upper aqueous phase was transferred to a clean 1.5 *mL* micro centrifuge tube. The aqueous phase contained purified DNA and was stored at $-20^{o}C$ for further use. The quantity and purity of extracted DNA from bacteria and yeast samples (1.5 $\mu L$) was analysed on a ND-Nanodrop (2000c, Thermo Fisher, Germany) spectrophotometer.

*5.2.4. DNA amplification using Polymerase Chain Reaction (PCR)*

DNA that was extracted from bacteria was amplified using standard PCR by two set of primers: Ec338f (5'-ACT CCT ACG GGA GGC AGC AGC AG-3') corresponding to nucleotide positions 349 to 368 and Ec518r (5'-ATT ACC GCG GCT GCT GG-3') targets the V3 region of the 16S ribosomal DNA with expected 400 bp corresponding to nucleotide positions 529 to 545 and acetic acid and lactic acid bacteria primers:WBAC1 (5'-GTC GTC AGC TCG TGT CGT GAG A-3') corresponding to nucleotide positions 1069 to 1090 and WBAC2 (5'-CCC GGG AAC GTA TTC ACC GCG-3'), corresponding to nucleotide positions 1374 to 1394 on the *L. plantarum* 16S rDNA gene sequence (GenBank accession

number AJ271852) for both set of the above primers (Lopez et al., 2003) targets the V7 to V8 regions of the 16S rDNA genes which produce amplicons of approximately 330 bp (Camu et al., 2007; Di Cagno et al., 2014). The PCR reactions for WBAC1 and WBAC2 were run for 30 cycles: Initial denaturation at 95$^{o}C$ for 5 min and final extension at 72$^{o}C$ for 5 minutes, denaturation was at 95$^{o}C$ for 60s, annealing at 67$^{o}C$ for 30s and extension at 72$^{o}C$ for 60s (Lopez et al., 2003). While for Ec338f and Ec518r the PCR reactions were run for 25 cycles: Initial denaturation at 94$^{o}C$ for 5 min and final extension at 72$^{o}C$ for 10 minutes, denaturation was at 94$^{o}C$ for 60s, annealing at 52$^{o}C$ for 60s and extension at 72$^{o}C$ for 60s (Amper et al., 1999; Omar & Amper, 2000; Lopez et al., 2003).

The DNA extracted from yeast was amplified by PCR using the forward primer NL1 (5'-GCC ATA TCA ATA AGC GGA GGA AAA G-3') and the reverse primer LS2 (5'- ATT CCC AAA CAA CTC GAC TC-3') targets the D1 to D2 region of the 26 ribosomal DNA corresponding to nucleotide positions 266 to 285 on the S. cerevisiae 26S RNA gene (GenBank accession number M19229) (Cocolin et al., 2000). The reactions were run for 30 cycles: Initial denaturation at 95$^{o}C$ for 5 min and final extension at 72$^{o}C$ for 7 minutes, denaturation was at 95$^{o}C$ for 60s, annealing at 52$^{o}C$ for 45s and extension at 72$^{o}C$ for 60s (Cocolin et al., 2000). The PCR was set to hold at 4$^{o}C$. The reaction master mix for both bacteria and yeast was performed in a total volume of 25 $\mu L$ for each reaction with the following reagents: PCR master mix 12.5 $\mu L$ (1x), forward and reverse primers (100 $\mu M$) for yeast and bacteria, 2.5 $\mu L$ (0.5 $\mu M$) and 1.0 $\mu L$ (0.2 $\mu M$) each respectively, DNA template 5 $\mu L$ ($\leq$ 500 $ng$) and nuclease free water 2.5 $\mu L$. PCR products were run on 2% agarose gels, stained with ethidium bromide and the DNA bands on the agarose gel were visualized using a UV trans-illuminator.

The un-purified PCR-products were sent for sequencing to Inqaba biotech (Pretoria, South Africa). The obtained sequences from Inqaba biotech (Pretoria, South Africa) were edited with Chromas lite (Technelysium Pty Ltd, Australia). The cleaned sequences in Federal Acquisition STreamlining Act (FASTA) format was subjected to Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1990) for similarity identification. Multiple alignment and sequence similarity with that of available sequences of reference strains from GenBank database found in National Centre for Biotechnology Information (NCBI) (www. ncbi.nlm.nih.gov).

## 5.3. Results and discussions

### *5.3.1. LAB and Yeast PCR*

After DNA isolation and quantification (section 5.2.3), the isolated DNA was used in the standard PCR (section 5.2.4) to amplify the targeted gene fragments for LAB and yeast. The LAB PCR (Figure 8) product analysed through gel electrophoresis on a 2% agarose gel shows the presence of two different amplicons; the amplicons generated with Ec338f-Ec518r primer combination was between 400-300 bp (lane C,D,G,H,I,J,L,M and N) and while that which was amplified with WBAC1-WBAC2 primer combination (O,P,Q,R,S,T,U,V,W,X,Y,Z) for LAB was 350-300 bp. Meanwhile the yeast PCR results (Figure 9) in lane A, D, G, H, I and K show amplified PCR products with 300-200 bp size amplified using the NL1-LS2 primer. The length of base pair given by the amplification of Ec338f-Ec518r and WBAC1-WBAC2 primer combination is in agreement for the expected base pair as reported by Lopez et al. (2003).

**Figure 8:** PCR products for lactic acid bacteria separated using gel electrophoresis on a 2% agarose gel. M: 100bp DNA ladder, lane C,D,G,H,I,J,L,M and N (Ec338f-Ec518r primer), O,P,Q,R,S,T,U,V,W,X,Y,Z (WBAC1-WBAC2 primer).

**Figure 9:** PCR products for yeast separated using gel electrophoresis on a 2% agarose gel. M: 100bp DNA ladder, lane A, D, G, H, I and K amplified PCR products with 300-200 bp size amplicons with NL1-LS2 primer.

*5.3.2. LAB and Yeast identification*

Identification of LAB and yeast in *oshikundu* was done through multiple alignment of the sequence by comparing to the GenBank (Figure 10). The identified LAB species from *oshikundu* that have ≥ 98% similarity includes *L. plantarum, L. pentosus, L. acidifarinae, L. paraplantarum, L. spicheri, L. namurensis, L. zymae, L. fermentum, L. brevis, Leuconostoc gurlium, L. delbrueckii* subsp *bulgaricus, L. buncheri* and *Pediococcus acidilactici* (figure 11). The LAB *L. fermentum, L. pentosus, L. plantarum*, in *oshikundu* have been previously reported by Embashu (2014) using API 50 CH/CHL medium, in which *L. delbrueckii subsp. delbreckii* was reported meanwhile *L. delbrueckii subsp. bulgaricus* was identified in this study. Similar

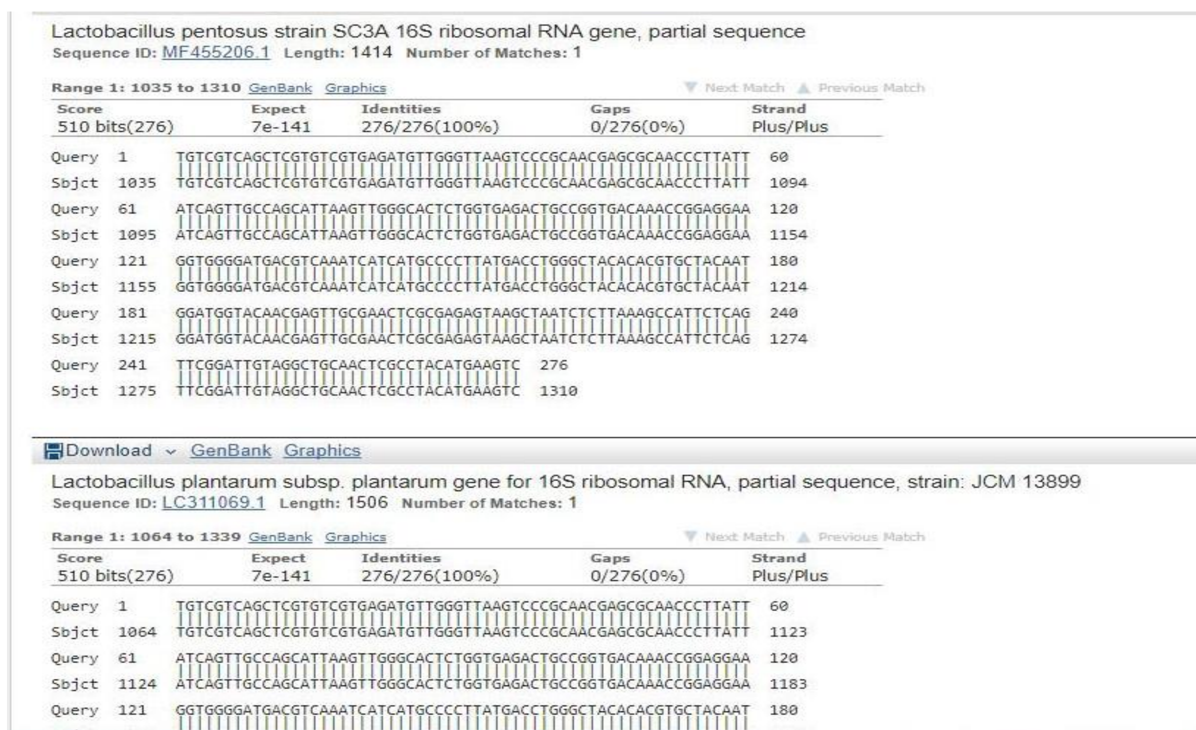

**Figure 10:** Multiple alignment and sequence similarity with that of available sequences (from *oshikundu* isolates) of reference strains from GenBank database of NCBI.

LAB *L. plantarum* and *L. brevis* (Lopez et al., 2003), *L. fermentum* and *P. acidilactici* (Vieira-Dalod`e et al., 2007) were also reported in fermented product using different identification molecular methods.

The LAB from *oshikundu* was clustered in four groups (Figure 4) based on their similarity. The overall sequence similarity within different strains of a species ranged between 0.99 to 0.7 (boot strapping). The closest (0.99) was obtained in group III with a lower similarity obtained in divergence of group IV (Figure 11). There were a low number of changes per 100 nucleotides site which was 0.2 (20%) (dissimilarity) among the groups, which showered a 0.99 value (boot strapping) in the divergence blanches. Given this, a high similarity value (0.96 and 0.91) in group I and II, may suggest a strong exclusion of any other to the node clusters.

Meanwhile for yeast only *Saccharomyces cerevisiae, Saccharomyces paradoxus* and *Pichia kudriavzeii* were identified. *S. cerevisiae* (Stringini et al., 2009; Greppi et al., 2013) from similar fermented product have beer also reported, although using different molecular methods. The pH of *Oshikundu* was reported to be between pH 3.33-3.60 by Embashu (2014).

Generally, yeast grow on a wide range of pH but a pH 4-4.5 is favoured and LAB grow at pH lower than 4 (Taylor, 2016). According to the review by Blandino et al., (2003) some of the fermented beverages, food, weaning food for baby, gruel made from sorghum and millet to be fermented by *Lactobacillus, Leuconostoc* and *Saccharomyces* spp. that are similar to fermenting microorganisms found in *oshikundu*. These millet and sorghum fermented products include boza (*Lactobacillus, Saccharomyces cerevisiae, Leuconostoc*), buza (*Lactobacillus, Saccharomyces*), burukutu (*Saccharomyces cerevisiae, S. chavelieri, Leuconostoc mesenteroides, Candida, Acetobacter*), chikokivana (*Saccharomyces cerevisiae*), doro (yeasts and bacteria), kaffir beer (yeasts, LAB) merissa (*Saccharomyces*), ogi (*Ped. pentosaceus, L. fermentum, L. plantarum, Saccharomyces cerevisiae, Candida kruseii*) (Franz et al., 2014) and sorghum beer (LAB and yeast) (Blandino et al., 2003). The identified bacteria from *Oshikundu* of which are hetero-fermentative (Adams & Moss, 2008;

Pr¨uckler et al., 2015) that are likely to produce multiple by-products such as lactic acid, ethanol/carbon dioxide ($CO_2$), acetic acid and possibly other organic acids during *Oshikundu* fermentation. The Saccharomyces cerevisiae may also produce ethanol and carbon dioxide ($CO_2$) during fermentation of *Oshikundu*. The bubbling and ethanol (< 2% *v/v*) reported by Embashu (2014) could possibly be the end product of fermentation by the identified yeast: *Saccharomyces* spp., and *Pichia kudriavzevii* hetero-fermentative bacteria such *Leuconostoc L. brevis, L. fermentum, L. plantarum, L. pentosus* (Adams & Moss, 2008; Pr¨uckler et al., 2015). Nonetheless, it remains unclear whether the alcohol reported by Embashu (2014) is the by-product of hetero-fermentative bacteria or yeast (*Saccharomyces*) in *Oshikundu* or product of both, thus further investigations are required.

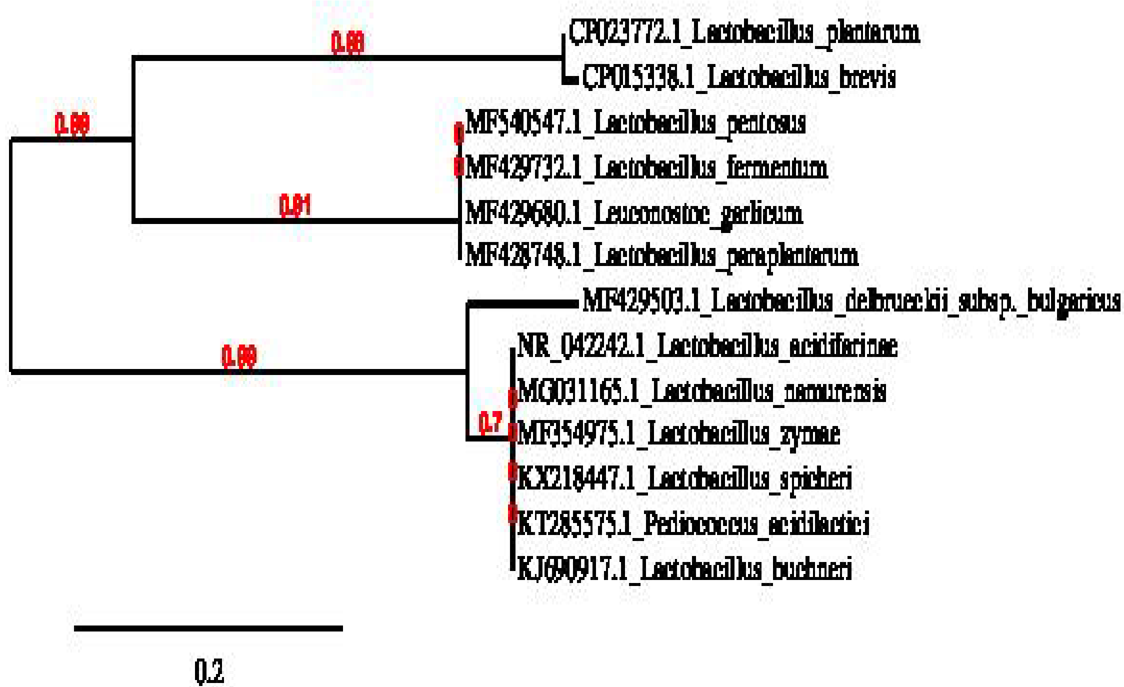


**Figure 11:** Phylogenetic tree for the identified LAB strains showing the genetic relatedness of LAB from *Oshikundu*, obtained from multiple alignment and sequence similarity with that of available sequences of reference strains from GenBank database of NCBI, with number of changes per 100 nucleotide sites 0.2.

## 5.4. Conclusions

Like most of the traditional fermented products, *Oshikundu* appears to be the result of fermentation by LAB and *Saccharomyces cerevisiae*. Nonetheless it also appears that hetero-fermentative LAB is responsible for its alcoholic content. The interaction between LAB and yeast in *oshikundu* need further investigation.

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# Chapter 6

## Effect of pre-cooking pearl millet (*Pennisetum glaucum*) flour on the reduction of dregs in *Oshikundu*

Chapter 6 addresses objective 4.

To determine the effect of pre-cooking on dreg reduction in brewing *Oshikundu*.

Chapter is published in the the International Science and Technology Journal of Namibia.

**Abstract**

*Oshikundu* or *Ontaku* is a low- or non-alcoholic fermented drink commonly brewed in over half of Namibian households on a daily basis. It is prepared using water, pearl millet (*Pennisetum glaucum*) flour and sorghum (*Sorghum bicolor*) malt flour. Like many African fermented drinks, *Oshikundu* is a dynamic drink with live fermenting microorganisms that can lead to the spoilage due to possible microorganism proliferation and sedimentation of dregs. Thus, the optimal shelf life is not known. The amount and quality of ingredients and conditions of processing are not standardised. *Oshikundu* has a lot of insoluble solids which tend to settle and form sediment (dregs) at the bottom. To reduce the amount of dregs, this study used smaller amounts of sorghum malt and pearl millet flour (42 % less) that produced the same volume of *Oshikundu*, as that produced using traditional amounts. The duration of heating pearl millet flour adjunct was also varied (prolonged, up to 10 minutes) to improve the solubilisation of starch but not to change the consistency of *Oshikundu* from the way it is prepared traditionally in households. The amount of total solids in *Oshikundu* prepared in this study was significantly lower than common household-made *Oshikundu*.

Keywords: *Oshikundu*, pearl millet, total solids, *Sorghum bicolor*, dregs.

## 6.1. Introduction

*Oshikundu* is a traditional cereal based fermented beverage popularly consumed in Namibia. It is prepared in approximately over half of Namibian homes almost every day for own consumption. It is also sold at the informal markets. It is apparently a sour-tasting, mildly effervescent gruel of low or no alcohol content (Taylor, 2004) and contains suspended matter (dregs), which tends to settle to the bottom during storage. This can affect its stability (Briggs, Boulton, Brookes & Stevens, 2004). It is prepared by using water, pearl millet flour and sorghum malt. Pearl millet malt flour can also be used instead of malted sorghum flour. Similar products have been reported in Africa (Taylor & Emmambux 2008). For example *togwa* in Tanzania, is made from maize meal and finger millet (Hellstr¨om, V´azques-Ju´arez, Svanberg & Andlid, 2010) and in Nigeria, *kunun-zaki* is made using pearl millet and white *fonio* (Akoma, Jiya, Akunmka & Mshila, 2006). For pearl millet, food additive such as sugar can be added. The amount of ingredients, the conditions of preparation and hygiene is as variable as the number of households that makes *Oshikundu*. Generally, 500 mL of water is boiled and added to about 200 g of pearl millet flour (Taylor, 2004) and mixed thoroughly and uniformly using a wooden spatula to avoid lumps formation. This results in a relatively thick paste. The paste is cooled to about 40$^{o}$C. Milled sorghum malt (about 50 g) is then added and mixed well into the paste. This mixture is intermittently stirred while left to cool further for about 30 min or more. Thereafter, the paste can either be diluted with water (1 to 1.5 L) (Taylor, 2004) or left overnight and then diluted the following morning. The diluted mixture is left to ferment spontaneously (without back-slopping, addition of small amount of previously fermented *Oshikundu* and/or dregs) or it is back-slopped and left to ferment. The amount of dregs (*ehete*), i.e. the suspended particulate matter in *Oshikundu*, which settles to the bottom ranges between 2 and 10% (Embashu, 2014), as is. This amount of dregs is a problem to some consumers. Not everyone who drinks *Oshikundu* likes the presence of dregs.

One may thus find that some people especially children would scoop off the watery part of *Oshikundu* and leave the dregs in the container. The dregs then go to waste because it is relatively thick to consume on its own without it being part of the sour-tasting and effervescent liquid component. Therefore, this study investigated the effect of time of pre-cooking the pearl millet adjunct (flour) on the amount of dregs in *Oshikundu*. Preliminary results indicated that innovative control of adjunct pre-cooking can reduce the amount of dregs.

## 6.2. Materials and methods

### *6.2.1. Experimental materials*

Pearl millet and malted sorghum flours used was purchased from Oshakati open market in April 2015 and was stored at ambient conditions in a storage room at the University of Namibia before use within 4 months.

### *6.2.2. Pre-gelatinisation of pearl millet and oshikundu samples preparation*

Pearl millet flour was subjected to four different hydrothermal treatments over time. The amounts used in the present study were 42% lower than those reported by Taylor (2004) and Embashu (2014). This is to minimise the amount of sediment in the product. About 58 g of pearl millet meal was added to 400 ml of boiled water and mixed my stirring. This was left to cool for roughly 30 minutes, to about 45$^{o}$C. The same amount of pearl millet meal was treated as above but was removed from the heat source as soon as it started to boil. The above was repeated but paste was boiled for further 5 minutes before being removed from the heat source. The last treatment was done as the others except that the paste was boiled for further 10 minutes. About 2.4 g of sorghum malt was added before diluting with 600 ml of water. This was followed by back-slopping i.e. the addition of a 15 ml of previously fermented *Oshikundu*. All samples were then incubated at 30$^{o}$C for 24 hours. After *Oshikundu* was left

to ferment, 15 ml aliquots were frozen till moisture and total solid analysis (conducted within 2 weeks).

*6.2.3. Traditionally-prepared Oshikundu*

Traditional *oshikundu* samples (2) were collected from two different households in Windhoek. These were used to compare total solids between *Oshikundu* samples made with the precooked pearl millet paste and the traditionally-processed *Oshikundu*.

*6.2.4. Moisture and total solids contents determination*

Clean crucibles were placed in the oven at 105$^{o}$ C for 2-3 hours to dry. These were then placed in a desiccator to cool for about 45 min. Each crucible was weighed, appropriately labelled. Samples were vigorously shaken to mix them well and about 5 g aliquot of each oshikundu samples was put into the dried crucibles. The crucibles were then placed in the oven set at 105$^{o}$ C. They were left to dry for 24 hours. Thereafter they were removed and placed in a desiccator to cool for about 45 min and weighed. The moisture and total solids were calculated (James, 1995; Bradley Jr, 2010). Total solids were calculated as:

Total solid (%) = 100 - % Moisture.

*6.2.5. Statistical Analysis*

For each treatment, three independent samples were prepared. For moisture content determinations, duplicates were analysed per sample. The average values and their respective standard deviations ($n$ = 6) were computed in Statistica 12 software using one-way analysis of variance. To detect the differences between the means, Fisher least significance difference test was performed at a p-value of 0.05.

## 6.3. Results and discussions

Moisture (%) of *Oshikundu* achieved by pre-cooking pearl millet flour increased with prolonged cooking time (Table 9). Nonetheless, prolonged cooking time did not statistically ($p>0.05$) effect the moisture content. However, moisture of laboratory treatments were

significantly higher ($p<0.05$) than the traditional *Oshikundu*. The sample prepared by addition of just boiled water had a moisture content of 97.5%, whereas the one made from paste that was cooked for 10 min had 98.5%. This then resulted in a total solids content that decreased with prolonged heating time. But, prolonged heating was not statistically significant ($p>0.05$) to total solid content. On the other hand, there was a significant difference ($p<0.05$) between the traditional and prolonged heated *Oshikundu* total solids. This represents a 61% decrease in total solids between the laboratory and traditional *Oshikundu*. The increase in moisture content with prolonged heating can be attributed to pre-gelatinization. Water is presumably retained by the pearl millet starch granules due to continued water intake and swelling of the granules (Beleia, Varriano-Marston & Hoseney, 1980) favoured by exposure to increased temperatures.

**Table 9:** Moisture and total solids of *Oshikundu* made by pre-cooking pearl millet flour and traditional *Oshikundu*

| | ***Oshikundu* boiled water added** | ***Oshikundu* boiled paste instant** | ***Oshikundu* boiled paste for 5 minutes** | ***Oshikundu* boiled paste for 10 minutes** | **Traditional *oshikundu*** |
|---|---|---|---|---|---|
| **Moisture (%)** | $97.54 \pm 0.9^{a}$ | $97.84 \pm 1.2^{a}$ | $98.04 \pm 0.1^{a}$ | $98.51 \pm 0.1^{a}$ | $94.77 \pm 0.5^{b}$ |
| **Total solids (%)** | $2.46 \pm 0.9^{a}$ | $2.16 \pm 0.9^{a}$ | $1.96 \pm 0.1^{a}$ | $1.49 \pm 0.1^{a}$ | $5.24 \pm 0.5^{b}$ |

‡Values are means ± standard deviation. Means with same letter superscript are not significantly different ($p \geq 0.05$).

Dregs or suspended particles mostly comprise pearl millet adjunct, sorghum malt and possibly yeast and bacteria cells. The major component in *Oshikundu* besides water is pearl millet flour and sorghum malt flour (Embashu, Cheikhyoussef, Kahaka & Lendelvo 2013; Embashu, 2014). The sorghum malt comprises starch hydrolysis products including fermentable sugars. It also provides hydrolytic enzymes such as alpha- and beta-amylases which hydrolyse starch molecules (Taylor, 2004). This comes about during the malting of sorghum grain.

Alpha-amylases hydrolyse starch into lower molecular weight starch products such as oligosaccharides, dextrins and thereby solubilising the starch granules and molecules (Taylor, 2004). Beta-amylases effectively hydrolyse the products into smaller products with the primary release of maltose (Taylor, 2004). However, in food processing starch has to be gelatinised for its molecules to be readily accessible for hydrolysis by these enzymes (Dewar & Taylor 1999; Taylor, 2004). Pearl millet flour primarily comprises starch (Taylor, 2004) which in the *Oshikundu* making process most likely just partially undergoes gelatinisation upon its mixing with boiling water (Annor, Marcone, Bertoft & Seetharaman, 2014). However, this short-lived exposure of starch granules to hot water and in concentrated amounts will not disorganise the starch granules to expose the polymers to hydrolytic enzymes. Thus, the dregs presumably consist of mostly raw starch and some partially gelatinised starch. To the best of the authors' knowledge, there is no literature on the approach to reduce the amount of *Oshikundu* dregs, and this is the first study to investigate how to reduce the dregs in *Oshikundu* to improve the beverage quality and ultimately prolong the shelf life of this product. While it is true that the Namibian people possess different indigenous knowledge systems (Shapi, Mu Ashekele & Cheikhyoussef 2012; Mu Ashekele, Embashu & Cheikhyoussef 2012) and that their traditional practices especially in rural areas have an important role and values for these communities' daily life activities, it is noteworthy that such practices have been an art. This contribution therefore highlights the importance of the intersection of traditional practices and cuisines with science and technology. This intersection can potentially lead to improved quality of indigenous products and to their transformation into high quality products that can be commercially manufactured. This would ultimately contribute to the economic development of Namibia. This study provides two strategies to reduce the dregs in *Oshikundu*. The total solids and thus amount of dregs can be reduced by using lower amounts of dry ingredients while producing the same yield and by

pre-gelatinisation of adjunct. These approaches can positively contribute to the stability and possibly wider consumer acceptability of *Oshikundu*.

### 6.4. Conclusions

Use of smaller amounts of dry ingredients and pre-gelatinisation of pearl millet flour in the process of making *Oshikundu* can significantly decrease total solids in *Oshikundu*. This suggests that the amount of suspended particles in *Oshikundu* that tends to settle during storage can also be reduced though this route. For enhanced stability and possibly wide consumer appeal, this is a positive contribution towards processing of *Oshikundu* at an industrial and commercial level.

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# Chapter 7

## Innovative formulation of powder mixture for fermentation of *Oshikundu* and its preliminary sensory evaluation

Chapter 7 address objective 5.

To formulate an innovative ready to use dry ingredients of fermenting microbes for *oshikundu* brewing.

**Abstract**

There is a successful industrialisation of African traditional cereal based beverages. This is the result of ingredient treatment standardisation methods and flow process control. The fermentation of *Oshikundu* is still confined to household settings without empirical improvement on ingredients treatment and flow process. The preparation method of mixing dry ingredients to water is laborious. It requires acquired skills to know at which stage to add ingredients. This creates may contribute to the inconsistency of the final product. Thus, it results in fermentation with varying characteristics. Therefore, the study looked at the formulation of a dry ingredients powder mixture with an acceptable ease preparation method of *Oshikundu* fermentation. *Macia* and *Kantana* malts were used in formulation due to their superior qualities as set in chapter 3. The dry ingredients powder premix was brewed into *Oshikundu* and evaluated for consumer acceptability using the home use test (HUT) model. Preliminary sensory evaluation found the attribute 'smell' statistically different ($p \leq 0.05$) between *Oshikundu* brewed using *Kantana* and *Macia* malts. The easiness of preparation which requires the addition of water by the consumer was rated 9, the highest using a 9-point Hedonic scale. Panellists found the use of the premix easy to process as it does not require any skill or training. Therefore, the formulated dry ingredient powder premix can be used for brewing *Oshikundu*.

Keywords: *Oshikundu*, pearl millet, sensory, premix.

## 7.1. Introduction

There are successfully industrialised cereal based African traditional beverages such as mageu (Holzapfer & Taljaard, 2004), ogi (Onyekwere, Koleoso, Teniola & Akinrele, 2004), opaque beers: chibuku (Haggblader & Holzapfer, 2004; Kutyauripo, Parawira, Tinofa, Kudita & Ndengu, 2009) from Zimbabwe, while those trading as *tiokwe, ijula* and *zebra* are from South Africa (Haggblader & Holzapfer, 2004). Industrial productions of these traditional beverages are due to standardisation of ingredient treatment methods and flow process control. For continuous and successful industrialisation of any traditional product efficient, cheap and creative ways are employed in the flow process chain value. *Oshikundu/Ontaku* is a non-alcoholic, fermented and acidic beverage comprising of pearl millet (*Pennisetum glaucum (L.) R. Br.*) meal, sorghum (*Sorghum bicolor*) or pearl millet malt, water and/or pearl millet bran (Embashu & Nantanga, 2019a). Although *oshikundu* is sold in the informal market, the fermentation remains at the household level. Embashu and Nantanga (2019a) have highlighted the two processing methods of brewing *Oshikundu* and its drawbacks to industrial production. The ratio of ingredients (meal/flour: malt: water) during brewing is subjected to the brewers experience and thus variation in the final product. Furthermore, there is no control on the malting process to ensure quality malt for the brewing of *Oshikundu*. However, Embashu and Nantanga (2019b) have established malting conditions to produce quality malt in brewing *Oshikundu* and other nonalcoholic beverages as well as low-alcoholic beverages like *omalovu* (opaque beer). The preparation method of mixing dry ingredients to water is laborious. It requires acquired skills to know at which stage to add ingredients. This creates may contribute to the inconsistency of the final product. Thus, it results in fermentation with varying characteristics. Therefore, the study looked at the formulation of a dry ingredients powder mixture with an acceptable ease preparation method of *Oshikundu* brewing.

## 7.2. Materials and methods

### *7.2.1. Formulation of a dry powder premix*

Ingredients and flowprocess used in formulation are not provided in this section.

### *7.2.2. Preliminary sensory evaluation*

Sensory panel of 10 untrained members consist of 5 female and 5 male was randomly selected to evaluate *Oshikundu* brewing using the dry formulated ingredient powder premix (section 7.2.1) using consumer model home use test (HUT) (Lawless & Heymann, 2010). Panellists were informed of the purpose of the sensory evaluation test in the English language. Consent was obtained from the panellists. Panellists were instructed to add water to the premix up to a marking on the bottle (200 mL) and evaluate its acceptability when *Oshikundu* is ready to drink. *Oshikundu* was evaluated on a score sheet (Appendixes C) on taste, smell, colour and overall liking and ease of preparation using a 9-point hedonic structure scale (1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like or dislike, 6= like slightly, 7= like moderately, 8= like very much, 9= like extremely).

### *7.2.3. Statistical analysis*

Experiments were repeated once, except that of sensory evaluation. Data mean were compared using 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).

## 7.3. Results and discussions

### *7.3.1Preliminary sensory evaluation*

Preliminary sensory evaluation results are shown in Table 10. The use of a dry ingredients premix that requires the addition of water only was rated on the hedonic scale. The formulated premix resulted in a fermented *Oshikundu*, characterised by the effervescent, sweet smell and acquired taste. Panellists showed that *Oshikundu* smell differed significantly

($p \leq 0.05$) between those brewed using pearl millet or sorghum malts. However, pearl millet and sorghum malt *Oshikundu* taste, colour and overall liking were statistically the same ($p \leq 0.05$). The ease of preparation was rated the highest (9) by panellists. The panellists found that the use of dry powder ingredients premix which requires only the addition of water to be less time consuming and little effort needed compared to the traditional method of preparation. Furthermore, the powder was easy to process, it does not require skills or job training to brew *Oshikundu*.

**Table 10:** Panellists sensory of *Oshikundu* brewed using a dry powder ingredients premix of pearl millet or sorghum malts.

| **Consumer attributes** | **Malts** | |
|---|---|---|
| | Pearl millet | Sorghum |
| Overall liking | $6 \pm 1.8^{a}$ | $5 \pm 1.6^{a}$ |
| Smell | $7 \pm 1.3^{a}$ | $5 \pm 2.1^{b}$ |
| Taste | $6 \pm 2.2^{a}$ | $5 \pm 2.4^{a}$ |
| Colour | $7 \pm 1.6^{a}$ | $6 \pm 1.8^{a}$ |
| Ease of preparation | $9 \pm 0.8^{a}$ | $9 \pm 0.8^{a}$ |

Note: Values are mean ±standard deviation; Values with the same letter in a row are not significantly different ($p > 0.05$).

### 7.4. Conclusions

The study demonstrated the creative formulation of a dry ingredients powder premix for brewing *Oshikundu* that is ready to use. This is an improvement in the flow process of brewing *Oshikundu* The formulated premix was accepted by the panellists on the ease preparation method compared to the traditional lengthy and time-consuming.

## 7.5. Reference

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## Chapter 8

## Conclusions

Pearl millet and sorghum grain varieties had good germinative energy, above the recommended 90% for sorghum to be used in brewing. On the other hand, malting loss was unacceptably high up to 30%. Crude protein and fibre content were affected by malting since they improved. Malts of sorghum, *Macia* variety and that of pearl millet *Kantana* displayed the best quality (reducing sugars, FAN, alpha and beta amylase activity). Considering all the above, *Macia* and *Kantana* can be candidates for industrial malting for brewing low alcoholic and nonalcoholic beverages such as *Oshikundu*. For the content of phenolics, *Kantana* and Red sorghum had higher amounts of phenolic compounds and can potentially be vectors of delivering phenolics into human diets. Furthermore, malts are not of safety concern when it comes to coliforms and mycotoxins. However, total aerobic count exceeded the specification and this could potentially compromise the safety of the malt. None the less, this does not guarantee the safety of *Oshikundu* on coliforms and mycotoxins since there is scanty literature on this. Safety can only be ensured when all processes such as treatment, handling of ingredients, brewing flow process as well as the final product *oshikundu* are investigated and set. It appears that *oshikundu* is LAB (*Lactobacillus. plantarum, L. pentosus, L. acidifarinae, L. paraplantarum, L. spicheri, L. namurensis, L. zymae, L. fermentum, L. brevis, L. delbrueckii* subsp., *bulgaricus, L. buncheri, Leuconostoc gurlium* and *Pediococcus acidilactici*) and yeast (*Saccharomyces cerevisiae* and *S. paradoxus*) fermented. However, the dynamics of LAB and yeast during fermentation are not known. LAB detected in *oshikundu* could potentially be used in developing a defined starter culture for commercial production. Lactic acid bacteria are associated with preventing the growth of pathogenic bacteria and this could pontentialy contribute to the safety of *Oshikundu*. This study provides two strategies to reduce the dregs in *Oshikundu*. The total solids and thus amount of dregs

can be reduced by using lower amounts of dry ingredients while producing the same yield and by pre-gelatinisation of adjunct. This suggests that the amount of suspended particles in *Oshikundu* that tends to settle during storage (dreg) can also be significantly reduced through this route. The dry ingredients powder premix composited of pearl millet or sorghum malt was developed. The use of dry ingredients premix powder compared to the usual laborious method of mixing. The formulated premix was accepted by the consumer on the ease preparation method compared to the traditional lengthy, time-consuming and requires skills. The study contributed to new knowledge of malting conditions for the production of acceptable quality malt, safe from mycotoxins, identified potential fermenting LAB and yeast, as well as the formulation of a dry ingredients premix for *Oshikundu* brewing that eases the preparation method as it only requires the addition of water.

# Chapter 9

## Recommendations

Due to limited studies that aim at the value addition of *Oshikundu*, huge gaps exist. During the malting process, extra care is needed to minimise pearl millet grains loss, which contributes to a high malting loss due to their small size. The performance of these cereals varieties at different temperature and duration on their malt quality needs to be investigated. The use of 0.2% (m/v) NaOH could be employed in reducing microbial load during the malting process, especially the total aerobic count. Mycotoxins and microbial load should be looked at in *Oshikundu* to guarantee the safety of the product. Further investigation can look at the potential of identified microbes responsible for the fermentation of *Oshikundu* to develop a defined starter culture for commercial production. Also, future studies can look at consumer acceptability on the new dreg reduced sample in comparison to the traditional *Oshikundu*. The shelf life of the dry ingredients powder premix needs to be determined. Consumer test for the formulated powder for descriptive sensory analysis and at least 150 consumer panellist needs to be carried. The sensory evaluation should include a control product to compare the new product. Also, a descriptive sensory to be carried out to profile liked sensory parameters and inform the flavour compounds of the liked and descriptive sensory profiled samples. In addition, for the improved safety and quality of *Oshikundu* sensory quality, flavour compounds profile and safety aspects (e.g. methanol level) of *Oshikundu* need investigation in addition to the results of this study. The aspects such as starch and proteins in the pearl millets and their roles in the quality of *Oshikundu* may be studied. They are the major components of the solids in the products.

## APPENDIX A

## DETERMINATION OF REDUCING SUGARS: NELSON-SOMOGYI METHOD.

Nelson, N. (1944). Determination of reducing sugars: Nelson-Somogyi method. *Journal of Biological Chemistry*, 153, 375–380.

### Somogyi reagent

*Copper reagent A*

Dissolve 2.5 g of anhydrous sodium carbonate, 2.0 g sodium bicarbonate, 2.5 g potassium sodium tartrate and 20 g anhydrous sodium sulfate in 80 mL distilled water. Mix well before addition of distilled water to the make 100 mL. Filter if necessary. This solution should be stored where temperatures do not go below $20^{o}$ C. Cover the entire bottle with foil.

*Copper reagent B*

A 15% $CuSO_4.5H_2O$ solution with one or two drops of concentrated sulphuric acid per 100 mL. Dissolve 15 g copper sulfate in a small volume of distilled water. Add 1 or 2 drops of sulfuric acid and made up to 100 mL.

*Working Copper reagent (solution C)*

Mix 4 mL of Copper reagent B and 96 mL Copper reagent A before use.

### Nelson reagent (Asernomolybdate reagent)

Dissolve 25 g Ammonia molybdate [$(NH_4)_2MoO_4$ X $2H_2O$] in 450 mL of distilled water. Add 21 mL Sulfuric acid to the solution and mix well. Then add 3 g Disodium hydrogen arsenate [$Na_2HASO_4$ $_X$ $7H_2O$] dissolved in 25 mL distilled water. Mix well. Place the mixture in a bottle and incubate for 24 hrs at $37^{o}$C.

### Standard solution

*Stock glucose standard solution*

Weigh 100 mg/0.100 g of glucose and dissolve in 100 mL distilled water.

*Working glucose standard solution*

Dilute 10 mL of stock to 100 mL of distilled water (100 µg/mL). Pipette out 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution into a series of clean and dry test tube (Absorbance reading must be between 0.200 and 1.20). Make up the volume to 1 mL for test tube with working standard solutions of less than 1 mL.

**Extraction of reducing sugars**

Weigh 100 mg/0.1 g of sample in a 50 mL centrifuge tube and extract the sugar with hot 80% ethanol twice (5 mL each). Mix by shaking, leave it to stand for 5 minutes. Centrifuge at 4000 rpm for 3 minutes at 25$^{o}$C and decant. Collect and pool the supernatant in a 50 mL centrifuge tube. Evaporate the extracting solvent on a water bath at 80$^{o}$C. Add 10 mL distilled water to dissolve the sugar. Pipette 200 µL of malt but 100 µL unmalted of the sugar solution per sample into different test tubes. Amounts pipetted can be increased or decreased based on whether the absorbances are reasonable. That is, extent of dilution required determines the amounts used. Make up the volume to 1 mL for each sample.

*Method for sugar analysis*

Add 2 mL of working copper solution (solution C) to each of the test tubes containing a various standard solution or samples. For a blank test tube, add 2 mL of distilled water and then 1 mL of solution C. Close the test tube with parafilm. Vortex, and warm the test tubes in a boiling water bath (with cover) for 30 min. Cool to room temperature under tap water. Add 2 mL arsenomolydbate reagent and vortex. Let the samples stand for 5 minutes. If necessary,

centrifuge the sample (especially sample with high MW material). Measure absorbance at 540 nm against a blank without sugar.

## APPENDIX B

## DETERMINATION OF FREE AMINO NITROGEN IN SORGHUM MALT

Dewar, J. Taylor, J.R.N . and Joustra, S.M. (1995). Determination of free amino nitrogen in sorghum malt. Method No. 5. *In Accepted Methods of Sorghum Malting and Brewing Analysis*. Pretoria, CSIR Food Science and Technology. METHOD NO.5
Method accepted September 1994

### 1. INTRODUCTION

Free amino acids and small peptides (free a-amino nitrogen or FAN) are the source of nitrogen for yeast in brewing. Pre-formed FAN in the sorghum malt is a major source of FAN in the wort, the presence of which is necessary for normal yeast growth and fermentation.

### 2. APPARATUS

Analytical balance

Water bath at 30°C

Bench-type centrifuge

Spectrophotometer covering the visible light range

10 mm cuvettes

### 3. REAGENTS

#### Extractant

Prepare 1 litre of a 5% *(m/v)* solution of trichloroacetic acid in distilled water.

*Caution:* Care must be taken when handling this acid in its solid and diluted form as it can cause severe burns.

#### Ninhydrin colour reagent

*Dissolve in distilled water:* 100 g disodium hydrogen phosphate (Na2HP04·12H20); 60 g potassium dihydrogen phosphate (KHP04); 5 g ninhydrin (Merck cat. 6762); 3 g fructose (Merck cat. 5323) and make up to 1 litre. This colour reagent will keep for 2 weeks if stored at 0-4 °C in an amber or foil-covered bottle.

**Diluent**

Dissolve 2 g potassium iodate (KI03 , Merck cat. 5051) in 600 ml distilled water and add 400 ml 96% ethanol.

**Standard for ninhydrin assay**

Dissolve 107.2 mg glycine (Merck cat. 4201) in 100 ml distilled water in a volumetric flask. This is a stock solution and will keep for 1 week if stored at 0-4 °C. For each set of analyses carried out, 2 ml of this stock solution is diluted to 100 ml with distilled water in a volumetric flask.

**4. METHOD**

**Extraction of FAN from malt**

The 5% trichloroacetic acid solution is warmed to temperature in the 30 °C water bath. One gram of milled malt (milled according to the specification in SASS Method 235) is weighed into a 100 ml glass Erlenmeyer flask. Exactly 40 ml of the warmed 5% trichloroacetic acid is added to the malt. The flask is then tightly stoppered. Extraction is carried out for 1 hour with the flasks immersed in the 30°C water bath. At 20 minute intervals the flasks are swirled so as to suspend the contents. After extraction, a portion of the supernatant (10 ml) is centrifuged in the bench centrifuge for 10 minutes. After centrifugation, 1 ml of clear supernatant is diluted to 25 ml in a volumetric flask with distilled water.

**Ninhydrin assay**

Pipette 2 ml of the diluted standard into a test tube (do in triplicate). Pipette 2 ml of the distilled water (the Blank) into a test tube (do in triplicate). Pipette 2 ml of the diluted sample into a test tube (do in triplicate). Add 1 ml ninhydrin colour reagent to all the test tubes and mix well. Cover the test tubes with glass marbles to prevent evaporation and heat for exactly 25 minutes in a constantly boiling water bath. Cool the test tubes for 20 minutes in a water bath at room temperature, then add 5 ml of the diluent to each tube and mix well. Within 1

hour read the samples and standards against a blank (which should be virtually colourless) at 570 nm. The contents of each test tube should be mixed thoroughly by inversion (wear disposable gloves), prior to placing the contents in a cuvette. The standard should give an absorbance between 0.9 and 1.1.

**5. CALCULATION**

The results are calculated as mg FAN/1 00 9 dry mass of malt, which can be obtained from the following expression:

$$\frac{\text{Absorbance of sample x 400}}{\text{Mean of absorbance of standard}} \times \frac{100}{(100 - \text{moisture})}$$

The results are reported as:

Malt FAN = x mg/100 g (dry basis)

**6. REPEATABILITY AND REPRODUCIBILITY**

Repeatability: ± 5 mg FAN/1 00 g malt

Reproducibility: ± 10 mg FAN/1 00 9 malt

**7. BIBLIOGRAPHY**

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**Appendix C: Panellist consent form and score sheet form for oshikundu sensory evaluation.**

# SENSORY PANELIST CONSENT FORM

Sensory evaluation of *oshikundu/ontaku*

Thank you for willingness to potentially participate in a sensory evaluation project at the University of Namibia. The purpose of this study is to determine your response to products containing pearl millet and sorghum. You will be asked to evaluate how much you like or dislike them. This information will help us determine the best way to make new products.

**Date of Participation:** November 2019

**Voluntary Nature of Participation:** I understand the participation in this project is completely voluntary. I do not have to participate in this sensory project. If I do not agree to participate I can withdraw my participation any time, without penalty.

**Risks to the Individual:** I understand that I will evaluate *oshikundu/ontaku* prepared from pearl millet and sorghum using consumer sensory evaluation. The risks involved in drinking, is no greater than drinking *oshikundu/ontaku* prepared using purchased cereal flour/meal that is currently available in the Namibian market.

**Medical Liability**: I understand that no financial compensation will be paid to me in connection with any physical injury or illness in the unlikely event of physical injury or illness in the unlikely event of physical injury or illness as an indirect result of my participation in this sensory project.

**Confidentiality:** Participants are not required to reveal any confidential information. All responses to questions will be treated in confidential manner. Responses to sensory questions via the evaluation form are tracked using numbers only. These numbers are not in any way related to the participant's name.

If you have any question about this sensory project, please contact Dr. komeine KM Nantanga at 0813400242, knantanga@unam.na or Werner Embashu at 0811474764, wembashu@unam.na, University of Namibia.

I HAD THE OPPORTUNITY TO READ THIS FORM, ASK QUESTIONS ABOUT THIS SENSORY PROJECT AND I AM PREPARED TO PARTICIAPTR IN THIS PROJECT.

Participant's signature: Date:

Participant's name (*please print clearly*):

Sensory Panel leader signature: Date:

University of Namibia
Multidisciplinary Research Center/Department of Food Science and Technology
SCORE SHEET FOR CONSUMER SENSORY EVALUATION (OSHIKUNDU/ONTAKU)
Panel leader: Dr. Komeine KM Nantanga Date: November 2019

Panelist name: ________________________________
Gender: _____________
Ager group
20-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ >61 ☐

**Instructions**

1. Fill with tap water to the mark on the bottle, do this between 20h00 to 21h00. Mix by shaking. Leave the bottle on a table until the next day morning. When oshikundu/ontaku is ready to drink, processed to point number 2
2. Rinse your mouth with water before and after each sample.
3. Indicate how much you like or dislike each sample by placing an X in the box next to the appropriate term in relation with your perception.

| | Overall liking | | Smell | | Taste | | Colour | | Ease of preparation | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample code | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... | Sample #.......... |
| 1= Dislike extremely | | | | | | | | | | |
| 2= Dislike very much | | | | | | | | | | |
| 3= Dislike moderately | | | | | | | | | | |
| 4= Dislike slightly | | | | | | | | | | |
| 5= Neither like or dislike | | | | | | | | | | |
| 6=Like slightly | | | | | | | | | | |
| 7= Like moderately | | | | | | | | | | |
| 8= Like very much | | | | | | | | | | |
| 9= Like extremely | | | | | | | | | | |

How long it took to get ready____________ hours

Comments:______________________________________________________________________________