UNIVERSITY OF RWANDA

EFFECT OF EUCALYPTUS ASH ON MICROBIAL FLORA AND AFLATOXINS IN RED SORGHUM MALTS AND ON PHYSICO-CHEMICAL AND ORGANOLEPTIC CHARACTERISTICS OF IKIGAGE BEER

2025

JEAN BOSCO SHIMIRWA

EFFECT OF EUCALYPTUS ASH ON MICROBIAL FLORA AND AFLATOXINS IN RED SORGHUM MALTS AND ON PHYSICO-CHEMICAL AND ORGANOLEPTIC CHARACTERISTICS OF IKIGAGE BEER

BY

JEAN BOSCO SHIMIRWA Ref. Number: 223027401

Dissertation submitted in fulfilment of the requirements for the degree:
MASTER OF SCIENCE IN BIOTECHNOLOGY
In the Department of Biology, School of Science
College of Science and Technology
at
The University of Rwanda

Supervisor: Prof. François LYUMUGABE

Co-Supervisors: - Dr. Benjamin MANIRAKIZA
- Mr. Ephrem HABUMUGISHA

Kigali-Rwanda, 2025.

Declaration

I, Jean Bosco SHIMIRWA, hereby declare that this research project submitted to the University of Rwanda for the degree of Master of Science in Biotechnology is my own original work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements to the award of any degree or any other qualification.

Jean Bosco SHIMIRWA

Date: 31/08/2025.

Dedication

This dissertation is dedicated to:

- The University of Rwanda, College of Science and Technology, School of Science, Department of Biology
- The European Union and ENABEL, a Belgian international cooperation agency, through the KWIGIRA project, which funded a Master of Science in Biotechnology at the University of Rwanda.
- The Académie de Recherche et d'Enseignement Supérieur (ARES), which funded my three- month stay in Belgium at the Université Catholique de Louvain.
- My supervisors (Prof. François LYUMUGABE, Dr. Benjamin MANIRAKIZA and Mr. Ephrem HABUMUGISHA).
- The entire research team at the Brewery and Food Industries Laboratory (INBr) at the Université Catholique de Louvain: Prof. Sonia COLLIN (Coordinator) and other staff, doctoral and master's students.
- Professor Antoine NSABIMANA, Coordinator of Master of Science in Biotechnology program at the University of Rwanda.
- Professors from various universities who participated in teaching activities as part of the Master of Science in Biotechnology program at the University of Rwanda. Among them, there are professors from the Université Libre de Bruxelles (ULB), the Université Catholique de Louvain and the Université de Liège (Belgium), Université de Grenoble Alpes (France), the University of Gothenburg (Sweden), New York University, Abu Dhabi campus (United Arab Emirates), and the University of Rwanda.
- Professors and the entire research team at the International Center for Genetic Engineering and Biotechnology (ICGEB)

Sincerely, I can offer nothing more in return for what you have done for me to pursue my master's degree. May Almighty God bless you!

Acknowledgements

The success of this dissertation is the result of the contribution and commitment of various individuals and institutions, and therefore they deserve to be highly recognized. First and foremost, I thank Almighty God for his incomparable love and help throughout my life, especially during my master studies and research. I would like to warmly thank the work of my supervisor Prof. François LYUMUGABE for his support to my research project and Professor Sonia COLLIN for coordinating the INBr at UCLouvain and for leading the cooperation between the INBr and the students and researchers from other universities and higher education institutions, future researchers and workers, who completed their internships in her laboratory. On this occasion, I would like to warmly thank all the doctoral and master's students, as well as the other laboratory staff members at INBr-UCLouvain, for their invaluable assistance since my first day in the laboratory. I also acknowledge Mr. Ephrem HABUMUGISHA, a Ph.D student at the Université Catholique de Louvain, who always supported me in day-to-day activities in the laboratory during my research. My thanks also go to Dr. Benjamin MANIRAKIZA who always followed my laboratory activities at UR-CST.

I warmly thank all the professors from the various universities who participated in teaching activities in the Master of Science in Biotechnology program at the University of Rwanda for their immeasurable skills, knowledge, advice, guidance, and mentorship. In the same vein, I extend my sincere thanks to the European Union and ENABEL, a Belgian international cooperation agency, through the KWIGIRA project, which funded a Master of Science in Biotechnology program at the University of Rwanda, as well as the Académie de Recherche et d'Enseignement Supérieur (ARES), which funded my three- months stay in Belgium at the Université Catholique de Louvain. My thanks also go to the Ministry of Health, Rwanda and BK Foundation for their big support to the program. My last but not least thanks go to the management of the University of Rwanda, College of Science and Technology, School of Science, Department of Biology, for their staff development initiative by helping staff to obtain study leave to undertake internships and training abroad in accordance with their daily duties and responsibilities.

List of Figures

List of Tables

List of Abbreviations and Acronyms

UR: University of Rwanda

CST: College of Science and Technology

MSc: Master of Science

UCLouvain: Université Catholique de Louvain

INBr: Brewery and Food Industries Laboratory

ARES: Académie de Recherche et d'Enseignement Supérieur

ULB: Université Libre de Bruxelles

LAB: Lactic Acid Bacteria

SMC: Malted Sorghum with Ash

SMNC: Malted Sorghum without Ash

NABLAB: Non-Alcoholic Beer Brewed with Low Alcohol by Brewing process

EBC: European Brewery Convention (unit for color/turbidity)

FAN: Free Amino Nitrogen

α-AA: Alpha-Amino Acid

pH: Potential of Hydrogen (measure of acidity)

CO_2: Carbon Dioxide

ANOVA: Analysis of Variance

SAFE: Solvent-Assisted Flavor Evaporation

GC-MS: Gas Chromatography–Mass Spectrometry

°P: Degrees Plato (sugar concentration)

BU: Bitterness Units

CFU: Colony Forming Units

PDA: Potato Dextrose Agar

PCA: Plate Count Agar

ISO: International Organization for Standardization

$Ca(OH)_2$: Calcium Hydroxide

NaOH: Sodium Hydroxide

KMS: Potassium Metabisulfite

ASBC: American Society of Brewing Chemists

POF(+): Phenolic Off-Flavor positive yeast

4-VG: 4-Vinylguaiacol

AFB_1, AFB_2, AFG_1, AFG_2: Types of Aflatoxins

°C: Degrees Celsius

% v/v: Percent Volume per Volume

mg/L: Milligrams per Liter

µg/kg: Micrograms per Kilogram

Abstract

Ikigage is a traditional Rwandan beer brewed primarily from red sorghum. However, its quality and safety can be compromised by microbial contamination and aflatoxin accumulation during production. These issues not only threaten public health due to the toxicity and carcinogenic nature of aflatoxins but also compromise the safety, sensory quality, and economic viability of traditional beverages. Current solutions to reduce these contaminants are often costly and unsuitable for small-scale, artisanal brewers. While traditional brewing practices have been studied, limited research exists on the potential of eucalyptus ash as a natural additive to improve microbial safety and extend shelf life. This study aimed to evaluate the effect of eucalyptus ash on the microbial flora, physico-chemical characteristics, aflatoxin content, and organoleptic properties of Ikigage beer. Red sorghum grains were malted with and without 5% eucalyptus ash and used to produce beers fermented with S-33 and LA-01 yeast strains in a controlled microbrewery. Sorghum samples were analyzed for microbial counts (total viable count, yeasts and molds) and Total aflatoxin while Ikigage samples were analyzed for physico-chemical parameters (pH, Turbidity, Total polyphenols, Total flavonoids, FAN, alcohol content) and organoleptic characteristics. Statistical analysis was conducted using ANOVA, and mean comparisons were performed at a 95% confidence level ($p < 0.05$). Results showed that eucalyptus ash addition significantly reduced total aflatoxin levels from 30.58 µg/kg for untreated sorghum to 0.47 µg/kg for sorghum treated with 5% ash. Physico-chemical changes included an increase in pH from 4.1 for Ikigage produced from son treated sorghum (SMNC) to 4.7 of Ikigage produced from sorghum treated with 5% ash, with a corresponding decrease in turbidity while rganoleptic evaluation indicated no adverse impact on taste, aroma, or consumer acceptability. The findings suggest that eucalyptus ash is a viable, low-cost solution to improve the safety and quality of Ikigage beer, aligning with public health priorities and supporting the modernization of traditional Rwandan brewing methods.

Key words: *Ikigage beer; Eucalyptus ash; Aflatoxins; Microbial flora; Physico-chemical characteristics; Organoleptic properties.*

Table of Content

CHAPTER I. INTRODUCTION

I.1. Background

For drinks like Ikigage, a traditional Rwandan beer brewed from cereals like red sorghum (*Sorghum bicolor*), malting is an essential step in traditional African brewing. Malting involves soaking, germinating, and drying grains, processes that have a major impact on the end product's microbial load, biochemical alterations, and sensory qualities (Stone et al., 2020). Sorghum malts, however, are especially vulnerable to microbial contamination, notably from fungi that can produce aflatoxins, which are strong mycotoxins that have serious health consequences (Wild & Gong, 2009). As aflatoxin contamination in cereals and related products is linked to immunosuppression, liver cancer, and childhood stunting, it is a serious public health concern throughout sub-Saharan Africa (Kensler et al., 2010).

Due to its essential oils and bioactive substances including eucalyptol, flavonoids, and tannins, eucalyptus species, which are extensively spread throughout Africa, have long been recognized for their antibacterial and antifungal qualities (Batish et al., 2008). Eucalyptus ash, the mineral-rich residue left over after burning Eucalyptus wood or leaves, has drawn more attention recently as a possible antibacterial agent. Ash materials are alkaline and high in metal oxides, such as calcium, potassium, and magnesium, which may prevent microbial growth or make the environment unfavorable for fungal growth.

Aflatoxin levels and microbiological contamination may be decreased by using eucalyptus ash during the malting process, increasing the safety of conventional sorghum malts without the need for costly chemical treatments. Furthermore, because traditional Ikigage beer drinkers are sensitive to alterations in flavor profiles that depart from accepted standards, it is crucial to evaluate the organoleptic consequences, such as taste, fragrance, and mouthfeel, even though antimicrobial effects are desirable (Stone et al., 2020).

Consequently, the purpose of this study is to determine how the eucalyptus ash treatment affects the microbial flora and aflatoxin concentration of red sorghum malts as well as the organoleptic properties of Ikigage beer. The results could support traditional brewing methods that are safer and more widely accepted, supporting cultural culinary heritage and public health objectives.

I.2. Problem Statement

The use of sorghum in brewing is limited due to fungal contamination leading to a risk of mycotoxin production, the low β-amylase and β-glucanase activities of the malt, the filterability of the wort and the subsequent stability of the beer (Lyumugabe et al., 2012).

A crucial phase in making Ikigage, a culturally significant Rwandan beer, is the traditional malting of red sorghum (*Sorghum bicolor*). Sorghum grains are extremely susceptible to microbial contamination during malting, especially from fungi like *Aspergillus species*, which can produce aflatoxins, which are extremely poisonous and carcinogenic secondary metabolites (Wild & Gong, 2009). Aflatoxin contamination endangers food security and the commercial viability of traditional beverages in addition to posing major public health threats, such as hepatocellular cancer and immunosuppression (Williams et al., 2004).

The current approaches to managing the microbial load and aflatoxin contamination during malting are frequently costly, chemically demanding, and unsuitable for conventional small-scale breweries (Shephard, 2008). Affordable, natural, and culturally acceptable interventions that can improve food safety without sacrificing the sensory attributes, taste, scent, and appearance that determine whether or not Ikigage is accepted by consumers are becoming more and more necessary (Stone et al., 2020). Because of bioactive substances like eucalyptol and tannins, eucalyptus species, which are common in Rwanda and other parts of Africa, have been shown to possess antibacterial qualities (Batish et al., 2008; Lyumugabe et al., 2012). Burning eucalyptus wood produces alkaline, mineral-rich ash that may prevent microbial growth and lessen the production of aflatoxin (Thiex et al., 2012).

The impact of *Eucalyptus sp.* ash on microbial flora, aflatoxin levels, and taste characteristics in sorghum malts and Ikigage beer has not been thoroughly studied, despite its historical applications in other food preservation contexts. The potential of eucalyptus ash as an easy, affordable, and sustainable intervention to enhance the safety and quality of red sorghum malts and the consequent Ikigage beer is thus critically lacking. Closing this gap could help preserve and modernize traditional brewing methods while also providing major public health advantages.

I.3. Justification of the Study

At its 75th session in March 2021, the United Nations General Assembly proclaimed 2023 the International Year of Millets, a group of species that includes sorghum. This reflects the growing interest in this cereal, which can be grown in all soils. In the brewing sector, sorghum is gaining renewed interest, both for the production of gluten-free beers and for its richness in polyphenols.

Apart from their socio-cultural importances, African sorghum beers are very rich in calories, vitamin B and essential amino-acids and constitute an income source for the local brewers who produce them at household scale by using the traditional technologies (Lyumugabe et al., 2012). In Rwanda, the production of the sorghum based beers depends largely on socio-economic imperatives such as the wages of agricultural workers, payment of school fees and purchase of school materials for children, payment of the mutual health insurance, beverages for all traditional and non-traditional festivals such as dowry, marriage, etc (Musabanganji et al., 2023). In that way, they guarantee the stability of the family economy and punctuate the social life of the average peasant.

Utilizing Eucalyptus ash as additive in local beer production could contribute to the stability, shelf life and contamination prevention of local beer especially Ikigage and therefore promote the development of higher-quality, health-promoting traditional beers. This study will promote the Rwandan government policy called "Made in Rwanda" and will contribute to the Economic Development and Poverty Reduction Strategy in Rwanda (EDPRS 3) in its part of broader goals of food security and economic development.

I.4. Research Objectives

I.4.1. Main objective

To investigate the effect of Eucalyptus ash on the microbial flora and aflatoxin content of red sorghum malts, and on physico-chemical and organoleptic characteristics Ikigage beer.

I.4.2. Specific objectives

1. To assess the impact of Eucalyptus ash treatment on the microbial load (bacterial and fungal counts) of red sorghum malts.
2. To evaluate the influence of Eucalyptus ash treatment on aflatoxin levels in red sorghum malts.
3. To determine the organoleptic (sensory) characteristics of Ikigage beer produced from

treated and untreated sorghum malts.

4. To evaluate the impact of ash treatment on the physico-chemical characteristics of the beers produced from red sorghum malts

5. To establish correlations of microbial reduction, aflatoxin reduction, and physico-chemical parameters between beers produced from sorghum malted with Eucalyptus ash and sorghum malted without ash.

I.5. Research Hypotheses

I.5.1. Null Hypothesis

Eucalyptus ash treatment has no significant effect on the microbial load and aflatoxin levels in red sorghum malts and the organoleptic characteristics (taste, aroma, appearance, and acceptability) of Ikigage beer.

I.5.2. Alternative Hypothesis

Eucalyptus ash treatment significantly reduces the microbial load and aflatoxin levels in red sorghum malts and influences the organoleptic characteristics of Ikigage beer.

CHAPTER II. LITERATURE REVIEW

Beer is one of the oldest fermented beverages in the world, consumed for thousands of years on all continents. Its production has always been closely linked to local resources and cultural traditions, which explains the great diversity of raw materials and manufacturing processes throughout the world. If barley malt has gradually established itself as the reference cereal in the modern brewing industry, it is mainly due to its enzymatic richness, its easily degradable starch composition and its wide adaptability to temperate climates. However, its cultivation remains limited in tropical and subtropical zones, where climatic conditions are unfavorable for its development (Yeo & Liu, 2014). In a context of economic pressure, development of new products and food sovereignty, many breweries, particularly in Africa and Asia, are resorting to the use of raw grains (Bogdan & Kordialik-Bogacka, 2017). These alternative raw materials make it possible to significantly reduce production costs, limit dependence on malted barley imports and offer beers with different sensory profiles (Dabija et al., 2021).

Sorghum (*Sorghum bicolor),* a cereal widely cultivated in tropical areas, is the most widely used raw grain in Africa, both for the production of traditional opaque beers and for the production of industrial beers as a partial or total adjuvant to malted barley (Taylor et al., 2013). Due to its chemical composition relatively close to that of barley, particularly in starch (65–74%) (*Traité de Brasserie*, 2022), sorghum has real brewing potential. However, the use of malted sorghum presents several major technological challenges: its low enzymatic activity, mainly amylolytic, limits starch hydrolysis; its higher gelatinization temperature than that of barley complicates the extraction of fermentable sugars (Espinosa-Ramírez et al., 2013). In addition, red and brown sorghum varieties are rich in polyphenols, particularly tannins, which can inhibit certain brewing enzymes, alter colloidal stability and cause flocculation and filtration problems (Taylor & Daiber, 1988; Aron & Shellhammer, 2010).

To overcome these limitations, current industrial solutions rely on the addition of exogenous enzymes (α-amylase, β-glucanase, proteases) and the optimization of mashing steps (Espinosa-Ramírez et al., 2013). However, certain local traditional practices, such as the addition of ash during malting, particularly in Rwanda and Burundi, are of significant technological interest. Some studies have shown that ash treatment improves the quality of sorghum malt by significantly reducing the total polyphenol and tannin content, while increasing amino acid availability and starch digestibility (Claver et al., 2011; Kageruka et al., 2024). More recently, Kageruka et al., 2024) also demonstrated that the addition of 5 % eucalyptus ash during red

sorghum malting helps raise wort pH, thereby promoting enzyme activity and improving wort clarity by reducing the polyphenol load. However, very few studies have been carried out on the impact of the addition of eucalyptus ash on the physicochemical and sensory characteristics of red sorghum beers.

II.1. The use of Raw Grains in Brewing

The global beer market is extremely competitive, a trend that has intensified in recent years. Europe, North America, and Japan, in particular, have seen their sales decline, partly due to the popularity of lager in these markets. To counter this trend, many breweries have embarked on the development of new and innovative products, particularly through the use of unmalted grains (Yeo & Liu, 2014; Hager et al., 2014). Thus, many breweries today use various adjuncts in their beer production. In Europe, 10% to 30% of malt is replaced by unmalted grains, while in Africa, where climatic conditions are not optimal for growing barley, this figure is between 50% and 75%, making sorghum the primary raw material used for beer production, both in its malted and raw grain form (Bogdan & Kordialik-Bogacka, 2017). These other raw grains are often used in the brewing industry as an alternative and cost-effective source of extracts, as well as for the organoleptic and physicochemical properties (flavor, foam, colloidal stability) they contribute to the brewing process and finished beers (Yorke et al., 2021; Steiner et al., 2012).

II.2. Sorghum

II.2.1. Origin and Geographic Distribution of Sorghum

Sorghum is a cereal grain native to North-East Africa and was first cultivated 3,700 to 4,000 years ago (Berenji et al., 2011). It is widespread in tropical and subtropical regions of Asia, the Americas, and Europe (Venkateswaran et al., 2018). Over the years, red sorghum has established itself in many countries, particularly in tropical and subtropical regions, where it has adapted to diverse environments. Today, red sorghum is cultivated in over 100 countries. Sub-Saharan Africa remains one of the largest producers of red sorghum, with Nigeria being the largest producer with 6.5 million tonnes produced in 2023-2024, representing 11% of global production (Zannou et al., 2025). The table below shows global red sorghum production through 2024.

Table 1: Ranking of the world's largest red sorghum producers (Zannou et al., 2025)

SN	Market	World production (%)	Total production (2023/2024, millions of tones)
1	USA	14	8.0
2	Nigeria	11	6.4
3	India	8	4.7
4	Mexico	8	4.5
5	Brasil	8	4.4
6	Ethiopia	7	4.0
7	Soudan	5	3.1
8	China	5	3.0
9	Argentina	4	2.5
10	Australia	4	2.2

II.2.2. Sorghum Grain Taxonomy

Sorghum (*Sorghum bicolor*) is a monocotyledonous plant native to Africa, belonging to the Poaceae family and the Panicoideae subfamily (Taylor & Kruger, 2018). This family includes more than 12,000 species divided into over 700 genera, including wheat, rice, barley, and maize. From an end-use perspective, four types of sorghum are distinguished:

- Grain sorghum (*Sorghum bicolor* (L.) Moench): Used in human and animal food
- Sweet sorghum (Sorghum saccharatum): with a sweet stem, i.e., whose sap is rich in sucrose, like that of sugarcane. It is used for the production of sorghum syrup.
- Forage sorghum: which is a hybrid variety derived from grain sorghum and sweet sorghum, the perennial plant of which is used for animal feed.
- Paper sorghum: used for the manufacture of kraft paper (*Traité de Brasserie*, 2022).

II.2.3. Sorghum Grain Structure

Sorghum grains are generally spherical in shape and come in various sizes and colors. Typical sorghum seeds are generally 4 mm long, 2 mm wide, and 2.5 mm thick, with colors ranging from black to red, purple, brown, yellow, and blue, to yellow to white (Ratnavathi, 2018).

Its basic anatomical components are the pericarp (outer layer), the germ (embryo), and the endosperm (storage tissue). The distribution of these components varies depending on the variety and environment, with an average of 8 %, 82 %, and 10 % (put a distance between a number and unit, and btn a number and %, check whole document) for the pericarp, endosperm, and germ, respectively (Ratnavathi, 2018).

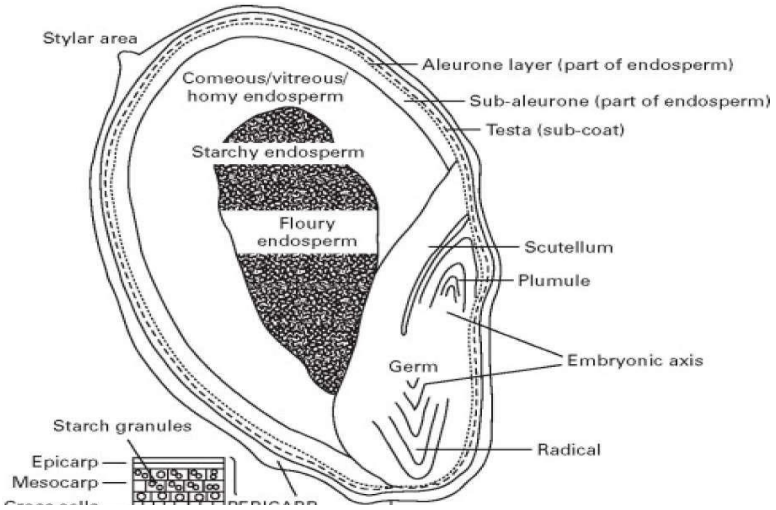

Figure 1: Structure of a sorghum seed (Taylor & Emmambux, 2007)

The pericarp, which varies in thickness from 8 to 160 mm, is structured into three distinct layers: the epicarp, the mesocarp, and the endocarp (Ratnavathi, 2018). Below the pericarp is a layer known as the seed coat, or testa. This layer plays a key role in the storage of tannins and pigments in certain sorghum genotypes (Mezgebe et al., 2018). The endosperm, on the other hand, is where almost all of the starch granules are stored. It consists of the aleuronic layer, the peripheral endosperm, the vitreous (hard) endosperm, and the floury (soft) endosperm. The aleuronic layer is a single layer of cells located just beneath the seed coat. This layer is rich in proteins and enzymes, oils, vitamin B complexes and minerals (Ratnavathi, 2018).

II.2.4. Chemical Composition of Sorghum Grain

Sorghum grain is rich in starch, protein, micronutrients, and crude fiber, but low in fat, which gives it good nutritional properties. Sorghum is more closely related to corn, but contains more protein than corn, less fat, and approximately the same amount and proportion of carbohydrates (Ratnavathi, 2018). The table below shows the nutrient composition of a sorghum grain.

Table 2: Nutritional Composition of a Sorghum Grain

Constituants	Proportions
Main components (%)	
Proteins	4.40 – 21.10
Starch	55.60 – 75.20
Amylose	21.20 – 30.20
Soluble sugars	0.70 – 4.20
Reducing sugars	0.05 – 0.53
Fibers	1.00 – 3.40
Grass materials	2.10 – 7.60
Ashes	1.30 – 3.30
Minerals (mg/100g)	
Calcium	11.00 – 586.00
Phosphorus	167.00 – 751.00
Iron	0.90 – 20.0
Vitamins (mg/100g)	
Thiamine	0.24 – 0.54
Niacine	2.90 – 6.40
Riboflavine	0.10 – 0.20
Antinutritional factors	
Tannins (%)	0.1 – 7.22
Phytic acid (mg/100g)	70 – 314

II.2.4.1. Starch

Starch is the dominant carbohydrate in the sorghum grain and is stored as granules in the endosperm. It occurs as starch granules consisting of two consecutive polymers: amylose (20–3 0% of sorghum grain starch) and a highly branched polymer called amylopectin (70–80 % of sorghum grain starch) (Endalamaw et al., 2025). Amylose (106–108 kDa) is a linear sugar consisting of 50 to 300 glucose units linked together in an alpha-(1,4) chain. Amylopectin (108–109 kDa) has a more branched structure than amylose, with alpha-(1,6) chains every thirty residues (Endalamaw et al., 2025; (*Traité de Brasserie*, 2022). The good starch content in sorghum grain makes it a cereal of choice as a raw grain in brewing, as a supplement to

barley despite their different starching temperatures (70 to 90 °C for sorghum versus 51 to 60 °C for barley) (Traité de Brasserie, 2022).

II.2.4.2. Proteins

After starch, proteins are the second most abundant component in sorghum grain. Their content can vary from 6 to 20 %, depending on genetic and environmental influences (Bean et al., 2019). They are classified according to their solubility in different solvents. Thus, a distinction is made between albumins (soluble in water), globulins (soluble in salt), kafirins or prolamins (soluble in aqueous alcohol), cross-linked kafirins (soluble in aqueous reducing alcohol), cross-linked glutelins (soluble in detergent reducing agent at alkaline pH), and unrestricted structural protein residues (Ratnavathi, 2018).

II.2.4.3. Lipids

The crude fat content of sorghum grown during the rainy season, analyzed from year to year, ranges from 2.5 % to 3.5 %, which is higher than that of wheat and rice, and higher than that of maize. The germ and aleurone layers are the main contributors to fat content, with the germ accounting for 80 % of the total fat in the sorghum grain (Ratnavathi, 2018).

II.2.4.4. Vitamins and Minerals

Sorghum is rich in minerals such as potassium (K), magnesium (Mg), Fe, Zn, and copper (Cu), mainly concentrated in the pericarp, aleurone layer, and germ (Kumar et al., 2022). The mineral content of sorghum is strongly influenced by environmental conditions (Endalamaw et al., 2025). Sorghum is also rich in vitamins. It mainly contains riboflavin and pyridoxine, but also more pantothenic acid, nicotinic acid, and biotin, which are essential for human nutrition (Ratnavathi, 2018).

II.2.4.5. Antinutritional Factors

All sorghums contain phenols, which can affect the color, appearance, and nutritional quality of grains and products (Ratnavathi, 2018). Phenolic compounds can be divided into three categories: flavonoids, phenolic acids, and tannins, which are considered major antinutritional factors in sorghum (Endalamaw et al., 2025).

II.2.4.5.1. Flavonoids

The most abundant group of flavonoids in sorghum is flavans, of which anthocyanidins are the most abundant compounds (Ratnavathi, 2018). These anthocyanidins, specific to sorghum, are called 3- deoxyanthocyanidins. Their distinctive feature lies in the absence of the hydroxyl group in position 3 of the C ring, a unique characteristic among plant anthocyanidins (Awika & Rooney, 2004). Among these 3-deoxyanthocyanidins, luteolinidin and apigeninidin are the major pigments responsible for the red color of sorghum (Zannou et al., 2025). Besides anthocyanidins, sorghum flavanoids also include flavan-3-ols ((+)-catechin and (-)-epicatechin)), flavan-4-ones (naringenin and eriodictyol), dihydroflavonols (taxifolin), flavones (apigenin and luteolin), and flavonols (kaempferol) (Bröhan et al., 2011).

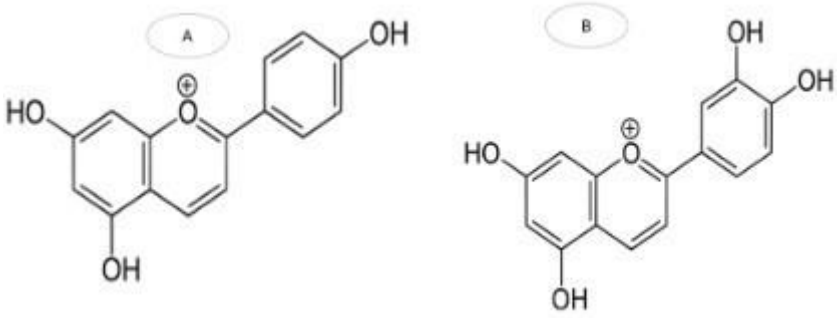

Figure 2: Structures of the major anthocyanidins in sorghum (Zannou et al., 2025)

(A) apigenidin (B) luteolinidin

II.2.4.5.2. Phenolic Acids

There are two main families of phenolic acids in sorghum: hydroxybenzoic acids and hydroxycinnamic acid (Ratnavathi, 2018). Ferulic acid is the most abundant phenolic acid in sorghum, while other phenolic acids, including syringic acid, protocatechuic acid, caffeic acid, p-coumaric acid, and sinapic acid, are also more abundant. These phenolic acids in sorghum are mainly concentrated in the bran and in bound form.

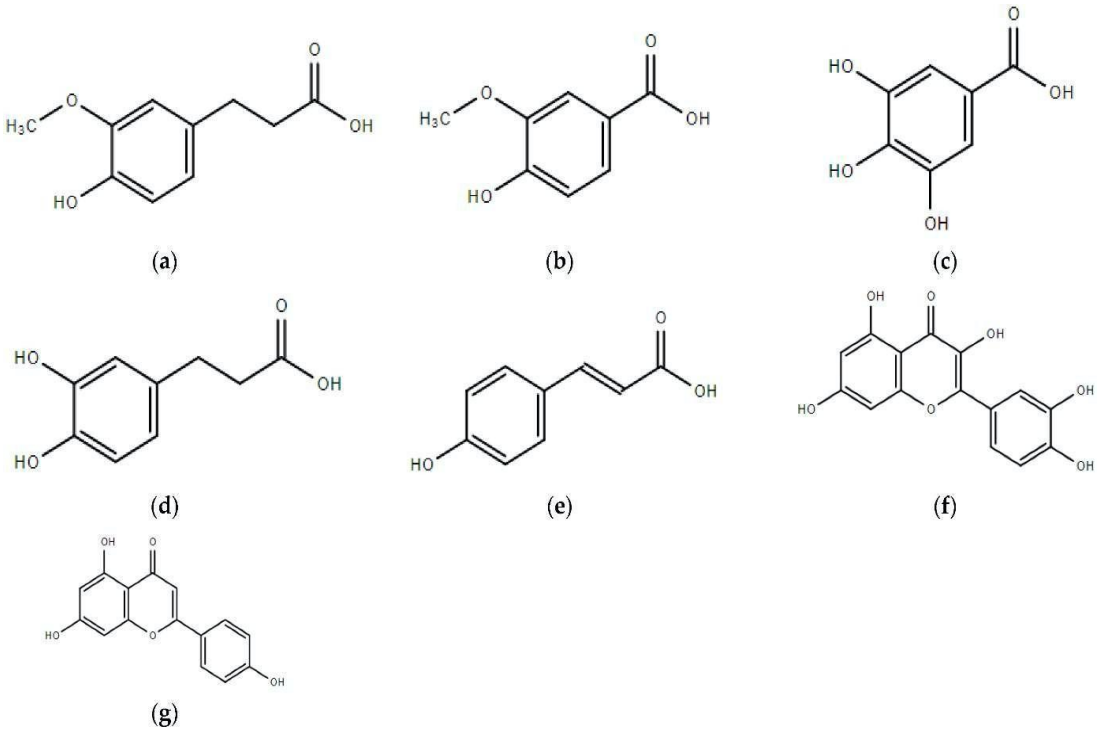

Figure 3: Structure of Phenolic acids in sorghum grain

(a) ferulic acid; (b) vanillic acid; (c) gallic acid; (d) caffeic acid; (e) p-coumaric acid; (f) luteolin; (g) apigenin (Stefoska-Needham, 2024)

II.2.4.5.3. Tannins

Grain sorghum contains tannins with a high molecular weight and a high degree of polymerization compared to other cereals, and these are the most studied polyphenols in sorghum. Tannin content ranges from 10.0 to 68.0 mg/g dry weight in tannin-rich sorghum compared to other cereals and legumes (0.5-3.8 mg/g for tannin-free sorghum, 3.6-13.1 mg/g for millet, 1.7 mg/g for buckwheat groats, and 1.8-2.9 mg/g for cowpea) (Dykes et al., 2005). Tannin concentrations in sorghum varieties vary considerably depending on their color. For example, red and brown grain sorghums contain more bioactive compounds, such as tannins, which are considered beneficial to human health and are widely used in the brewing and food industries (Abraha et al., 2015).

II.2.4.5.4. Stilbenes

Stilbenes are a small family of phenolic compounds derived from the phenylpropanoid pathway (Chong et al., 2009). They play an important role in both plant disease resistance and

human health. A positive relationship is observed between the number of stilbenes and grain color, as they are present in lower amounts in white varieties. For example, white sorghum contains low traces of trans-piceid, up to 0.1 mg/kg, but no trans-resveratrol. In contrast, red sorghum contains both substances (Bröhan et al., 2011).

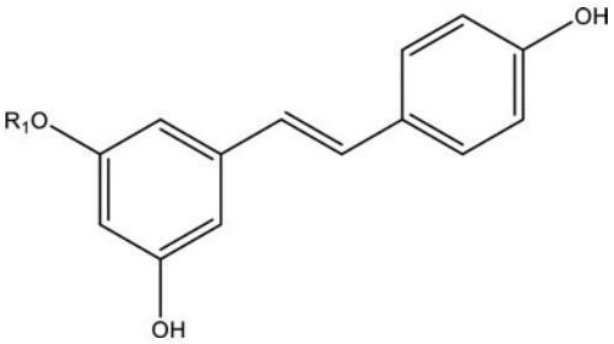

Figure 4: Structure of Stilbenes present in sorghum grain (Bröhan et al., 2011)**.**

trans-Resveratrol (R1 = H); trans-Piceid (R1 = Glucose)

II.2.5. Uses of Sorghum

Sorghum is one of the main food grains in Africa, as well as in parts of India and China. Approximately 500 million people worldwide consume sorghum in their daily diet (Che et al., 2018). Sorghum is a cereal whose behavior is very similar to that of wheat in certain baking applications, making it an easy- to-work, whole-grain, inexpensive, and gluten-free alternative to wheat. It can therefore be used for the production of bread, porridge, pasta, and alcoholic beverages (McGinnis & Painter, 2020).

Sorghum-based bread: Sorghum is a good substitute for wheat flour in gluten-free and whole-grain breads. Since it does not contain gluten, additives, such as egg, xanthan gum, or a soluble fiber supplement, are needed to simulate the characteristics of gluten in bread made with wheat (Taylor et al., 2006).

Pasta: As with bread production, sorghum grains are also used for noodle production due to their amylose content (Suhendro et al., 2000). Sorghum flour can also be combined with other flours (rice, corn, potato) and in different ratios to improve the quality of the noodles produced (Ferreira et al., 2016).

Sorghum syrup: Sorghum syrup is generally produced from a variety of sorghum called sweet sorghum or sorghum. Unlike other grain products, it is obtained by crushing the stalk and then concentrating its sap through heating, similar to maple syrup. It is the primary product

extracted from the stalk, not a by-product like molasses. Less processed than common sweeteners, it retains a richness in nutrients and antioxidants (Debebe et al., 2018).

II.2.6. Sorghum in the Brewing Industry

Sorghum can be used in brewing either as an adjunct in lagers or as the primary raw grain for brewing 100 % sorghum beers. Thus, pale lagers made from sorghum have been produced in many parts of the world. It has been used in the United States as an adjunct in lagers since the 1980s. However, the most significant advances in sorghum brewing have been made in Nigeria. Following the government's ban on the importation of barley malt in 1988, Nigerian brewers had to turn to local grains such as maize and sorghum to replace malted barley (Dabija et al., 2021).

II.2.6.1. Example of beers brewed with Sorghum

Traditional Beers: In Africa, various types of traditional fermented beverages have been described, commonly referred to as opaque beers or sorghum beers. These beverages have socio-cultural and nutritional value. They are known as Dolo in Burkina Faso, Ikagage in Rwanda (Lyumugabe et al., 2012), Amgba in Cameroon (Ronald & Roger, 2017), Pito or Bburukutu in Nigeria and Ghana, Merissa in Sudan, Doro or Chibuku in Zimbabwe, Bili Bili in Chad, Mtama in Tanzania, Tchapalo in Côte d'Ivoire, Togo, and Benin, and Kaffir in South Africa (Dabija et al., 2021). These beverages constitute an essential element of African culture. Indeed, in these countries, sorghum beer is commonly consumed during festivals, weddings, rituals, birth ceremonies, and funeral rites (Dabija et al., 2021).

Modern Beers: In Africa, many contemporary beers are brewed primarily from sorghum alone or in combination with other grains (malted barley, millet, maize, etc.). This practice is evident in Uganda, Botswana, Ghana, Nigeria, Burkina Faso, Sierra Leone, Tanzania, Zambia, and Zimbabwe, with iconic brands such as Eagle Lager, Eagle, Star, Gulder, Libs Sorghum, Harp, and Tusk have emerged (Wani & Minja, 2025; Tétédé Rodrigue KONFO et al., 2015). Furthermore, even in Europe, Asia, and America, sorghum beer is an option for people with celiac disease due to the absence of gluten in the grain (Bogdan & Kordialik-Bogacka, 2017).

Figure 5: Images of modern beers made from sorghum (Noel, 2015)

II.2.6.2. Limitations of sorghum use in brewing and possible solutions

Limitations: The use of sorghum malt in beer production has posed some challenges, mainly due to its low amylolytic activity (which is insufficient for complete saccharification), its high gelatinization temperature, and its low free amino nitrogen content. Sorghum malt exhibits higher α-amylase activity but lower β-amylase activity than barley malt. Reduced enzyme activity can lead to insufficient production of fermentable sugars (which, combined with low FAN content, makes the wort less fermentable), high dextrin content, and increased viscosity, thus creating problems with wort filtration and beer stability (Espinosa-Ramírez et al., 2013). In addition, the presence of huge amounts of polyphenols with a significant effect on the work of enzymes, and the formation of cloudiness both in hot and cold conditions (Bwanganga Tawaba et al., 2013). To overcome these problems, it is recommended to use exogenous enzymes during brewing.

Possible Solution: Use of Exogenous Enzymes

A few exogenous enzymes are currently used in brewing. Beta-glucanases are used for the degradation of beta-glucans and xylanases for the removal of pentosans when raw grains are used ("Essays in Brewing Science," 2007). Proteinases and amylases are used in high-gravity brewing. Amyloglucosidases are used in both brewing and fermentation. Acetoacetate decarboxylase is used in fermentation to bypass the production of diacetyl, papain, and propyl endopeptidase during maturation to eliminate polypeptides that cause turbidity in beer. Glucose oxidase/catalase is used in conditioned beers to eliminate oxygen. Some studies have shown that the use of exogenous β-amylase and amyloglucosidase would increase the fermentable sugar content of red sorghum wort (Espinosa-Ramírez et al., 2013). Companies such as

Customized Brewing Solutions (CBS BREW) have developed exogenous enzymes that can be used for brewing sorghum beers, including those used in this study. These include:

Sorgainase: This is a mixture of plant endoproteases and fungal exopeptidases, standardized to 160 tyrosine units, in association with a protein hydrolyzate. It facilitates proteolysis, which consists of the degradation of endosperm membrane proteins into smaller peptides or amino acids (CBS BREW | BREWING ENZYMES | Colloidal Stability, Beer Attenuation, Foam Stabilizer | BELGIUM, n.d.).

Liquamyl T: It is a thermostable alpha-amylase, ensuring the liquefaction of starch by endohydrolysis of alpha-1,4 bonds at high temperatures (90-100°C). It rapidly cleaves amylose and amylopectin chains, generating fragments of approximately 30 glucose units, which is manifested by a purple iodine color. This exogenous enzyme has an optimal pH between 4.5 and 6.5 and is only destroyed after several minutes at 100°C. The objectives of using Liquamyl T in the brewing process refer to its importance of rapidly liquefying gelatinized starch by working at high temperatures; preparing starch for subsequent saccharification with Sorgamyl, allowing higher maltose levels to be achieved and improving wort filtration efficiency when non-liquefied starch is causing filtration problems (CBS BREW | BREWING ENZYMES | Colloidal Stability, Beer Attenuation, Foam Stabilizer | BELGIUM, n.d.).

Sorgamyl: It is a mixture of plant and fungal maltogenic amylases capable of producing high levels of maltose by saccharifying sorghum starch. It is introduced after cooling the mash, before pitching, to increase its fermentable sugar content (CBS BREW | BREWING ENZYMES | Colloidal Stability, Beer Attenuation, Foam Stabilizer | BELGIUM, n.d.).

II.2.7. Use of Eucalyptus Ash in Malting

In East African countries, sorghum malting may involve the addition of wood ash (particularly eucalyptus) or wood ash extracts, either during steeping or just before germination. However, the effect of these ash remains poorly studied. However, (Kageruka et al., 2024) demonstrated that the addition of 5 % eucalyptus ash during malting affects various wort parameters, including an increase in the pH of wort, which is crucial for optimal enzyme activity during mashing; and a significant decrease in polyphenol content (up to 76%) which causes a subsequent increase in mashing yield (up to 69%). Addition of ashes has also impacted other characteristics of the wort, including color, colloidal stability, and taste.

II.3. History and Types of Ikigage Beer

Red sorghum (*Sorghum bicolor*) is the main ingredient in ikigage, a traditional fermented beverage from Rwanda, however other cereals like finger millet or maize are occasionally used. Ikigage has always played a significant role in Rwandan social gatherings, rites, and festivities. It is a significant symbol of hospitality and solidarity in addition to being a source of nourishment. Ikigage has centuries- old roots, with generations passing along the brewing techniques verbally. In order to malt sorghum grains, early brewers first soaked during sorghum for 12 hours, germinated with addition of either eucalyptus or banana ashes during 2-4 days, and dried the grains before mashing. The resulting wort undergoes spontaneous fermentation during 2-4 days at room temperature and Ikigage is produced (Muyanja et al., 2003).

Depending on regional variations and preparation techniques, several types of Ikigage can be distinguished:

Traditional Ikigage: Brewed purely from red sorghum with no additives; mildly alcoholic, slightly sour, and viscous.

Mixed Cereal Ikigage: Incorporates maize, millet, or cassava flour to supplement sorghum, often affecting the flavor and alcohol content.

Sweetened Ikigage: Sometimes enhanced with honey or sugarcane juice to produce a sweeter flavor and higher alcohol levels (Lyumugabe Loshima, 2013).

II.4. Formation of Chemical Reactions and Formulation of Ikigage Beer

The biochemical transformation in Ikigage brewing is driven by enzymatic and microbial activity. The major chemical reactions include:

1. **Malting Phase:**

   Enzymatic Activation: During germination, enzymes such as amylases and proteases are activated, breaking down starches into fermentable sugars and proteins into amino acids.

   Microbial Activity: Lactic acid bacteria (LAB) such as Lactobacillus spp. begin colonizing the mash, producing lactic acid and lowering the pH, which favors yeast growth.

2. **Mashing and Fermentation Phase:**

   Saccharification: Amylases convert complex carbohydrates into simpler sugars like

maltose and glucose.

Alcoholic Fermentation: Wild yeasts, mainly Saccharomyces cerevisiae and Candida species, ferment sugars into ethanol and carbon dioxide.

Acidification: LAB continue producing organic acids, contributing to the beverage's slight sourness and improving preservation.

These biochemical processes are vital for the flavor development, alcohol content, and safety of Ikigage.

1. **Formulation of Ikigage Beer:** Formulation typically follows malting of sorghum (soaking, germination, drying), grinding of malted sorghum, mixing with water to form mash, natural fermentation (2–5 days), optional addition of sweeteners (honey, sugarcane juice) and filtration and consumption.

CHAPTER III. MATERIALS AND METHODS

III.1. Samples Collection

III.1.1. Sorghum Grain Sampling

Red sorghum (*Sorghum bicolor*) grains were collected from local markets and farmers in Kirehe, Rusizi, Gicumbi and Gisagara districts from Rwanda. A composite sampling method was used. Random sub-samples (approximately 1kg each) were collected from different vendors and combined to form a representative batch in a district. Samples were be stored in the UR-CST laboratory, at 4°C until processing to avoid fungal growth and contamination.

III.1.2. Eucalyptus Ash Collection

Fresh Eucalyptus wood was burnt to collect ash. Ash was sieved (mesh size 2 mm) to remove large debris and stored in sterile, dry containers at room temperature for use during malting.

III.1.3. Ikigage Beer Collection

Microbial load and Aflatoxin content were analyzed from ikigage beer produced by traditional brewers from Kirehe, Rusizi, Gicumbi and Gisagara districts from Rwanda, and samples of produced beers were aseptically collected from local markets in the identidied areas and brought to the laboratory for analysis.

III.2. Laboratory Analysis of Raw Samples and Beer

III.2.1. Enumeration of the Microbial Flora

Twenty-five grams of solid sorghum were aseptically grinded and diluted with 225ml of diluent (sterile peptone water) followed by serial dilution by peptone water to the dilution factor of 10-6. All sorghum samples were isolated for total aerobic mesophilic flora and yeasts & molds. A duplicate of 0.1 ml from all dilution factors from 10^0 (original sample) to 10^{-6} (last dilution factor) was aseptically inoculated on appropriated culture media. For Total Aerobic Plate Count (APC), serial dilutions were plated on Plate Count Agar (PCA) and incubated at 37°C for 48 hours (*ISO 4833-1:2013 - Microbiology of the Food Chain — Horizontal Method for the Enumeration of Microorganisms — Part 1: Colony Count at 30 °C by the Pour Plate Technique*, n.d.). For Fungal (Yeast and Mold) Count, samples were plated on Potato Dextrose Agar (PDA) acidified with tartaric acid and incubated at 25°C for 5 days (Pitt & Hocking, 2009).

III.2.2. Determination of Total Aflatoxin

A ground sample, equivalent to 50 g dry matter, was blended with 5 g of sodium chloride and 100 ml mixture of methanol and water (4:1 v/v) at high speed for 1 min using a blender. The mixture was filtered through fluted filter paper (Whatman 2 V, Whatman, Middlex). The filtrate (15 ml) was diluted (1:4) with distilled water, re-filtered through glass microfiber filter paper (Whatman, Middlex, UK). 10 mL filtered extract into a clean vessel were diluted with 20 mL purified water, mixed well and filtered through 1.5µm glass microfiber filter into a clean vessel. AflaTest column was prepared by removing both end caps and gently shaking the buffer solution from the top of the column. By using a micro-pipettor, 1 mL of filtered extract was added to the column headspace and then the column was attached to a VICAM pump stand in order to pass the filtered extract completely through the column at a rate of about 1 drop/second until air comes through column. We washed the column with 1 mL of purified water at a rate of 1-2 drops/seconds and repeated once more until no bubbles came through the column. Aflatoxin was eluted from the column with 1 ml HPLC grade methanol at the rate of 1-2 drops/second into a glass cuvette, mixed with freshly made 1 ml Aflatest developer and its fluorescence measured in a pre-calibrated fluorometer. Limit of detection (LOD) was interpolated at 1.0 µg/kg. All samples that gave greater than 300 µg/kg aflatoxin were repeated.

III.3. Microbrewery Production of 4 Malted Red Sorghum Beers with and without Ashes, Fermented with S33 and LA-01 Yeasts, with the Addition of Exogenous Enzymes

Phsico-chemical and organoleptic characteristics were analyzed from Ikigage beer which was produced by a modern method in a microbrewery located at the Brewery and Food Industries Laboratory (INBr), Université Catholique de Louvain. The specific objectives of brewing were the production of 12% wort from ash-red sorghum malt from Rwanda, production of 12% wort from ash- free red sorghum malt from Rwanda, production of an alcoholic beer from ash-red sorghum malt using S33 yeast, production of an alcoholic beer from ash-free red sorghum malt using S33 yeast, production of a non-alcoholic beer from ash-red sorghum malt using LA-01 yeast, production of a non-alcoholic beer from ash-free red sorghum malt using LA-01 yeast and analysis of physicochemical and organoleptic properties of the beers produced.

III.3.1. Raw Materials

The raw materials used were malt (15 kg of ash-colored red sorghum malt and 15 kg of ash-free red sorghum malt from Rwanda) and water (Stock saline solution, diluted 100x in the wort to obtain 700 ml for 70 L of final wort).

Table 3: Chemical ingredients required for microbrewery beer production

Products	Preparation for 5 liters of concentrate	Concentration in (g/L) of concentrate	Final concentration (ppm) in the must
$CaSO_4.2H_2O$	7.25 g	1.45	14.5
Na_2SO_4	82.75 g	16.55	165.5
NaCl	169.5 g	33.90	339
$MgCl_2.6H_2O$	6.25 g	1.25	12.5
Na_2CO_3	229. 45 g	45.89	458.9
KCl	9.15 g	1.83	18.3
$FeCl_3.6H_2O$	0.1 g	0.02	0.2
KNO_3	0.45 g	0.09	0.9

Hops: 4g of supercritical hop extract (15 BU), at the start of the boil to reduce the risk of beer contamination.

Yeasts: S-33 (10 x 10^6 live cells/ml) and LA-01 (10 x 10^6 live cells/ml)

Adjuncts: ascorbic acid (60 ppm, 6 g/hL)**,** 60 ppm SO2 (10.5 g/hL KMS or potassium metabisulfite), 7.35 g KMS in 70 L of must**,** 25 ml Sorgainase: 0.02% w/w at mash (50°C)**,** 13 ml Liquamyl T: 6.5 ml (0.1% w/w) at 75°C, 5 ml (0.08% w/w) at 93°C and 1.5 ml (0.02% w/w) at 100°C and 1 ml of Sorgamyl (0.05% w/w) at the end of boiling, before fermentation.

III.3.2. Procedure

III.3.2.1. Preparation of Brewing Equipment

The day before production, all brewing equipment, both in the laboratory and the microbrewery, was prepared. In the microbrewery, the brewing equipment underwent a thorough 30-minute cleaning with a 2% chlorinated alkaline solution to remove organic

deposits. It was then rinsed with cold water, followed by an additional 30-minute cleaning with a 0.5% peracetic acid solution to remove mineral deposits. After this, it underwent another cold-water rinse, followed by a complete hot water rinse. Additionally, brewing equipment was prepared: saline solution, pH meter, ascorbic acid, KMS, refractometer, exogenous enzymes.

III.3.2.2. Brewing

On the day of brewing in the microbrewery, 15 kg of malted red sorghum with and without 5% eucalyptus ash from Rwanda were ground using a two-roller mill (roller pitch 0.9 mm) to obtain a fine grind suitable for filtration. Stock saline solution, then heated to mashing temperature. Mashing was carried out at 50 °C for 30 min (Figure 8) with 45 L of water in the presence of 300 mg/L of calcium at 50 °C and 25 ml of Sorgaïnase (0.02% w/w). In addition, 7.35 g of KMS (10.5 g/hL of potassium metabisulfite for 70 L of final must) and 4.2 g of ascorbic acid (6 g/hL for 70 L of final must) were added at the start of mashing to reduce lipoxygenase activity and therefore limit lipid oxidation. After this proteolytic phase, the mashing temperature was gradually increased to 93 °C (at a rate of 1 °C/min). When the temperature was increased to 75°C, 6.5 ml of Liquamyl T (0.1% w/w) was added. At 93°C, 5 ml of Liquamyl T (0.08% w/w) was added and a 15-minute pause was observed. The temperature was then increased at a rate of 1°C per minute up to 100°C, followed by a 5-minute pause in the presence of 1.5 ml of Liquamyl T (0.02% w/w).

Figure 6: Malted red sorghum with 5% eucalyptus ash and ashless malted red sorghum used for brewing

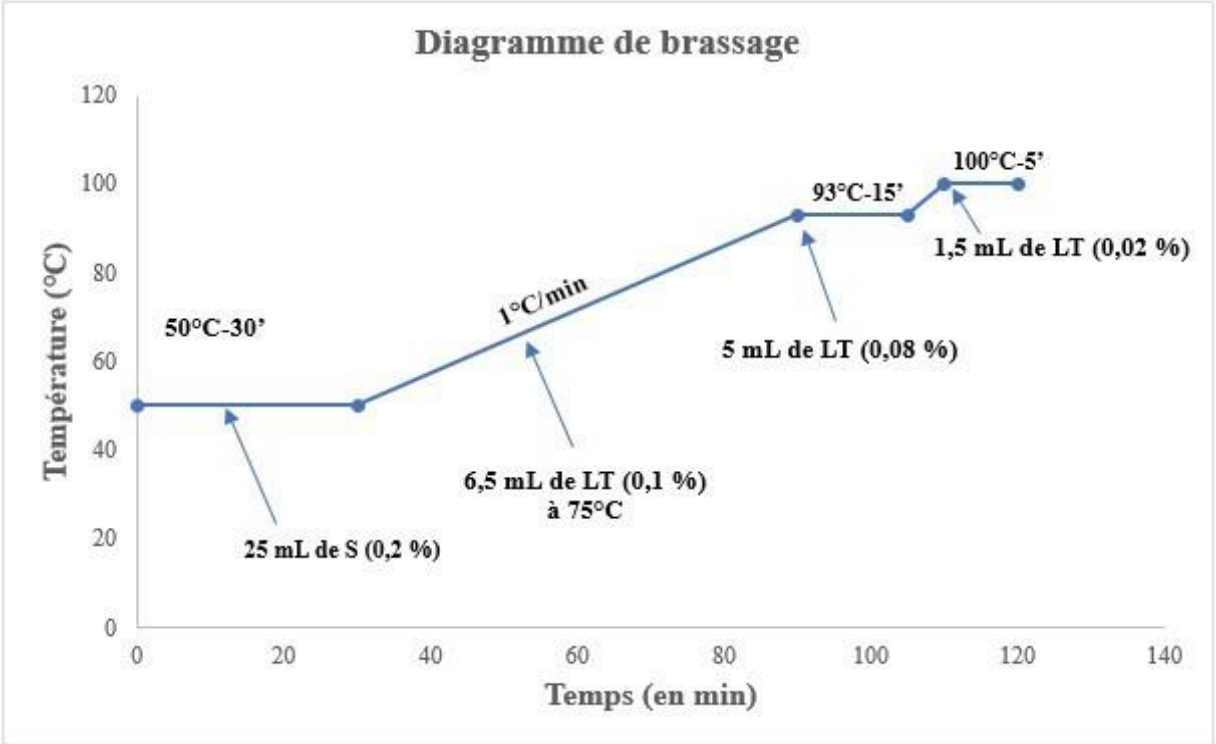

Figure 7: Diagram of the brewing flowchart adopted for the production of beers

S : Sorgainase / **LT** : Liquamyl T

III.3.2.3. Filtration

After brewing, the mash was filtered in the lauter tun with 2 kg of rice straw and 1.8 L of water preheated to 95°C, then left to stand for 20 minutes to allow the filter cake to form. The wort was then recirculated for 10 min through the cake until a clear wort was obtained. Once this objective was achieved, filtration was started and lasted 1 h. The density of the filtrate was regularly measured during filtration. The spent grains were then washed with 15.7 L of water preheated in the Brouwland of 20 L at 95 °C, bringing the malted sorghum wort with ashes to 13.0 °P for a volume of 62.5 L and the malted sorghum wort without ashes to 11.0 °P for the same volume before boiling. 10 L of demineralized water was used to dilute the malted sorghum wort with ashes to obtain 72.5 L of wort with an 11.2 °P, while 5.5 L of demineralized water was used to dilute the malted sorghum wort without ashes to obtain 68.0 L of wort with a 10.1 °P.

III.3.2.4. Boiling

The wort was then boiled for 60 minutes in the mash tun. At the beginning of boiling, 4g of supercritical CO2 hop extract was added to protect the wort from any contamination and to preserve the aromas brought by the sorghum. At the end of boiling, 68 L of ash-treated malted sorghum wort of 11.9 °P and 62 L of ash-free malted sorghum wort of 11.1 °P were obtained. The ash-treated malted sorghum wort was further diluted with 12 L of demineralized water to obtain 80 L of wort of 10.1 °P.

III.3.2.5. Whirlpool/Clarification

We then proceeded to clarify the wort using a whirlpool for 10 minutes. We then let the wort rest for 30 minutes to allow the trub cone to form.

III.3.2.6. Transfer and Cooling

The filtered and clarified wort was then transferred to previously rinsed and disinfected cylindrical- conical tanks for inoculation and to initiate fermentation. At the end of the previously observed resting time, 60 L of malted red sorghum wort with 5% ash was transferred to CCT1 for the production of an Ale (Ale SMC). Then, 1 ml of Sorgamyl was added (0.05% w/w) to saccharify the dextrins released during mashing by the Liquamyl T. The remaining wort was diluted with 14 liters of demineralized water to obtain 34 liters of wort at 6°P. 20 liters of this must were transferred into a plastic Brouwland fermenter for the production of a NABLAB (NABLAB SMC). For the ashless malted red sorghum wort, 40 liters of this wort were transferred to CCT2 for the production of an Ale (Ale SMNC) followed by the addition of Sorgamyl. The remaining wort was diluted to obtain a wort at 6°P, however we were only able to achieve 7°P after measurement. The diluted wort was transferred to a second plastic Brouwland fermenter for the production of an ashless malted red sorghum NABLAB (NABLAB SMNC)

III.3.2.7. Fermentation/ Storage/ Conditioning

Inoculation was carried out at 25°C in a sterile manner at a rate of 10 million live cells/ml for our four beers. Our different Ales underwent 6 days of fermentation, while the NABLABs underwent 2 days. The following table presents the yeast strains selected for our study and their characteristics.

Table 4: Characteristics of the yeast strains used

	Strains	IT (°C)	FT (°C)	FTe (days)	Beer
SafAle^{TM} S- 33	*Saccharomyces cerevisae*	18-25	25	6	Ale
SafBrew^{TM} LA – 01	*Saccharomyces cerevisiae* var. *chevalieri*	18-25	25	2	NABLAB

Note: IT : Inoculation temperature FT: Fermentation temperature / FTe: fermentation time

III.3.2.8. Maturation and Saturation

At the end of fermentation, our various beers were placed at 4°C for 14 days to mature. At the end of this maturation period, the Ales were saturated on site in their respective fermenters, while the NABLAB beers were transferred to kegs for saturation. All beers were saturated using CO_2 up to 6 g/L, followed by bottling. Samples of each beer were also taken to measure physicochemical parameters such as pH, color, turbidity, bitterness, FAN content, total polyphenols and total flavanoids, and fermentation aromas.

III.3.3. Characterization of Wort and Beer Samples

III.3.3.1. Measurement of Wort and Beer pH

The pH of the samples was measured using a pH meter according to the protocol described in EBC method 9.35.

III.3.3.2. Measurement of Wort Specific Gravity, Beer Alcohol Content, and Extracts

The wort specific gravity, alcohol content, and extracts (primary, actual, and apparent) of the samples were measured using the Anton Paar Beer Alcolizer (EBC method 9.2.6).

III.3.3.3. Measurement of Total Polyphenol Content in Wort/Beer

The total polyphenol content of our wort and beer samples was determined spectrophotometrically according to the protocol described in EBC method 9.11.

III.3.3.4. Measurement of Total Flavanoid Content in Wort/Beer

The total flavanoid content of our wort and beer samples was determined spectrophotometrically, according to the protocol described in EBC Method 9.12.

III.3.3.5. Measurement of α-Amino Acid Content in Wort/Beer

The α-amino acid content of our wort and beer samples was determined spectrophotometrically, according to the protocol described in EBC Method 8.10.1.

III.3.3.6. Measurement of Beer Turbidity

The turbidity of our beer was measured with a Hach nephelometer using EBC method 9.24.

III.3.3.7. Beer Bitterness Measurement

The bitterness of our beer samples was measured using a spectrophotometer, according to the protocol described in EBC method 9.8.

III.3.3.8. Wort/Beer Color Measurement

The color of our samples was measured using the ASBC spectrophotometric method.

III.3.3.9. Aroma Analysis Using the SAFE Method

SAFE (Solvent-Assisted Flavor Evaporation) is a non-selective method for extracting volatile molecules. It is based on a gentle distillation process at a temperature of approximately 40°C and a very low pressure, around 10^{-4} mbar. In this process, volatile molecules are entrained by a solvent, such as water or dichloromethane, and then condensed in a device cooled with liquid nitrogen.

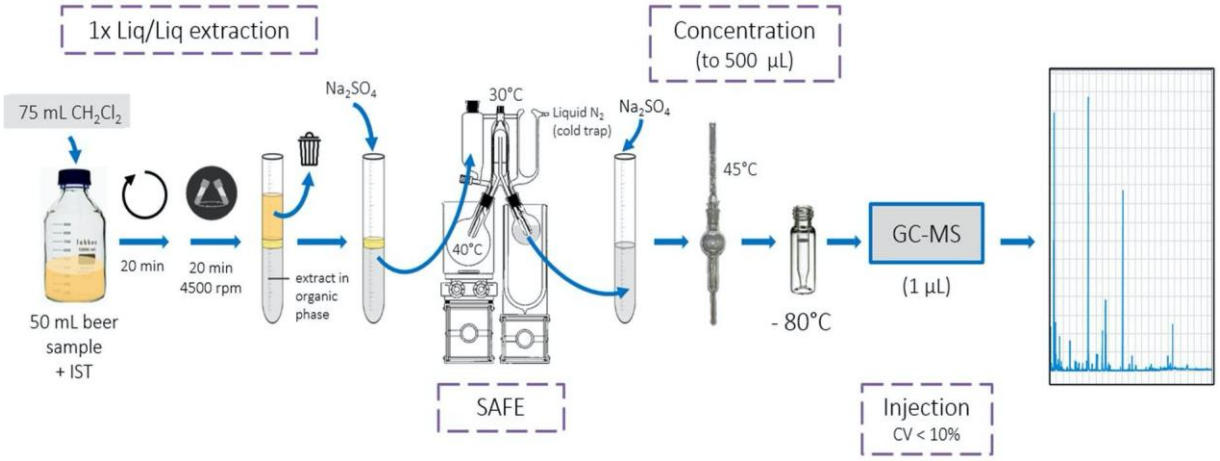

Figure 8: Principle of aroma extraction using the SAFE method

Procedure: Degassed 50 ml samples were spiked with 150 µL of 2-acetylthiophene solution, which had a concentration of 8 mg/L, serving as an internal standard, to obtain a final concentration of 24 mg/L in the beer. These samples were then extracted using 75 ml of double-distilled dichloromethane on a stir plate at 1000 rpm for 20 minutes. The mixture was then placed in a separating funnel to separate the organic phase from the aqueous phase. The remaining organic phase was then dried with a spoonful of anhydrous sodium sulfate.

Finally, the non-volatile compounds were separated by vacuum distillation using a SAFE system (Glasblaeserei Bahr, Manching, Germany). The conditions for SAFE analyses were as follows: water bath temperature at 40 °C, pressure less than 10^{-4} Pa and the apparatus body at 30 °C. The distillate was continuously collected in the liquid nitrogen-cooled SAFE flask for 15 minutes. Then, the distillate underwent extraction with demineralized water (3 × 17 ml), the objective being to eliminate any residual alcohol. The various extracts obtained were then dried over anhydrous sodium sulfate. To measure recovery rates, 50 µL of n-decane solution (50 mg/L) was added as an external standard (EST) to achieve a final concentration of 5 ppb in the extract, before concentration to 500 µL in a Danish-Kuderna apparatus at 45°C. To ensure maximum stability, the extracts were stored at -80°C until GC-MS analysis.

CHAPTER IV. RESULTS AND DISCUSSION

IV.1. Results

IV.1.1. Evaluation of the Effect of Eucalyptus Ashes on Physico-Chemical Characteristics of the Wort.

After boiling, wort samples were taken to characterize them before inoculation. The wort results are summarized in the table below:

Table 5: Characteristics of the wort after boiling

Parameters	SMNC wort	SMC wort
pH	5.8	6.4
Total Polyphenol Content (mg/L)	1492	242
FAN Content (mg/L)	313	399
Total Flavanoid Content (mg/L)	509	13

IV.1.2. Monitoring the Impact of Eucalyptus Ashes on the Physico-Chemical Parameters of Wort during Fermentation

After inoculating our musts, different parameters were monitored over a 6-day period. These include pH, apparent extract (Ea), true extract (Er), and Alcohol content (%v/v).

Table 6: Changes in parameters during fermentation

Parameters	0 h	12 h	1st day	2nd day	3rd day	4th day	5th day	6th day
pH								
ALE SMC	6.4	4.9	4.7	4.7	4.7	/	/	4.7
ALE SMNC	5.8	4.1	4.1	4.1	4.1			4.1
NABLAB SMC	6.4	5.6	5.3	5.0				
NABLAB SMNC	5.8	4.8	4.8	4.8				
Ea (°P)								
ALE SMC	10.1	5.1	4.4	3.8	3.5	/	/	3.3
ALE SMNC	11.1	6.5	5.4	4.8	4.2			3.9
NABLAB SMC	6.0	5.2	5.2	5.2				
NABLAB SMNC	7.0	6.0	6.0	6.0				

Er (°P)								
ALE SMC	10.1	6.1	5.5	5.0	4.7	/	/	4.7
ALE SMNC	11.1	7.3	6.5	5.6	5.5			3.2
NABLAB SMC	6.0	5.3	5.3	5.3				
NABLAB SMNC	7.0	6.2	6.2	6.2				
Density (Alcohol (% v/v))								
ALE SMC	0.0	2.6	2.9	3.3	3.5	/	/	3.6
ALE SMNC	0.0	2.3	3.1	3.3	3.6			3.8
NABLAB SMC	0.0	0.4	0.4	0.4				
NABLAB SMNC	0.0	0.5	0.5	0.5				

IV.1.3. Evaluation of the Effect of Eucalyptus Ashes on Physico-Chemical Characteristics of the Produced Beers

Physicochemical characteristics such as Color, Bitterness, Turbidity, FAN, Total Polyphenols, and Total Flavonoids were measured after the beers had matured. The table below presents the results obtained.

Table 7: Physicochemical Characteristics of the Finished Beers

Parameters	ALE SMNC	ALE SMC	NABLAB SMNC	NABLAB SMC
pH	4.3	4.9	5.3	6.2
Color (°EBC)	93.8	86.3	79.3	52.8
Bitterness (BU)	13.9	11.6	6.8	6.0
Turbidity (EBC)	197	249	497	588
FAN (mg/L)	30	25	24	30
Total Polyphenols (mg/L)	974	163	832	71
Total Flavanoids (mg/L)	191	25	306	19

IV.1.4. Effect of Eucalyptus Ashes on the Aroma Profile of the Produced Beers using SAFE Method

Aroma profile of the beers was performed to determine the aromas that red sorghum malt can contribute to the beer, but also to see if ash could influence the aroma profile of our beers. The table below presents the different compounds revealed by this analysis.

Table 8: Aromas contributed by malted red sorghum (with and without added ash) in two Ales and two NABLAB beers

Types	IK	tr (min)	Name of compound	Perception thresholds (ppm)	Ale SMC (ppb)	Ale SMNC (ppb)	NABLAB SMC (ppb)	NABLAB SMNC (ppb)
Esthers		6.292	Ethyl Propanoate		25	32	9	n.d
	780	8.649	Ethyl Butanoate		10	12	n.d	n.d
	854	11.668	Isoamyl Acetate	0.5	101	102	9	8
	857	11.822	2-methylbutyl Acetate		10	11	n.d	n.d
	926	15.640	Methyl Thioisovalerate		20	21	n.d	n.d
	932	16.082	3-methylpentyl Acetate		9	9	n.d	n.d
	952	17.495	Isoamyl Propanoate		42	89	11	11
	979	19.346	Ethyl Caproate	0.2	48	53	n.d	n.d

	1202	42.104	Phenylethyl Acetate		211	48	n.d	n.d
	1306	54.915	Methyl Geranate		19	19	n.d	n.d
	1381	64.265	Ethyl Caprate		104	33	9	n.d
	1581	79.893	Ethyl Dodecanoate		43	n.d	n.d	n.d
	1632	82.679	Isoamyl Decanoate		61	n.d	n.d	9
	1980	97.252	Ethyl Palmitate		108	16	25	n.d
Phenols	961	18.111	Phenol		22	116	101	169
	1069	27.461	2-Guaiacol	0.07	37	42	n.d	16
	1150	36.141	4-Ethylphenol	0.3	13	19	n.d	n.d
	1294	53.496	4-Vinylguaiacol	0.3	217	250	424	603
	1420	68.216	2-Acetylphenol		48	65	n.d	34
	1543	77.568	4-Vinylsyringol		28	31	n.d	9
Alcohols		5.431	Butan-1-ol		16	13	n.d	n.d
		5.870	2-Methylbutan- 2-ol		34	31	37	37
	723	7.024	Isoamylic Alcohol	50-70	7955	7763	979	936

	725	7.084	2-Méthylbutanol		679	784	925	910
	907	14.268	3-Isopropylpentan-1-ol		13	12	14	14
	952	17.489	Methionol		249	599	n.d	n.d
	1100	30.363	2-Phenylethanol	40-125	16841	17846	1586	2629

IV.1.5. Effect of Eucalyptus Ash on Microbial Flora

Table 9: Effect of eucalyptus ash on microbial flora and aflatoxins in red sorghum malt

Sample type	Tested samples (n)	Total Mesophilic bacteria (CFU/ml)	Yeasts (CFU/ml)	Moulds (CFU/ml)
Unmalted Sorghum	4	2.1×10^6	2.0×10^5	6.5×10^5
Ashless malted sorghum	6	6.83×10^7	1.88×10^7	8.65×10^6
Malted sorghum with 5% eucalyptus ash	6	8.87×10^6	5.7×10^6	2.2×10^5

IV.1.6. Effect of Eucalyptus Ashes on Total Aflatoxin in Malted Red Sorghum Samples

Table 10: Total aflatoxin contamination in sorghum malt with and without the addition of Eucalyptus ash during the malting process

Sample type	Tested samples (n)	Positive samples (%)	Min – Max (µg/kg) (Positive samples)	Average (µg/kg)
Ashless malted sorghum	6	100	5.5 – 47	30.58
Malted sorghum with ashes	6	83.33	0.5 – 1.2	0.47

IV.2. Results Discussion

IV.2.1. Evaluation of the Effect of Eucalyptus Ashes on Physico-Chemical Characteristics of the Wort

The pH of the ashless malted red sorghum wort (wort of malted red sorghum without ashes) was 6.5 at the beginning of mashing and stabilized at 6.4 until the end of the boil. The one of the ash-malted red sorghum (wort of malted red sorghum with ashes) was 5.9 at the beginning of the mash and 5.8 at the end of the boil. These results are consistent with those obtained by

(Kageruka et al., 2024), showing that the addition of 5 % ash during malting or mashing would contribute to raising the pH and thus promoting the action of enzymes involved in mashing. In addition, the total polyphenol and flavanoid contents were higher in the SMNC wort than in the SMC wort. These results are similar to those obtained by (Kageruka et al., 2024) who demonstrated that the addition of 5% eucalyptus ash during malting of red sorghum significantly reduced the content of total polyphenols and total flavanoids in the wort obtained after brewing.

Finally, the alpha amino acid content of the wort was higher in the SMC wort compared to that obtained in the SMNC wort. These results are similar to those obtained by (Claver et al., 2011), who demonstrated that soaking and malting sorghum grains in the presence of wood ash extract increases their essential amino acid content (Claver et al., 2011).

IV.2.2. Monitoring the Impact of Eucalyptus Ashes on the Physico-Chemical Parameters of Wort during Fermentation

The evolution of pH during fermentation varied depending on the type of beer and the malting method. Overall, a rapid drop in pH is observed during the first 24 hours, followed by a stabilization from the second day onward. For Ales, the pH dropped from 6.4 to 4.7 (SMC) and from 5.8 to 4.1 (SMNC) within the first 48 hours. This trend continued during the first days of fermentation, with slightly higher pH values for beers brewed with malted red sorghum with ash. The same observation is made for NABLAB beers, where the NABLAB SMNC had a lower pH than the NABLAB SMC at each measurement point. This result can be explained by the production of organic acids during fermentation by the yeast, which gradually lowers the pH. This difference could also be explained by the effect of the ash, which increases the pH of the wort, as discussed before. These results are similar to those (Lyumugabe et al., 2010) who showed that traditional Ikigage and Merissa beers (brewed with malted red sorghum) have relatively low pH (3.9 ± 0.46) (Lyumugabe et al., 2010).

The evolution of apparent extract (Ea), true (real) extract (Er), and alcohol content (% v/v) showed differences between beer types and the presence/absence of ash in sorghum malt. For Ales, initial extract values were 10.1 °P for SMC and 11.1 °P for SMNC and gradually decreased. A sharp drop is noted from the first day of fermentation, confirming good yeast activity. On day 6, apparent extract reached 3.3 °P (SMC) and 3.9 °P (SMNC), while true extract droped to 4.7 and 3.2 °P, respectively, with alcohol levels reaching 3.6 and 3.8% v/v. This result is consistent with observations that yeasts consume fermentable sugars (maltose,

maltotriose), generating ethanol and CO_2, which lowers the apparent extract and increases the alcohol (% v/v). These results suggest that ashless malted red sorghum has a slightly more advanced fermentation, this probably due to the difference in the primitive extract of the two musts (the primitive extract of the SMNC must being higher than that of the SMC). (Lyumugabe et al., 2012) indicate in their work that traditional sorghum-based beers have alcohol contents between 2 and 4.5% v/v (Lyumugabe et al., 2012). For NABLAB beers, fermentation was interrupted after two days before being warned, and alcohol production therefore remains very low (≤ 0.50 % v/v), which corresponds to the standards for alcohol-free beers (*Traité de Brasserie*, 2022).

IV.2.3. Evaluation of the Effect of Eucalyptus Ashes on Physico-Chemical Characteristics of the Produced Beers

SMNC Ale beer had a color of 93.8°EBC compared to 86.3°EBC for SMC, while NABLAB SMNC beer had a color of 79.3°EBC compared to 52.8°EBC for SMC. SMNC-based beers have a stronger color than SMC-based beers, due to their high concentration of polyphenols responsible for this red (pink) color. Eucalyptus ash treatment reduces the polyphenol content in both wort and beers and therefore significantly reduced the color intensity of SMC-based beers.

The turbidity of the beers was influenced by the type of sorghum used. In both beer styles, samples made from ash malted sorghum (SMC) had higher turbidity than those made from ashless sorghum (SMNC), with significant differences in both NABLAB (588 EBC for SMC vs. 497 EBC for SMNC) and Ale (249 EBC for SMC vs. 197 EBC for SMNC). This increased turbidity may be explained by incomplete fermentation in NABLAB, which results in reduced yeast flocculation. The addition of ash appears to further enhance this phenomenon, thus affecting the colloidal stability of all SMC-based beers.

Free Assimilable Nitrogen (FAN) content varied slightly between the beers. The SMNC Ale beer had a FAN content of 30 mg/L compared to 25 mg/L for the SMC. In the NABLAB beer, the SMC also reached 30 mg/L, while the SMNC reached 24 mg/L. When compared to the FAN contents of the respective musts (for the SMNC and SMC musts) from which they were derived, these results show that the yeast was able to extract the maximum amount of free nitrogen for its metabolism, which led to good fermentation of our different beers.

From an antioxidant perspective, marked differences emerged. Total polyphenols were much more abundant in the ashen beers: 974 mg/L for the SMNC Ale compared to only 163 mg/L

for the SMC. NABLAB SMNC contained 832 mg/L of polyphenols, while NABLAB SMC contained only 71 mg/L. These results sufficiently demonstrate the effect of ash on the polyphenol content in beer. Such a reduction in the polyphenol content in beer improves other beer characteristics such as its brightness and taste profile (by reducing its astringency) (*Traité de Brasserie*, 2022).

Finally, total flavanoids followed similar but not strictly parallel trends to those of polyphenols. In Ale beers, SMC had a concentration of 245 mg/L compared to 191 mg/L for SMNC. In NABLAB beers, SMNC reached 306 mg/L compared to 19 mg/L for SMC. These results confirm the reducing effect of ash on polyphenols (and therefore the flavanoids that are part of them) in the beers produced.

IV.2.4. Effect of Eucalyptus Ashes on the Aroma Profile of the Produced Beers using SAFE Method

SAFE analysis identified several families of volatile compounds in the four beers, including esters, higher alcohols, and phenolics. Generally, beers produced from ash-free sorghum (SMNC) had slightly higher concentrations of aromatic compounds, higher alcohols, and certain esters. The detected esters, such as Isoamyl acetate, Ethyl propanoate, and Ethyl caprate, as well as higher alcohols such as Isoamyl alcohol and 2-phenylethanol, are generally associated with pleasant fruity notes in the beer. SMNC Ale beers generally had higher ester and higher alcohol levels than SMC beers, although these concentrations remained below the perception threshold. This trend was also observed in the NABLAB beers. These differences were not sufficiently pronounced and did not allow us to confirm the effect of ash on these compounds.

Furthermore, concentrations of 4-vinylguaiacol (4-VG), responsible for a typical clove aroma, are significantly higher in SMNC-based beers than in SMC-based beers (424 ppb for NABLAB SMC versus 603 ppb for NABLAB SMNC and 217 ppb for Ale SMC versus 250 ppb for Ale SMNC), with levels exceeding their perception threshold. This compound results from the enzymatic decarboxylation of ferulic acid, the main phenolic acid present in sorghum (Ratnavathi, 2018). This reaction is catalyzed by POF(+) yeasts, at an estimated rate of approximately 140 ppb per day (*Traité de Brasserie*, 2022). 4-VG can also be generated during wort boiling by thermal degradation of ferulic acid, when the temperature exceeds 100°C. These mechanisms explain the high 4-VG levels observed in beers made from ashless sorghum (SMNC).

Furthermore, our previous results have shown that ash treatment significantly reduces the total polyphenol content in beer. Ferulic acid, classified as a phenolic acid (and therefore a sorghum polyphenol), is therefore directly affected. This leads us to believe that the use of eucalyptus ash reduces the 4-VG content in SMC beers by reducing the content of its precursor, ferulic acid, in sorghum grains.

IV.2.5. Effect of Eucalyptus Ash on Microbial Flora

Untreated red sorghum malts exhibited higher counts of both bacteria and fungi, which can significantly contribute to aflatoxin accumulation and spoilage. Ash-treated malts showed significantly reduced microbial loads, indicating that eucalyptus ash is effective in reducing contamination during the malting stage. These observations are in line with the initial hypotheses of the study and confirm that Eucalyptus ash has practical potential as a natural antimicrobial agent in traditional brewing.

The incorporation of eucalyptus ash during the malting of red sorghum had a significant inhibitory effect on microbial flora, particularly total aerobic mesophilic bacteria and fungi (yeasts and molds). This is strongly supported by the results in Section IV.1.5 where malted sorghum treated with 5% eucalyptus ash consistently showed lower microbial counts than untreated sorghum. The antimicrobial efficacy of eucalyptus ash can be attributed to its alkaline nature and mineral composition. Eucalyptus ash is rich in metal oxides such as calcium, potassium, and magnesium, which raise the pH of the malting environment. This increased alkalinity creates an unfavorable condition for the growth of many bacteria and fungi, which typically thrive in slightly acidic to neutral environments.

Additionally, bioactive compounds such as eucalyptol, tannins, and flavonoids naturally present in Eucalyptus ash may exert direct antimicrobial and antifungal effects, contributing to the suppression of spoilage organisms and toxin-producing fungi like *Aspergillus spp.*

IV.2.6. Effect of Eucalyptus Ashes on Total Aflatoxin in Malted Red Sorghum Samples

The addition of eucalyptus ash during malting of red sorghum can be a good method in the traditional African context to reduce the level of mold and aflatoxin contamination without harming the quality of sorghum malt. In sample analyzed, the incidence of aflatoxin contamination in sorghum malted with addition of vegetable ash was very low, with aflatoxins being detected in 5 (0.5 and 1.2 µg/kg) of the 6 samples. However, the incidence of aflatoxin

contamination was high in the samples of sorghum malted without addition of vegetable ash samples, as aflatoxins were detected in all of them (5.5 – 47 μg/kg).

The susceptibility of food product to fungal attack occurs during pre-harvest, transportation, storage, and processing of the foods. Dietary exposure to aflatoxins may result in severe toxic and carcinogenic outcomes in humans and animals. Aflatoxin toxicity may result in nausea, vomiting, abdominal pain, convulsions, and other signs of acute liver injury. Long-term exposure also leads to various complications like growth retardation, cirrhosis, and hepatocellular carcinoma (Ayush R Aj Dhakal, n.d.) According to the Codex Alimentarius Commission (1995), the maximum limit level of aflatoxins (sum of AFB1, AFB2, AFG1, and AFG2) in foods is 10 μg /kg. In the present study, malted sorghum without ash addition exhibited higher aflatoxin levels (average of 30.58 μg/Kg) while all samples of malted sorghum with ash addition exhibited lower aflatoxin levels (average of 0.47 μg/kg), the average value lower than this maximum permissible level. The diminution of both microbial flora and aflatoxin content when the Eucalyptus ash is added during the malting process can be explained by the mineral's composition of ash. In fact, (Rufino et al., 2010) reported the high levels of calcium, potassium, magnesium, iron and zinc in the wood ash. The mixture of alkaline ions, Ca and K may result in attractive functional properties. Alkalis have been used as antimicrobial agents since ancient times and their antimicrobial ability is a function of the degree of dissociation and thus the interaction of the –OH and H+ in the cytoplasmic membrane of microbial cells, resulting in their death. The microbial proliferation, especially yeast and fungi, during barley malting was reduced by the use of alkaline washes. The addition of dilute calcium hydroxide [$Ca(OH)_2$)/ (lime)], produced when calcium oxide is added to water and sodium hydroxide (NaOH) during barley steeping, has been shown to inhibit microbial growth. The sorghum steeping in $Ca(OH)_2$ (2000 ppm) prevented bacteria and mold growth without affecting the malting loss, diastatic power, cold and hot water extracts of sorghum malt (Ogundiwin et al., 1991). (Lefyedi & Taylor, 2006)reported also that steeping sorghum grains in dilute NaOH reduces the levels of coliforms and mold contamination (to undetectable levels with some mold species) and improves the sorghum malt quality by increasing the diastatic power.

To assess aflatoxin exposure due to drinking of the traditional beer, it is also necessary to know the level of beer consumption in African communities. The research conducted by (Musabanganji et al., 2023) reported that the production of Rwandan traditional sorghum beer is profitable (net margin of RwF 9,663 per week, from weekly sales of 234 liters on average)

and the majority of the consumers started consuming when they were less than 15 years and reported also that they consume the sorghum beer two or three times per week (43.6 per cent), while 22.6 per cent take it every day. However, consumption of this traditional sorghum beer in rural may lead to a risk of aflatoxin exposure. To well evaluate the distribution of aflatoxin contamination, it will be necessary to determine the aflatoxin level in finished traditional sorghum beers.

CHAPTER V. CONCLUSION AND RECOMMENDATIONS

V.I. Conclusion

The aim of this study was to investigate the impact of adding eucalyptus ash to red sorghum on microbial load, aflatoxin content and on physicochemical and organoleptic properties of produced beers. It was found that this treatment significantly modified the wort composition and the quality of the resulting beers. The addition of ash increased the wort pH, thus promoting enzymatic activity during brewing. Furthermore, the ash increased the FAN content, which could promote better yeast activity, although the SMNC and SMC Ale beers had similar alcohol contents (3.8% and 3.6% v/v, respectively). Furthermore, it also led to a significant reduction in the polyphenol and flavonoid levels, and therefore in the total antioxidant capacity of the SMC beers.

Beers made from ashless sorghum (particularly Ale) generally presented a more marked aromatic richness, with a greater diversity and intensity of esters, higher alcohols and phenols, although all remained below the perception threshold. Most of these odorous compounds were also found and perceived in the SMC Ale beer, thus suggesting that ash did not significantly modify the aromatic profile of the beers. This study also highlighted the effect of ash on 4-vinylguaiacol, the concentration of which was significantly reduced in SMC-based beers.

V.2. Recommendations

Based on the results of this study, different recommendations are proposed. First of all, Eucalyptus ash in traditional brewing should be adopted. Small-scale and traditional brewers of Ikigage beer in Rwanda should consider integrating 5% eucalyptus ash during the malting process to naturally reduce microbial contamination and aflatoxin levels without compromising the organoleptic quality of the final product.

The Rwandan government and different institutions, through initiatives such as “Made in Rwanda” and EDPRS 3, should support the dissemination and training of local brewers on the use of eucalyptus ash as a sustainable method to improve food safety and beer quality and public health authorities should raise awareness about the risks associated with aflatoxins in traditional sorghum-based products and promote safe, affordable mitigation strategies such as the use of eucalyptus ash.

Lastly, regarding the standardization of ash usage protocols, further research should be undertaken to standardize eucalyptus ash preparation, dosage, and application techniques to ensure consistent quality and safety in traditional beer production.

REFERENCES

A ayush R aj Dhakal. (n.d.).

Abraha, T., Githiri, S. M., Kasili, R., Araia, W., Nyende, A. B., Abraha, T., Githiri, S. M., Kasili, R., Araia, W., & Nyende, A. B. (2015). Genetic Variation among Sorghum (Sorghum bicolor L. Moench) Landraces from Eritrea under Post-Flowering Drought Stress Conditions. *American Journal of Plant Sciences*, *6*(9), 1410–1424. https://doi.org/10.4236/AJPS.2015.69141

Aron, P. M., & Shellhammer, T. H. (2010). A discussion of polyphenols in beer physical and flavour stability. *Journal of the Institute of Brewing*, *116*(4), 369–380. https://doi.org/10.1002/J.2050-0416.2010.TB00788.X

Awika, J. M., & Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, *65*(9), 1199–1221. https://doi.org/10.1016/J.PHYTOCHEM.2004.04.001

Batish, D. R., Singh, H. P., Kohli, R. K., & Kaur, S. (2008). Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management*, *256*(12), 2166–2174. https://doi.org/10.1016/J.FORECO.2008.08.008

Bean, S. R., Wilson, J. D., Moreau, R. A., Galant, A., Awika, J. M., Kaufman, R. C., Adrianos, S. L., & Ioerger, B. P. (2019). *Structure and Composition of the Sorghum Grain*. 173–214. https://doi.org/10.2134/AGRONMONOGR58.C9

Berenji, J., Dahlberg, J., Sikora, V., & Latkovi, D. (2011). Sorghum bicolor (L.) Moench. *Economic Botany*, *65*(2), 190–208. https://doi.org/10.1007/S12231-011-9155-2

Bogdan, P., & Kordialik-Bogacka, E. (2017). Alternatives to malt in brewing. *Trends in Food Science and Technology*, *65*, 1–9. https://doi.org/10.1016/J.TIFS.2017.05.001

Bröhan, M., Jerkovic, V., & Collin, S. (2011). Potentiality of red sorghum for producing stilbenoid-enriched beers with high antioxidant activity. *Journal of Agricultural and Food Chemistry*, *59*(8), 4088–4094. https://doi.org/10.1021/JF1047755

Bwanganga Tawaba, J.-C., Khady Ba, Jacqueline Destain, Paul Malumba, François Béra, & Philippe Thonart. (2013). Vers une intégration du sorgho comme matière première pour la brasserie moderne (synthèse bibliographique). *Https://Popups.Uliege.Be/1780-4507*, *17*(4), 622–633. https://popups.uliege.be/1780-4507/index.php?id=10435

CBS BREW | BREWING ENZYMES | colloidal stability, beer attenuation, foam stabilizer | BELGIUM. (n.d.). Retrieved July 29, 2025, from https://www.cbsbrew.com/portfolio-item/brewing-enzymes/

Che, P., Anand, A., Wu, E., Sander, J. D., Simon, M. K., Zhu, W., Sigmund, A. L., Zastrow-Hayes, G., Miller, M., Liu, D., Lawit, S. J., Zhao, Z. Y., Albertsen, M. C., & Jones, T. J. (2018).

Developing a flexible, high-efficiency Agrobacterium-mediated sorghum transformation system with broad application. *Plant Biotechnology Journal*, *16*(7), 1388–1395. https://doi.org/10.1111/PBI.12879

Chong, J., Poutaraud, A., & Hugueney, P. (2009). Metabolism and roles of stilbenes in plants. *Plant Science*, *177*(3), 143–155. https://doi.org/10.1016/J.PLANTSCI.2009.05.012

Claver, I. P., Zhou, H. ming, Zhang, H. hua, Zhu, K. xue, Li, Q., & Murekatete, N. (2011). The Effect of Soaking with Wooden Ash and Malting upon Some Nutritional Properties of Sorghum Flour Used for Impeke, a Traditional Burundian Malt-Based Sorghum Beverage. *Agricultural Sciences in China*, *10*(11), 1801–1811. https://doi.org/10.1016/S1671-2927(11)60180-6

Dabija, A., Ciocan, M. E., Chetrariu, A., & Codină, G. G. (2021). Maize and sorghum as raw materials for brewing, a review. *Applied Sciences (Switzerland)*, *11*(7). https://doi.org/10.3390/APP11073139

Debebe, A., Temesgen, S., Redi-Abshiro, M., Chandravanshi, B. S., & Ele, E. (2018). Improvement in Analytical Methods for Determination of Sugars in Fermented Alcoholic Beverages. *Journal of Analytical Methods in Chemistry*, *2018*. https://doi.org/10.1155/2018/4010298

Dykes, L., Rooney, L. W., Waniska, R. D., & Rooney, W. L. (2005). Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. *Journal of Agricultural and Food Chemistry*, *53*(17), 6813–6818. https://doi.org/10.1021/JF050419E

Endalamaw, C., Nida, H., Tsegaye, D., van Biljon, A., Herselman, L., & Labuschagne, M. (2025). Genetics of sorghum: grain quality, molecular aspects, and drought responses. *Planta*, *261*(3). https://doi.org/10.1007/S00425-025-04628-X

Espinosa-Ramírez, J., Pérez-Carrillo, E., & Serna-Saldívar, S. O. (2013). Production of brewing worts from different types of sorghum malts and adjuncts supplemented with β-amylase or amyloglucosidase. *Journal of the American Society of Brewing Chemists*, *71*(1), 49–56. https://doi.org/10.1094/ASBCJ-2013-0125-01

Essays in Brewing Science. (2007). *Essays in Brewing Science*. https://doi.org/10.1007/0-387-33011-9

Ferreira, S. M. R., De Mello, A. P., De Caldas Rosa Dos Anjos, M., Krüger, C. C. H., Azoubel, P. M., & De Oliveira Alves, M. A. (2016). Utilization of sorghum, rice, corn flours with potato starch for the preparation of gluten-free pasta. *Food Chemistry*, *191*, 147–151. https://doi.org/10.1016/J.FOODCHEM.2015.04.085

Hager, A. S., Taylor, J. P., Waters, D. M., & Arendt, E. K. (2014). Gluten free beer - A review. *Trends in Food Science and Technology*, *36*(1), 44–54. https://doi.org/10.1016/J.TIFS.2014.01.001

ISO 4833-1:2013 - Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique. (n.d.). Retrieved April 30, 2025, from https://www.iso.org/standard/53728.html

Kageruka, H., Habumugisha, E., Simon, M., Vantsiotis, T., Declercq, N., & Collin, S. (2024). The Ancestral Use of Ashes in Red Sorghum Malting, an Efficient Tool for Avoiding Polyphenol-Related Enzymatic Inhibition During Mashing. *Journal of the American Society of Brewing Chemists*, *82*(4), 349–356. https://doi.org/10.1080/03610470.2024.2395107

Kensler, T. W., Roebuck, B. D., Wogan, G. N., & Groopman, J. D. (2010). Aflatoxin: A 50-Year Odyssey of Mechanistic and Translational Toxicology. *Toxicological Sciences*, *120*(Suppl 1), S28. https://doi.org/10.1093/TOXSCI/KFQ283

Kumar, R., Dhiman, M., Sharma, L., Dadhich, A., Kaushik, P., & Sharma, M. M. (2022). Nanofertilizers: The targeted nutrient supplier and enhance nutrients uptake by pearl millets (Pennisetum glaucum). *Biocatalysis and Agricultural Biotechnology*, *45*. https://doi.org/10.1016/J.BCAB.2022.102524

Lefyedi, M. L., & Taylor, J. R. N. (2006). Effect of Dilute Alkaline Steeping on the Microbial Contamination, Toxicity and Diastatic Power of Sorghum Malt. *Journal of the Institute of Brewing*, *112*(2), 108–116. https://doi.org/10.1002/J.2050-0416.2006.TB00240.X

Lyumugabe, F., Gros, J., Nzungize, J., Bajyana, E., & Thonart, P. U. de L.-Ul. > D. des sciences de la vie > B. et microbiologie industrielles. (2012). Characteristics of African traditional beers brewed with sorghum malt: a review. *Biotechnologie, Agronomie, Société et Environnement*, *16*(4), 509–530. https://orbi.uliege.be/handle/2268/142889

Lyumugabe, F., Kamaliza, G., Bajyana, E., & Thonart, P. H. (2010). Microbiological and physico-chemical characteristic of Rwandese traditional beer "Ikigage." *African Journal of Biotechnology*, *9*(27), 4241–4246. https://doi.org/10.5897/AJB10.053

Lyumugabe Loshima, F. (2013). *Characterisation and improvement of the quality of Rwandese traditional beer « ikigage » made from sorghum*.

McGinnis, M. J., & Painter, J. E. (2020). Sorghum: History, Use, and Health Benefits. *Nutrition Today*, *55*(1), 38–44. https://doi.org/10.1097/NT.0000000000000391

Mezgebe, A. G., Abegaz, K., & Taylor, J. R. N. (2018). Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum and malting quality. *Journal of Cereal Science*, *79*, 319–327. https://doi.org/10.1016/J.JCS.2017.11.015

Musabanganji, E., Maniriho, A., & Lyumugabe, F. (2023). Production, commercialization and consumption of traditional sorghum beer ("Ikigage") and its impact on Local Farmers' Welfare in Rwanda. *Agronomie Africaine*, *35*(2), 333–346.

https://www.ajol.info/index.php/aga/article/view/257044

Muyanja, C. M. B. K., Narvhus, J. A., Treimo, J., & Langsrud, T. (2003). Isolation, characterisation and identification of lactic acid bacteria from bushera: A Ugandan traditional fermented beverage. *International Journal of Food Microbiology*, *80*(3), 201–210. https://doi.org/10.1016/S0168-1605(02)00148-4

Noel, N. F. (2015). *Projection of Sorghum production response through multiple uses promotion strategies in selected districts of Tanzania*.

Ogundiwin, J. O., Ilori, M. O., Fessehatzion, B., Babalola, G. O., & Olajuyigbe, A. O. (1991). Effect of chemical treatments on the micro-organisms associated with malting of sorghum grains and sorghum malt. *Journal of Applied Bacteriology*, *71*(2), 139–143. https://doi.org/10.1111/J.1365-2672.1991.TB02969.X

Pitt, J., & Hocking, A. (2009). *Fungi and food spoilage*. https://doi.org/10.1007/978-0-387-92207-2.PDF

Ratnavathi, C. V. (2018). Grain structure, quality, and nutrition. *Breeding Sorghum for Diverse End Uses*, 193–207. https://doi.org/10.1016/B978-0-08-101879-8.00012-7

Ronald, B. J., & Roger, D. D. (2017). Traditional Processing and Quality Control of the "Red Kapsiki": A Local Sorghum Beer from Northern Cameroon. *Brewing Technology*. https://doi.org/10.5772/INTECHOPEN.69595

Rufino, M. do S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, *121*(4), 996–1002. https://doi.org/10.1016/J.FOODCHEM.2010.01.037

Shephard, G. S. (2008). Risk assessment of aflatoxins in food in Africa. *Food Additives and Contaminants - Part A*, *25*(10), 1246–1256. https://doi.org/10.1080/02652030802036222,

Stefoska-Needham, A. (2024). Sorghum and health: An overview of potential protective health effects. *Journal of Food Science*, *89*, A30–A41. https://doi.org/10.1111/1750-3841.16978

Steiner, E., Auer, A., Becker, T., & Gastl, M. (2012). Comparison of beer quality attributes between beers brewed with 100% barley malt and 100% barley raw material. *Journal of the Science of Food and Agriculture*, *92*(4), 803–813. https://doi.org/10.1002/JSFA.4651

Stone, H., Bleibaum, R., & Thomas, H. (2020). *Sensory evaluation practices*. https://books.google.be/books?hl=en&lr=&id=U2XRDwAAQBAJ&oi=fnd&pg=PP1&dq=Stone,+H.,+%26+Sidel,+J.+L.+(2004).+Sensory+Evaluation+Practices+(3rd+ed.).+Academic+Press.&ots=q8jHtrDhO4&sig=2Xfi5a-4oJp3J61Y3oIolNrctzk

Taylor, J. R. N., & Daiber, K. H. (1988). EFFECT OF CALCIUM IONS IN SORGHUM BEER

MASHING. *Journal of the Institute of Brewing*, *94*(2), 68–70. https://doi.org/10.1002/J.2050-0416.1988.TB04559.X;WGROUP:STRING:PUBLICATION

Taylor, J. R. N., & Emmambux, M. N. (2007). Products containing other speciality grains: Sorghum, the millets and pseudocereals. *Technology of Functional Cereal Products*, 281–335. https://doi.org/10.1533/9781845693886.2.281

Taylor, J. R. N., & Kruger, J. (2018). Sorghum and millets: Food and beverage nutritional attributes. *Sorghum and Millets: Chemistry, Technology, and Nutritional Attributes*, 171–224. https://doi.org/10.1016/B978-0-12-811527-5.00007-1

Taylor, J. R. N., Schober, T. J., & Bean, S. R. (2006). Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science*, *44*(3), 252–271. https://doi.org/10.1016/J.JCS.2006.06.009

Tétédé Rodrigue KONFO, C., Worou CHABI, N., DAHOUENON-AHOUSSI, E., CAKPO-CHICHI, M., Mansourou SOUMANOU, M., & Coco Kodjo SOHOUNHLOUE, D. (2015). IMPROVEMENT OF AFRICAN TRADITIONAL SORGHUM BEERS QUALITY AND POTENTIAL APPLICATIONS OF PLANTS EXTRACTS FOR THEIR STABILIZATION: A REVIEW. *Journal of Microbiology, Biotechnology and Food Sciences*, *5*(2), 190–196. https://doi.org/10.15414/JMBFS.2015.5.2.190-196

Thiex, N., Novotny, L., & Crawford, A. (2012). Determination of Ash in Animal Feed: AOAC Official Method 942.05 Revisited. *Journal of AOAC INTERNATIONAL*, *95*(5), 1392–1397. https://doi.org/10.5740/JAOACINT.12-129

Traité de brasserie. (2022). 551.

Venkateswaran, K., Elangovan, M., & Sivaraj, N. (2018). Origin, domestication and diffusion of Sorghum bicolor. *Breeding Sorghum for Diverse End Uses*, 15–31. https://doi.org/10.1016/B978-0-08-101879-8.00002-4

Wani, G., & Minja, J. (2025). Factors Affecting Sorghum Production in Tanzania. *Eastern Africa Journal of Official Statistics*, *1*(1). https://doi.org/10.63933/EAJOS.1.1.2025.7

Wild, C. P., & Gong, Y. Y. (2009). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis*, *31*(1), 71–82. https://doi.org/10.1093/CARCIN/BGP264,

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition*, *80*(5), 1106–1122. https://doi.org/10.1093/ajcn/80.5.1106

Yeo, H. Q., & Liu, S. Q. (2014). An overview of selected specialty beers: Developments, challenges and prospects. *International Journal of Food Science and Technology*, *49*(7), 1607–1618.

https://doi.org/10.1111/IJFS.12488

Yorke, J., Cook, D., & Ford, R. (2021). Brewing with unmalted cereal adjuncts: Sensory and analytical impacts on beer quality. *Beverages*, *7*(1), 1–20. https://doi.org/10.3390/BEVERAGES7010004

Zannou, O., Miassi, Y. E., Dossa, K. F., Houngbédji, M., Agani, O. B., Kpoclou, Y. E., Dabadé, D. S., Aïssi, M. V., Koca, I., Kayodé, A. P. P., & Galanakis, C. M. (2025). Red sorghum (Sorghum bicolor) leaf sheaths as source stable pigments. *Discover Food*, *5*(1). https://doi.org/10.1007/S44187-025-00275-Z