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Microbial food safety and sustainable resource management in cassava-based processing systems:

From food crop utilisation to residue valorisation in Mozambique

Andreia Massamby

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Andreia Massamby
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Cover: Conceptual illustration summarising the scope of the thesis, from cassava production and processing to microbial food safety and sustainable bio-based applications of cassava residues. Author-composed illustration based on original material and photographs taken during joint field work by the author and collaborators, including Mats Sandgren, used with permission (Andreia Massamby, 2026).

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Swedish University of Agricultural Sciences, Department of Molecular Sciences, Uppsala, Sweden

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Abstract

Cassava (*Manihot esculenta* Crantz) is one of Mozambique´s main staple crops, crucial for rural food security and livelihoods. However, its traditional processing into cassava roasted flour (*rale*) and other products generates considerable amount of solid and liquid residues that remain underutilised, while the associated handling practices may compromise food safety.

This thesis investigates three interlinked aspects of cassava processing and utilisation: (i) the microbial safety and hygienic quality of *rale* produced in smallholder processing units; (ii) the potential of cassava residues as feedstocks for microbial lipid and ethanol production; and (iii) the cyanide tolerance of yeasts from cassava-processing environments.

Microbial analyses showed that, although occasional microbial contaminants were detected, *rale* met microbial safety limits, with roasting acting as the key step in reducing both microbial loads and cyanogenic compounds. Enzymatic hydrolysis and yeast fermentation of cassava residues demonstrated efficient conversion of these into microbial lipids and ethanol by *Rhodotorula toruloides* CBS 14 and *Saccharomyces cerevisiae* J672, respectively. In parallel, isolates of microbes from cassava effluents, such as *Pichia bovicola* J709 and *Magnusiomyces ingens* J711, exhibited notable cyanide tolerance, suggesting potential for biological effluent detoxification.

Overall, the study integrates food safety, residue valorisation, and microbial detoxification within cassava processing systems, highlighting the feasibility of combining safe food production with bio-based waste conversion and management. The findings in the work contribute to sustainable cassava bioprocessing strategies and can support Mozambique´s transition towards a circular, residue-driven bioeconomy.

Keywords: cassava residues; food safety; microbial contaminants; cassava residues; microbial lipids; ethanol; cyanide tolerance

Mikrobiell livsmedelssäkerhet och hållbar resurshantering i kassavabaserade processningssystem

Sammanfattning

Kassava (*Manihot esculenta* Crantz) är en av Moçambiques viktigaste basgrödor och har stor betydelse för landsbygdens livsmedelssäkerhet och försörjning. Den traditionella bearbetningen av kassava till rostat kassavamjöl (*rale*) och andra livsmedelsprodukter genererar dock betydande mängder fasta och flytande reströmmar som i stor utsträckning är outnyttjade, samtidigt som kassava som inte processas på ett bra sätt kan äventyra livsmedelssäkerheten.

Arbetena presenterade i denna avhandling syftade till att undersöka tre sammankopplade aspekter av kassavautnyttjande: (i) den mikrobiologiska säkerheten och hygieniska kvaliteten hos *rale* som produceras i småskaliga bearbetningsenheter; (ii) potentialen med att utnyttja kassavarestprodukter som substrat för mikrobiell produktion av lipider och etanol; samt (iii) cyanidtoleransen hos jästarter från kassavabearbetningsmiljöer.

De mikrobiologiska analyserna visade att *rale* uppfyllde livsmedelssäkerhetskraven, även om enstaka mikrobiella kontaminanter påträffades. Rostningen visade sig vara det avgörande steget för att minska både de mikrobiella halterna och cyanogena föreningar. Enzymatisk hydrolys och jästjäsning visade att man kan uppnå effektiv omvandling av kassavarestprodukter till mikrobiella lipider och etanol med hjälp av *Rhodotorula toruloides* CBS 14 respektive *Saccharomyces cerevisiae* J672. Samtidigt uppvisade isolat funna i, restvätskor från kassavaprocessingen såsom *Pichia bovicola* J709 och *Magnusiomyces ingens* J711, en anmärkningsvärd cyanidtolerans, vilket tyder på potential för biologisk rening av dessa restvätskor.

Sammanfattat kan man säga att studien integrerar livsmedelssäkerhet, restvärdesförädling och mikrobiell detoxifiering inom kassavaprocessning och visar att säker livsmedelsproduktion kan kombineras med biobaserad resursåtervinning. De uppnådda resultaten kan bidra till utvecklingen av hållbara bioprocessstrategier för kassava och därigenom stödja Moçambiques övergång mot en mer cirkulär grön bioekonomi.

Nyckelord: kassavarestprodukter; processningssystem; mikrobiell kontaminantering; mikrobiella lipider; etanol; cyanidtolerans

Segurança microbiológica e gestão sustentável de recursos em sistemas de processamento da mandioca

Resumo

A mandioca (*Manihot esculenta* Crantz) é uma das principais culturas alimentares em Moçambique, desempenhando um papel central na segurança alimentar e subsistência das comunidades rurais. O processamento tradicional desta cultura para produção da farinha torrada (*rale*) e outros derivados produz quantidades consideráveis de resíduos sólidos e líquidos geralmente descartados sem reaproveitamento. Além disso, práticas inadequadas de manuseamento durante o processamento podem comprometer a segurança alimentar dos produtos finais.

Esta tese tem como objectivo, investigar três aspectos interligados do processamento da mandioca em Moçambique: (i) a segurança microbiológica e a qualidade higiénica do *rale* produzido em unidades de processamento familiar; (ii) o potencial biotecnológico dos resíduos de mandioca como substractos para produção de lípidos microbianos e etanol; e (iii) a tolerância de leveduras associadas à ambientes de processamento de mandioca ao cianeto.

As análises microbiológicas demonstraram que, apesar da detecção ocasional de microrganismos contaminantes, o *rale* apresentou cargas microbianas dentro dos limites de segurança alimentar, sendo a torrefação determinante na redução da carga microbiana e dos compostos cianogénicos. A hidrólise enzimática seguida de fermentação permitiu a conversão eficiente de resíduos de mandioca em lípidos microbianos e etanol, utilizando *Rhodotorula toruloides* CBS 14 e *Saccharomyces cerevisiae* J672, respectivamente. Adicionalmente, leveduras isoladas de efluentes de processamento da mandioca, nomeadamente *Pichia bovicola* J709 e *Magnusiomyces ingens* J711, demonstraram tolerância ao cianeto, indicando potencial para aplicação em processos biológicos de detoxificação de efluentes.

De forma integrada, este estudo articula segurança alimentar, valorização de resíduos agroindustriais e detoxificação microbiana, demonstrando a viabilidade de conciliar produção de alimentos seguros com a conversão biotecnológica de resíduos contribuindo para estratégias sustentáveis de bioprocessamento da mandioca em Moçambique.

Palavras-chave: resíduos de mandioca; segurança alimentar; contaminantes microbianos; lípidos microbianos; etanol; tolerância ao cianeto

Preface

This research was motivated by the need to strengthen food safety and promote sustainable resource management within cassava-based processing systems in Mozambique. By integrating microbiological assessment, bioprocess optimisation, and the physiological characterisation of yeasts associated with cassava processing environments, this thesis provides insights applicable to improving hygiene standards in small-scale food production, valorising agro-industrial residues, and supporting the development of circular bioresource systems in which safe food production and waste utilisation coexist in a single framework.

Dedication

To my lovely parents and sister.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Massamby, A., Leong, S. L., Müller, B., Tivana, L., Passoth, V., Macuamule, C., & Sandgren, M. (2025). Microbial Contamination and Food Safety Aspects of Cassava Roasted Flour ("Rale") in Mozambique. Microorganisms, 13(1), 168. https://doi.org/10.3390/microorganisms13010168

II. Massamby, A., Blomqvist, J., Leong, S. L., Nagaraj, Y., Müller, B., Passoth, V., Tivana, L., Macuamule, C., & Sandgren, M. Potential of conversion of cassava processing residues by yeasts to produce value-added bioproducts (Submitted).

III. Massamby, A., Tivana, L., Blomqvist, J., Leong, S. L., Müller, B., Passoth, V., Macuamule, C., & Sandgren, M. Cyanide tolerance among yeasts from cassava processing effluents and reference strains (Manuscript).

The contribution of Andreia Massamby to the papers included in this thesis was as follows:

I. Contributed to project planning and designing the experiments. Conducted the experiments and performed almost all laboratory work. Performed data analysis and statistical analysis and wrote the original manuscript draft.

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Abbreviations

acetyl-CoA	Acetyl coenzyme A
AMA	Adenosine monophosphate
ATP	Adenosine triphosphate
CDW	Cell dry weight
cfu	Colony forming units
COD	Chemical oxygen demand
FAO	Food and Agriculture Organization of the United Nations
IFAD	International Fund for Agricultural Development
LAB	Lactic acid bacteria
LPMOs	Lytic polysaccharide monooxygenases
MADER	Ministério da Agricultura e Desenvolvimento Rural
MUFAs	Monounsaturated fatty acids
NADPH	Nicotinamide adenine dinucleotide phosphate
PUFAs	Polyunsaturated fatty acids
PPP	Pentose phosphate pathway
RH	Relative humidity
SFAs	Saturated fatty acids
2G	Second generation
SCOs	Single cell oils
STEX	Steam expansion
TAGs	Triacylglycerols
TCA	Tricarboxylic acid
v/v	Volume per volume
w/w	Weight per weight

1. Introduction

Mozambique, located along the southeastern coast of Africa, is an agrarian-based country where more than 70% of the population depends on subsistence farming for food and income (Costa & Delgado, 2019; Bratley & Meyer-Cirkel, 2025). Agriculture is the cornerstone of rural livelihoods, providing employment and food security, yet productivity is constrained by climatic variability, weak infrastructure, and limited access to improved inputs (Dominguez-Torres & Biceño-Garmendia, 2011; Burns et al., 2012; Cambaza, 2023). The country´s tropical to subtropical climate, with alternating rainy and dry seasons, supports the cultivation of several staple crops, most notably cassava, which thrives in both fertile and marginal soils (Cambaza, 2023).

Cassava (*Manihot esculenta* Crantz) is Mozambique´s main source of dietary starch, accounting for approximately 30% of total caloric intake and exceeding maize in dietary importance. It plays a crucial role in food security by acting as a reliable famine reserve crop, as its roots can remain unharvested in the soil for up to 30 months without spoilage (Costa & Delgado, 2019). The crop´s tolerance to drought and poor soils makes it particularly valuable in regions frequently affected by climatic variability. Nevertheless, cassava also presents significant limitations: its roots contain cyanogenic glucosides, which are toxic if not properly processed; its protein content is very low; and once harvested, its fresh roots deteriorate rapidly, typically within a few days (Tivana, 2012; Costa & Delgado, 2019).

More than 100 cassava varieties are cultivated across Mozambique, including both sweet and bitter types that differ in taste, cyanogenic content, and adaptability to local agroecological conditions (Donovan et al., 2011). Approximately 90% of national cassava production consists of bitter varieties, which are more resistant to pests and diseases but contain cyanogenic glucosides that must be eliminated through proper post-harvest processing (Costa & Delgado, 2019). Sweet varieties, such as *Xinhembwe* and *Munhaca*, are mainly grown in the southern coastal region of the Inhambane province in the south of Mozambique and are preferred for their low cyanogenic content and desirable sensory proprieties (Donovan et al., 2011).

In Mozambique, average cassava yields are low compared to those in West Africa and are roughly one-half to one-third of the yields in Latin America and Asia (Costa & Delgado, 2019). Nationally, cassava production covers approximately 873,953 ha of Mozambique, producing an estimated 7.61 million tonnes of fresh cassava roots annually (MADER, 2023). Around 70% of the cassava produced is intended for human consumption, serving as the staple food for more than half of the population in Mozambique, particularly in the northern and central regions provinces of Cabo Delgado, Nampula and Zambézia (Zvinavashe et al., 2011; Dias, 2012; Figure 1).

Cassava is typically sold as fresh root, dried, or semi-processed into grated flour, depending on the region and local consumption patterns. In southern provinces of Mozambique, cassava roasted flour, also known as *rale*, serves also as staple food in many households (**Paper I**). Industrial-scale cassava processing for food, feed or other applications remains virtually absent in the country, limiting the crop´s economic potential and value-chain diversification (Zvinavashe et al., 2011; Salvador et al., 2014; Costa & Delgado, 2019).

In southern Mozambique, especially in the provinces of Gaza and Inhambane, farmers have associations equipped with semi-mechanised units to process cassava and are supported by technical training programs (**Paper I**). These initiatives have enhanced processing efficiency and product quality. However, cassava production remains predominantly artisanal, relying on simple, non-motorised tools for household use and small-scale trade. In Inhambane province which accounts for an annual production of approximately 163,798 tonnes of cassava (MADER, 2023), eighteen cassava-processing associations are actively engaged in *rale* production. Additionally, five associations are likewise involved in *rale* production in Gaza province. However, limited research has addressed the food safety and quality along the cassava roasted flour processing chain (**Paper I**).

Cassava processing generates substantial amounts of solid and liquid residues (Andrade et al., 2022; Ogbonna et al., 2025; Olaniyan et al., 2025; **Paper II**) that are often discarded untreated, contributing to environmental pollution because of their high organic load and cyanide content (Olukanni & Olatunji, 2018; Maciel et al., 2023). The biochemical composition and high organic load of cassava processing residues make them promising raw materials for microbial bioconversion into value-added products (**Paper II**; **Paper III**), offering a sustainable pathway to integrate waste reduction with bioresource valorisation and underscoring their potential contribution to a circular and sustainable bioeconomy (Zhang et al., 2016; Andrade et al., 2022).

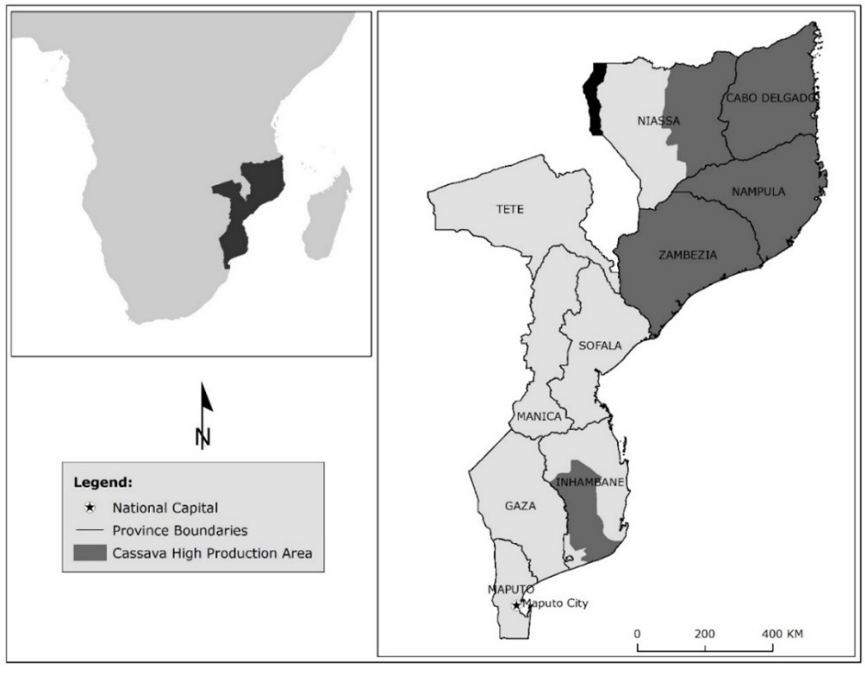

Figure 1. Cassava production areas in Mozambique (Modified from Salvador et al., 2014).

2. Cassava as a crop

2.1 Cassava global production and socio-economic relevance

Cassava (*Manihot esculenta* Crantz) is a perennial shrub belonging to the Euphorbiaceae family, which comprises more than 7 000 latex-producing species containing specialised laticifer cells (Konno, 2011; Shigaki, 2016). From a socio-economic perspective, cassava ranks among the most important crops in tropical and subtropical regions (Kosoe & Ogwu, 2024; Borku et al., 2025). It was domesticated in South America around a thousand years ago (McKey et al., 2010) and introduced to Africa during the 16th century, where it replaced native crops such as yam and pear millet and became a key staple food for low-income rural households (Pinto-Zevallos et al., 2016; Shigaki, 2016). Cassava is ranked as the fourth most important staple food crop worldwide, after rice, wheat, and maize, and contributes to the diet of more than one billion people due to its high carbohydrate content (Adebayo, 2023; Immanuel et al., 2024).

Global cassava production reached approximately 333.6 million tonnes in 2023, with Africa contributing around 65% of the total (FAOSTAT, 2025; Abirami et al., 2025). Nigeria remains the largest cassava producer, accounting for nearly one-fifth of global output in 2021 (Table 1). In Southeastern Africa, United Republic of Tanzania, Malawi, Zambia and Mozambique, dominate cassava production, although in Mozambique only 10–20% of harvests are commercially traded (Costa & Delgado, 2019; Abirami et al., 2025; FAOSTAT, 2025).

Table 1. Cassava Top Producing Countries in Africa and their contribution to African and World production in the early 2020´s.

Country	Position	Output (tons)	Africa production (%)	World production (%)
Nigeria	1	59,411,510	30.81	19.50
The Democratic Republic of Congo	2	40,050,112	20.85	13.19
Ghana	3	22,447,635	11.69	7.40
Angola	4	9,000,432	4.69	2.96
United Republic of Tanzania	5	8,184,093	4.26	2.70
Cameroon	6	6,092,549	3.18	2.00
Malawi	7	5,667,887	2.95	1.87
Cote d´Ivoire	8	5,238,244	2.73	1.73
Sierra Leone	9	4,588,612	2.39	1.51
Zambia	10	4,036,584	2.10	1.33
Mozambique	11	3,987,446	2.08	1.32

Source: Data adapted from FAO (2023), Adebayo (2023) and Borku et al., (2025).

2.2 Cassava cultivation, processing and utilisation

As described in Chapter 1, cassava´s ability to thrive under drought, low soil fertility, and minimal inputs underlies its widespread cultivation across Mozambique and other countries within Africa. Its perennial growth habit, and efficient photosynthesis between 25 ºC and 35 ºC (El-Sharkawy, 2004; Immanuel et al., 2024) make it particularly suited to marginal environments where it is cultivated by millions of rural households relying on family labour and basic tools (FAO & IFAD, 2000; Salvador et al., 2014; Immanuel et al., 2024).

Cassava can be harvested at any point between 6 and 30 months after planting and may be left in the ground as a food reserve. This flexibility ensures a continuous supply during droughts or periods of food scarcity, reinforcing its role as a cornerstone for food security and rural livelihood resilience (Anikwe & Ikenganyia, 2018; Costa & Delgado, 2019; Nadia et al., 2021).

Across Africa, cassava processing and consumption have evolved from simple household practices into culturally embedded food systems that sustain both food security and dietary diversity (Kolawole et al., 2010). The crop's labour-intensive cultivation and processing create substantial employment opportunities, particularly for women (Akpoghelie et al., 2025), making it both a subsistence and a commercial crop that contributes directly to household incomes and rural economic development (Amelework et al., 2021; Borku et al., 2025).

Driven by increasing demand for renewable feedstocks, cassava is now recognised as a strategic industrial raw material (Anyanwu et al., 2015; Zhang et al., 2016; Borku et al., 2025; Okolieuwa et al., 2025). Its high starch content (up to 90% on a dry-weight basis), combined with year-round availability and low-capital input requirements, positions it as an important feedstock for food, feed, and biotechnological applications (Howeler, 2000; Zhou & Thomson, 2009; Zhang et al., 2016; Borku et al., 2025).

Consumption and processing methods vary across regions. In Central and East Africa, cassava roots are typically boiled or fried, while the leaves are used as a nutrient-rich vegetable (Nweke, 2004; Chiwona-Karltun et al., 2015). In West Africa, fermented products such as *gari*, *atiéké*, *lafun*, and *fufu* are major staple foods, reflecting local detoxification and preservation traditions (Flibert et al., 2016; Halake & Chinthapalli, 2020; Obafemi et al., 2022). Common practices such as peeling, grating, boiling, drying, and

fermenting (Scaria et al., 2024), not only reduce cyanogenic compounds, but also enhance flavour, texture, and shelf life (Tivana, 2012; Adebayo et al., 2023). Differences in chemical composition, starch structure and anti-nutritional content further determine cassava´s safety, quality, and suitability for industrial conversion (Montagnac et al., 2009; Morgan & Choct, 2016). A detailed understanding of these properties is thus essential for optimising cassava processing and promoting its sustainable utilisation in food and bio-based systems.

2.3 Nutritional composition of cassava

The nutritional composition of cassava depends on the specific plant tissue (root or leaf) and on several factors such as variety, plant age, and environmental conditions (Tewe & Lutaladio, 2004). Cassava roots are characterised by a high carbohydrate content and low levels of lipids and proteins, which define their nutritional and energy profile (Montagnac et al., 2009; Morgan & Choct, 2016). Carbohydrates represent the main macronutrient of cassava root, accounting for approximately 25–35% of the fresh weight (Scaria et al., 2024). About 80% of these carbohydrates are starch, composed of 83% amylopectin and 17% amylose. Cassava starch is highly digestible and contributes substantially to caloric intake in many tropical developing countries (Rawel & Kroll, 2003; Morgan & Choct, 2016). It also contains dietary fibre which supports gastrointestinal function (Montagnac et al., 2009).

Cassava root protein levels range between 0.7% and 1.3% on a fresh-weight basis (Ngiki et al., 2014) and are usually deficient in essential amino acids (lysine, methionine, cysteine and tryptophan) but relatively rich in arginine, glutamic, and aspartic acids (Bradbury & Holloway, 1988; Gil & Buitrago, 2002). Roughly half of the nitrogen fraction occurs as free amino acids and non-protein nitrogen compounds such as nitrates and cyanogenic glycosides (Montagnac et al., 2009).

Lipid content in cassava roots is extremely low, at approximately 0.1% of fresh weigh (Morgan & Choct, 2016). The small lipid fraction consists mainly of glycolipids (52%) and non-polar lipids (45%), with palmitic and oleic acids as the dominant fatty acids (Hudson & Ogunsua, 1974; Gil & Buitrago, 2002). Consequently, cassava contributes marginally to the dietary intake of fat-soluble vitamins. In contrast, cassava is a notable source of

vitamin C, a water-soluble vitamin, with reported contents ranging from 15 to 45 mg per 100 g fresh weight (Okigbo, 1980; Charles et al., 2004) while B-vitamins are present in smaller amounts (Borku et al., 2025). The crop also provides minerals such as calcium, potassium, magnesium, and iron, with calcium levels (15–35 mg/100 g) higher than in many other tropical staples (Gil & Buitrago, 2002).

In contrast, cassava leaves are highly nutritious, containing 17–40% protein (Khieu et al., 2005) and notable levels of vitamin B_1, B_2, C, and carotenoids, together with calcium and magnesium (Adewusi & Bradbury, 1993). Young cassava leaves are also rich in vitamins A, B, C and K, which support vision, immune function, energy metabolism, and bone health (Shigaki, 2016; Rahman, 2025). These attributes make the cassava leaves a valuable dietary supplement in regions with limited access to animal protein, where low-alkaloid varieties are consumed as vegetable, providing essential nutrients (Borku et al., 2025).

Despite these nutritional advantages, cassava and its derivatives still pose important food safety challenges due to its anti-nutritional factors, particularly cyanogenic glycosides, which require adequate processing to ensure safe consumption (**Paper I**).

2.4 Anti-nutritional factors in cassava

Antinutrients are naturally occurring compounds in plants that reduce the bioavailability or absorption of essential nutrients in the human body (Shigaki, 2016). Fresh cassava roots have a short post-harvest shelf life of only 1–3 days and contain several anti-nutritional compounds – phytates, tannins (phenolics), oxalates, nitrates/nitrites, and saponins – that can be toxic and reduce nutrient digestibility and bioavailability (Montagnac et al., 2009; Salvador et al., 2014; Oresegun et al., 2016).

Among these, the most critical from a food safety perspective are the cyanogenic glycosides, naturally occurring compounds capable of releasing hydrogen cyanide (HCN) during processing or digestion (Salvador et al., 2014; Lateef & Ojo, 2016; Forkum et al., 2025). Cassava contains two principal cyanogenic glycosides: linamarin (93%) and lotaustralin (7%), whose concentrations range from 75 to 1000 mg HCN/kg of fresh root weight, varying depending on the variety, age, and environmental growth conditions (Burns et al., 2012; Ngiki et al., 2014).

When cassava tissues are disrupted during peeling, grating, and crushing, these compounds are enzymatically hydrolysed by endogenic linamarase, leading to the release of hydrogen cyanide (Tivana, 2012). Cyanide concentrations in cassava leaves are typically higher (up to six-fold) compared to the root, posing an additional health risk when leaves are consumed as vegetables (Ngiki et al., 2014). Depending on the content of cyanide glucosides, cassava varieties are classified into bitter and sweet. Farmers often prefer bitter varieties because of their superior yield, pest resistance, and prolonged in-ground storability (Tivana, 2012). However, these varieties are unsuitable for human consumption without extensive and adequate processing to eliminate cyanide (Salvador et al., 2014; Lateef & Ojo, 2016; Shigaki, 2016; Forkum et al., 2025).

In humans, chronic exposure to sublethal doses of cyanide, particularly from insufficiently processed cassava, can deplete sulphur-containing amino acids such as methionine and cysteine and result in severe health disorders including tropical ataxic neuropathy, goitre, cretinism, and epidemic spastic paraparesis (*konzo*), the latter being the most commonly reported cyanide-related illness in Mozambique (Cumbana et al., 2007; Nhassico et al., 2008; McKey et al*.,* 2010, Tivana, 2012; Nyaika et al., 2024).

This highlights the crucial role of post-harvest handling and processing of cassava to ensure health safety and nutritional quality of cassava-based foods. As fresh cassava has a rapid physiological deterioration that limits both food security and marketability (Tivana, 2012; Zainuddin et al., 2018), processing not only reduces the levels of cyanogenic glycosides and other anti-nutritional factors but also extends shelf life, improves palatability, and enables the production of diverse food and industrial products adapted to local and global markets.

2.5 Aims of the thesis

Cassava utilisation in Mozambique, and in all cassava utilising countries, represents a multidimensional challenge: ensuring food safety in traditional processing systems, managing the large quantities of residues generated during processing, and understanding microbial adaptation to cyanide-rich environments.

Building on this premise, this thesis investigates three interlinked perspectives covered in the chapters **3**–**5**:

- The microbial safety and hygienic practices of cassava roasted flour (*rale*), the most widely consumed cassava product in southern Mozambique.
- The biotechnological potential of cassava processing residues for microbial conversion into value-added products.
- Isolation and initial physiological characterisation of yeasts associated with cassava-processing environments.

Addressing these three interrelated dimensions is important to improve food safety, reduce environmental pollution and promote circular bio-based solutions that benefit both rural livelihoods and industrial sustainability. Through an integrated approach combining microbial ecology, food safety assessment, and biotechnology applications, this thesis aims to generate new insights that contribute to safer food production and sustainable bioresource management within cassava food-based systems.

To achieve these goals, the thesis is structured into three interrelated studies, each addressing specific objectives, which are:

Paper I. Microbial contamination and food safety aspects of cassava roasted flour (*rale*) in Mozambique.

- To evaluate the microbiological quality and safety of traditionally processed cassava flour (*rale*).
- To identify potential contamination sources, and to assess hygienic practices during processing and marketing.

Paper II. Potential of conversion of cassava-processing residues by yeasts to produce value-added bioproducts.

- To assess the ability of selected yeasts (*Rhodotorula toruloides* CBS 14 and *Saccharomyces cerevisiae* J672) to utilise cassava-derived substrates (peels, fibres, and process press water) for microbial growth and to produce lipids and ethanol, and to evaluate the effect of nitrogen supplementation on lipid synthesis by *R. toruloides*.

Paper III. Cyanide tolerance amongst yeasts from cassava processing effluents and reference strains.

- To isolate, identify, and characterise environmental yeasts naturally adapted to cyanide-rich cassava residues.
- To evaluate their physiological tolerance mechanisms as a basis for selecting robust strains for future bioconversion applications.

3. Cassava roots processing

3.1 Cassava processing and residues generation

Producing nutritious and safe food from cassava remains a significant challenge, as processing plays a crucial role in transforming the freshly harvested and highly perishable roots into safe, edible, and marketable products (Borku et al., 2025). As previously described in Chapter 2, cassava roots are highly perishable and contain toxic cyanogenic compounds; therefore, processing is indispensable for ensuring food safety and extending shelf life (Montagnac et al., 2009; Kolawole et al., 2010; Tivana 2012). The methods applied in Mozambique for cassava processing vary widely across regions and production scales, ranging from artisanal household techniques to semi-mechanised, and industrial operations.

Cassava processing generally begins with cleaning of the roots to remove soil and impurities, followed by manual peeling using knives or other simple tools (**Paper I**). This first step generates the major solid by-product – the cassava peels – which account for approximately 10–20% of the fresh root weight (Adetan et al., 2003; **Paper II**). The peels contain cyanogenic compounds that can pose health and environmental risks if not properly managed (Kolawole et al., 2010; Isimah et al., 2023; **Paper III**). Although rich in fibre and residual carbohydrates that could be used for animal feed, compost or other applications, the peels are often discarded and left to decompose, leading to localised pollution (Morgan & Choct, 2016).

After peeling, the roots are washed and grated or diced to release the starch and prepare the mash for fermentation. This stage, which typically lasts 1–3 days, improves product texture and flavour while substantially reducing cyanide levels. Fermentation is mainly driven by lactic acid bacteria (LAB) and yeasts. Linamarase released from disrupted plant tissues hydrolyses the cyanogenic glucosides linamarin and lotaustralin, releasing free hydrogen cyanide (HCN). Although this enzymatic hydrolysis initially increases free cyanide levels, it is a necessary step that enables subsequent detoxification through volatilisation and removal of HCN during fermentation, pressing, and roasting (Kostinec et al., 2005; Tivana, 2012). Once fermentation is complete, the mash is placed into porous bags and subjected to mechanical or manual pressing to remove excess liquid. This processing stage generates the second major by-product – the cassava

process press water – a liquid fraction rich in soluble organic matter and residual cyanide compounds (Olaniyan et al., 2025; **Paper I**; **Paper III**). Together with wastewater generated during washing and grating, this effluent often becomes a significant environmental pollutant when released untreated into surrounding ecosystems (Isimah et al., 2023). After pressing, the semi-dry mash is sieved and further processed according to the intended product through additional steps, such as roasting, drying or sedimentation.

Each processing stage thus generates considerable quantities of solid and liquid residues with distinct physicochemical characteristics. Solid residues include cassava peels and fibres, the latter being a side stream specifically generated during cassava starch production. Liquid residues mainly comprise washing wastewater generated during root washing and grating, as well as cassava process press water produced during the pressing stage. Although traditionally regarded as waste, these materials represent valuable substrates for biotechnological valorisation (**Paper II**), particularly through microbial fermentation to produce biofuels, biosurfactants, microbial lipids and protein for animal feed (Hierro-Iglesias et al., 2022; De Oliveira Schmidt et al., 2023; Hossain et al., 2025). Figure 2 illustrates the main cassava side streams generated along the processing chain, for both roasted flour (*rale*) and starch production, including peels, fibres, and wastewater fractions, as observed in processing units in southern Mozambique.

Figure 2. Cassava residues generated during *rale* and starch production in cassava processing facilities in Southern Mozambique: (a-b) reception and unloading of fresh cassava roots; (c) sampling of cassava process press water from *rale* production; (d) discharge of cassava effluents from starch processing into open channel; (e) accumulation of cassava after peeling; (f) storage of pressed cassava fibre remaining after starch or flour extraction. Adapted from **Papers II** and **III**.

3.2 Microbial contamination and hygienic risks during cassava processing

Several studies across Africa have documented bacterial and fungal contamination in cassava-based food products. *Aspergillus flavus*, *A. nomius*, *A. parasticus*, and *A. niger* have been isolated from dried cassava chips and roasted fermented roots in Malawi, Zambia, and Nigeria, producing aflatoxins and other mycotoxins that pose serious health risks to humans and livestock (Kolawole et al., 2010; Chiona et al., 2014). *Staphylococcus aureus* and *Escherichia coli* are also frequently detected in cassava flour, reflecting inadequate hygiene, excessive manual handling, and the use of contaminated water during processing or marketing (Adebayo-Oyetoro et al., 2013; Lateef & Ojo, 2016). In Kenya, cassava products sold in rural markets exhibited high bacterial and coliform loads, confirming poor post-harvest handling and storage conditions (Gacheru et al., 2015).

Similar patterns have been reported in Côte d'Ivoire, where spontaneously fermented products such as *attiéké* displayed diverse microbial contamination, demonstrating that fermentation alone does not ensure food safety (Djeni et al., 2015). Studies in Nigeria and Côte d'Ivoire further highlighted the presence of pathogenic bacteria and toxigenic fungi in stored and marketed cassava products (*lafún*, *attiéké* and *gari*), highlighting the need for improved sanitary practices, clean water supply, and modern drying systems (Adebayo-Oyetoro et al., 2013; Kouamé et al., 2013; Adjovi et al., 2015; Yusuf et al., 2024).

Beyond microbial contamination, cassava-based foods may accumulate mycotoxins – particularly aflatoxins produced by *Aspergillus* species – posing additional health risks (Roscoe et al., 2008; Ono et al., 2021; Matusse et al., 2024). In **Paper I** of this thesis, several mould species with toxigenic potential were identified and included species such as *A. flavus* (aflatoxin producer), *Penicillium citrinum* (citrinin), *A. niger* (ochratoxin A and fumonisins), and *Fusarium oxysporun* (fumonisins) (Marc, 2022; Pitt & Hocking, 2022). However, their abundance in *rale* samples was low, with *A. flavus* present at less than 3 cfu/g. These findings indicate that the risk of toxin production is minimal under normal storage conditions and would only become significant if *rale* were stored improperly, allowing mould growth and mycotoxin formation (**Paper I**).

Notably, toxigenic moulds were absent in the market samples collected within this study (**Paper I**), while non-toxigenic moulds, yeasts, and *S. aureus* were occasionally detected. This likely reflects variations in hygiene awareness and handling practices – such as the use of uncovered containers and exposure to airborne dust and soil (Okolo & Makanjuola, 2021; Figure 3). Matusse et al. (2024) detected low levels or even undetectable levels of aflatoxin in cassava flour samples from Gaza and Inhambane markets. These results indicate that, despite occasional contamination, the overall microbial and mycotoxin risks associated with artisanal *rale* production and trade appear to be low, provided that adequate hygiene and storage practices are maintained throughout the cassava value chain from cultivation to the household (**Paper I**).

Figure 3. Cassava manual peeling (a) and *rale* ready to sell in rural markets (b).

Bacterial pathogens such as *Listeria* spp., *S. aureus*, *Bacillus cereus*, and *E. coli* have been detected in fermented or roasted cassava products in Nigeria, reflecting suboptimal hygiene and handling conditions (Obadina et al, 2008; Lateef & Ojo, 2016). Although few cassava-related foodborne outbreaks have been reported across Africa, the occurrence of these microorganisms represents a significant public-health concern, emphasising the need for improved fermentation control, sanitation, and staff training for small-scale producing units (Omojokun, 2013). In **Paper I**, bacteria from the Enterobacteriaceae family – including *E. coli* – were detected at low abundance in several samples taken at several steps during cassava processing, as well as in *rale* collected from both markets and processing units. Their low occurrence suggests incidental contamination rather than active proliferation, reinforcing the need for better hygiene practices and water quality during traditional processing.

Despite cassava´s central role in diets across Africa, systematic studies on the microbial contamination and hygienic risks of roasted cassava flour (*rale*) under real processing and market conditions remain scarce, particularly in Mozambique (**Paper I**). Understanding microbial dynamics during processing is essential not only for ensuring safety but also for identifying beneficial microorganisms that may enhance flavour, texture, and shelf life.

3.2.1 Microbial contamination within the cassava processing chain: The case of Mozambique

During this thesis work, a comprehensive assessment of cassava processing and marketing was conducted to evaluate microbial indicators of hygienic quality in southern Mozambique, assessed both during the rainy and the dry seasons (**Paper I**). Overall, the representative processing unit followed five main stages of cassava processing:

a) delivery, peeling and washing of cassava roots;
b) chopping of cassava roots to obtain paste or mash;
c) pressing and/or fermentation of the paste which generates cassava process press water as a by-product;
d) sieving of the cassava paste to achieve the desired granule size, and
e) roasting, cooling, storage and packaging of the final product (*rale*) ready for marketing (Figure 4).

Across the processing chain, microbial counts varied according to both processing stage and season (Figure 5). During the warmer, rainy season, higher counts of moulds, LAB, and *Bacillus* spp. were observed, while yeasts and *S. aureus* predominated during the cooler, dry season. The most frequent yeast species identified were: *Wickeramomyces anomalus*, *Rhodotorula babjevae, Rhodotorula mucilaginosa*, *Pichia exigua* and *Meyerozyma caribbica*. Seasonal differences were attributed to higher temperature and humidity (~29 ºC; 75% RH) during the rainy season, which favour microbial growth and mould contamination. The highest microbial loads occurred during early stages of processing, particularly after washing and chopping, reflecting contamination sources associated with water, utensils, and manual handling. Pressing and fermentation further modified microbial profiles, with LAB and yeasts dominating some batches, suggesting spontaneous fermentation activity (Figure 5; **Paper I**).

Fermentation, typically driven by LAB and yeasts (Ray & Sivakumar, 2009), plays a dual role in *rale* production as it enhances sensory quality and product safety. The characteristic flavour and aroma of *rale* arise from microbial metabolism, while enzymatic and acidification processes simultaneously promote detoxification (Kostinec et al., 2005). In particular, the enzyme linamarase, released from both plant tissues and microbial cells, hydrolyses the cyanogenic glucosides linamarin and lotaustralin, releasing volatile hydrogen cyanide and thereby reducing the product´s toxicity (NRC, 1992; Kostinec et al., 2005).

Roasting markedly reduced total microbial viable counts, confirming its role as the critical control step for microbial inactivation. Beyond its effect on microbial viable counts, roasting also plays a decisive role in detoxification. The intense heat promotes the volatilisation of hydrogen cyanide released during fermentation and pressing, thereby reducing residual cyanogenic compounds to food safe levels. Indeed, the two most critical steps for cyanide elimination are the initial grating of cassava – where enzymatic hydrolysis of cyanogenic glucosides occurs – and the final roasting stage, which removes the liberated hydrogen cyanide through evaporation (Vasconcelos et al., 1990). However, occasional detection of moulds in the final roasted flour indicated possible post-roasting re-contamination during cooling, storage, and marketing (Okolo & Makanjuola, 2021; **Paper I**).

Overall, the findings from **Paper I** demonstrate that the artisanal cassava-processing systems in southern Mozambique are generally microbiologically safe, provided that basic hygiene and handling practices are maintained. The combined effect of fermentation and roasting ensures both detoxification and microbial safety, reflecting the effectiveness of traditional knowledge in securing food safety. Nonetheless, minor contamination risks particularly during post-roasting, handling and storage, indicate opportunities for improvement through targeted hygiene interventions. Beyond the food safety dimension, cassava processing also generates substantial amounts of by-products that hold potential for valorisation rather than disposal of the processing residues (**Paper II**). This dual perspective – ensuring safe food production (**Paper I)** while promoting the reuse of cassava residues – forms the conceptual bridge to next chapter, which explores the conversion of cassava processing residues into value-added bioproducts through yeast-based bioprocessing.

Figure 4. Main steps in cassava roasted flour (*rale*) processing at small-scale units in Inhambane: (a) root reception and peeling; (b) grating and pressing, (c) fermentation and liquid drainage; (d) sieving and drying; (e) roasting, packaging, and storage of final product (Adapted from **Paper I**).

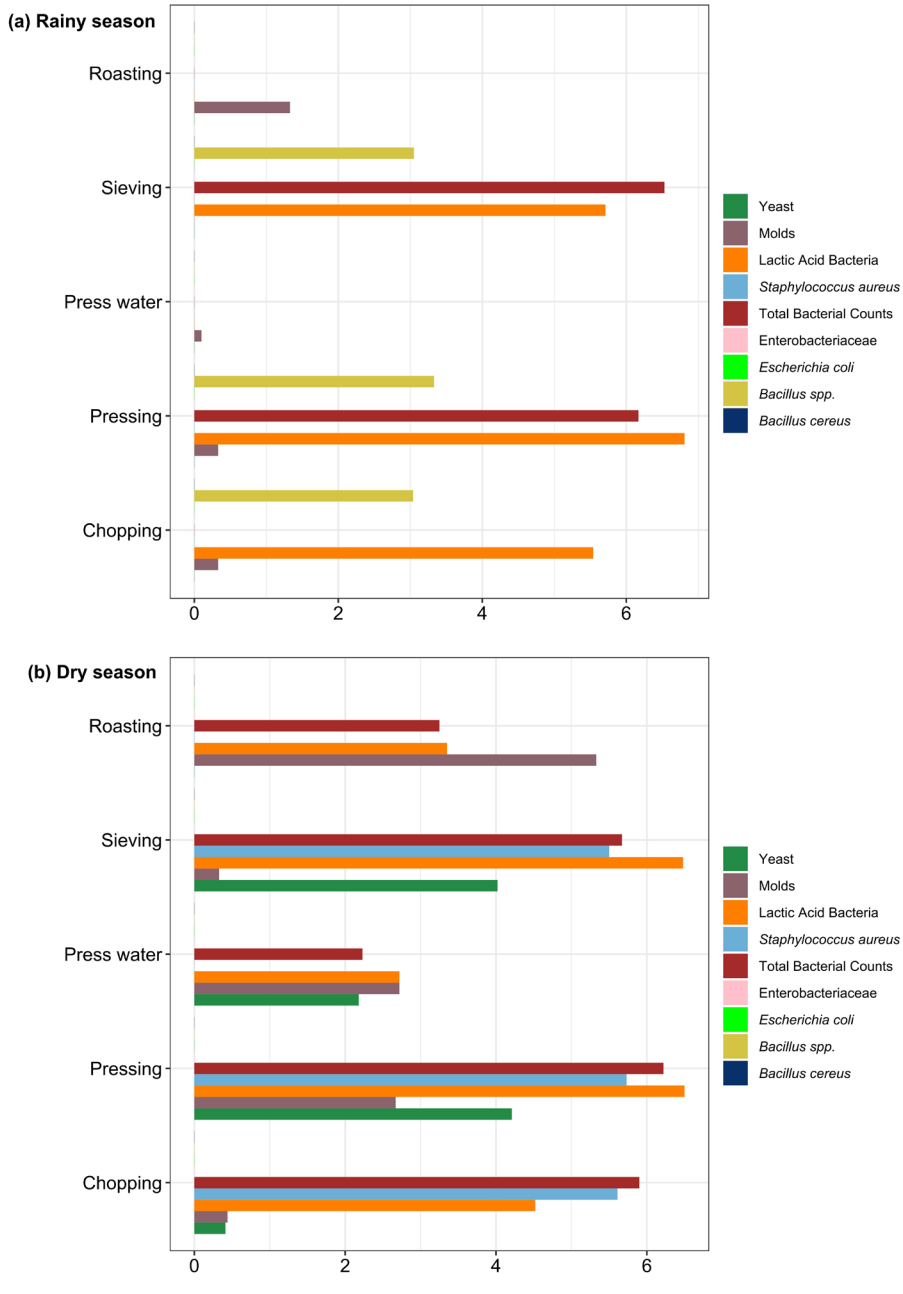

Figure 5. Enumeration (log cfu/g) of different microbial groups isolated within the representative processing unit during (a) rainy and (b) dry seasons (Adapted from **Paper I**).

4. Conversion of lignocellulosic biomass into value-added products

4.1 Lignocellulosic biomass and its structure

Lignocellulosic biomass is an abundant and renewable plant-derived material composed mainly of cellulose, hemicellulose, and lignin, with minor amounts of proteins, lipids, pectin and minerals (Guerriero et al., 2016; Ning et al., 2021; dos Anjos et al., 2025). It is the most promising feedstock for potential second-generation (2G) bioethanol production, offering a sustainable alternative to first-generation resources (e.g., maize, wheat, or sugarcane) that compete with food supply (Zoghlami & Paës, 2019; Devi et al., 2021).

The biochemical composition of lignocellulosic biomass depends on plant species, growing conditions, and tissue type (Zoghlami & Paës, 2019), but typically contains 35–55% cellulose, 20–40% hemicellulose, and 10–25% lignin (Sharma et al., 2022). Cellulose, a linear polymer of D-glucose connected by β-1,4-glycosidic bonds, forms highly ordered crystalline microfibrils embedded in an amorphous matrix. Hemicellulose, in contrast, is a branched heteropolymer composed of pentoses (xylose, arabinose) and hexoses (mannose, glucose, galactose), which connects cellulose to lignin through hydrogen and covalent bonds. Lignin, a hydrophobic aromatic heteropolymer derived from monolignols such as p-coumaryl, coniferyl, and sinapyl alcohols, provides rigidity and resistance to enzymatic degradation, but also hinders access to fermentable carbohydrates (Zoghlami & Paës, 2019; Devi et al., 2021) (Figure 6). This compact and interlinked architecture of lignocellulosic biomass, where lignin crosslinks cellulose and hemicellulose, creates the main barrier to efficient saccharification of the biomass, a phenomenon known as biomass recalcitrance (Zoghlami & Paës, 2019). Consequently, effective pretreatment strategies are required to breakdown this structure and release fermentable sugars for microbial conversion (Shukla et al., 2023).

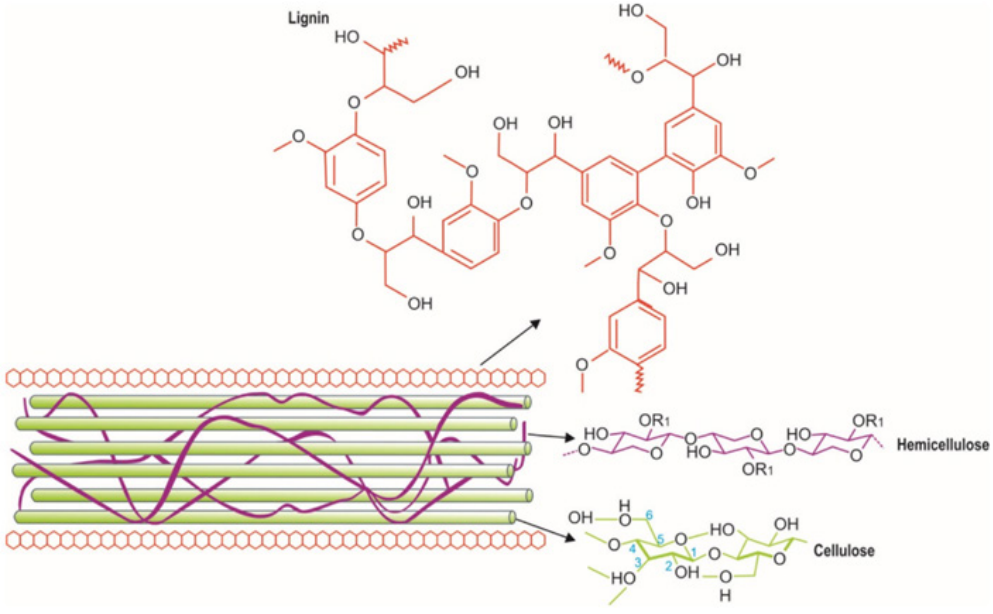

Figure 6. Lignocellulosic biomass structure and composition (Adapted from Tran et al., 2019; Ojo, 2023).

4.2 Pretreatment of lignocellulosic biomass

The complex and highly ordered structure of lignocellulosic biomass must be disrupted through pretreatment, a crucial step to break down the interlinked network of cellulose, hemicellulose, and lignin, and enhance enzymatic accessibility (Shukla et al., 2023; Abolore et al., 2024; Woźniak et al., 2025). Various pretreatments strategies have been developed, broadly classified as physical, physicochemical, chemical and biological methods, each differing in mechanisms and efficiency (Shukla et al., 2023). The efficiency of lignocellulosic biomass conversion largely depends on the choice of pretreatment method, which must be tailored to the biomass type, structural complexity, and polymer composition (Wi et al., 2015; Sharma et al., 2023; Woźniak et al., 2025). Recent advances in bioprocessing have focused on the efficient conversion of lignocellulose into bio-based products, including bioethanol, microbial oils, organic acids, and biopolymers (Ojo, 2023; Periyasamy et al., 2023; Alcocer-Garcia et al., 2025).

Physical pretreatments primarily aim to reduce particle size of the lignocellulosic biomass and increase the surface accessibility of polysaccharides through mechanical chopping, milling, extrusion, irradiation, or ultrasonication (Gallego-García et al., 2023; Sharma et al., 2023; Sant'Ana Júnior et al., 2025). Among physical and chemical approaches, thermo-pressure or hydrothermal pretreatments (e.g., steam expansion, previously known as steam explosion) involve exposing biomass to high temperature (160–240 ºC) and pressure (10–20 bar) followed by rapid decompression. This process effectively disrupts the lignocellulosic structure and improves enzyme accessibility, without requiring the use of external chemicals (Ziegler-Devin et al., 2021; Woźniak et al., 2025). These approaches are environmentally friendly and effective in solubilising hemicellulose and redistributing lignin, although they can be energy demanding and may lead to sugar degradation and inhibitor formation (Jönsson & Martín, 2016; Galbe & Walberg, 2019). Chemical pretreatments, in contrast, target the cleavage of structural bonds within the biomass matrix using agents such as acids, alkalis, ionic liquids, or organic solvents to depolymerise cellulose and hemicellulose into fermentable sugars. While highly effective, these methods often require costly chemical recovery and can generate inhibitory by-products such as furfural and hemifurfural (Jönsson & Martín, 2016; Sharma et al., 2023). Bio-pretreatment employs lignin-degrading microorganisms, mainly fungi and bacteria, to modify the cell wall components in an environmentally friendly manner, though typically at slower rates (Zoghlami & Paës, 2019; Devi et al., 2021).

Following physical and chemical pretreatments, enzymatic saccharification converts the exposed polysaccharides into fermentable sugars (Zhu et al., 2020; Tang et al., 2024). This process involves synergistic enzyme systems composed of cellulases, including endo-β-1,4-glucanase, cellobiohydrolase, and β-glucosidase, and hemicellulases such as xylanases, β-xylosidase, glucuronidase, and acetylesterase, which collectively hydrolyse cellulose and hemicellulose into monomeric sugars. More recently, auxiliary enzymes such as lytic polysaccharide monooxygenases (LPMOs), laccases, and manganese peroxidases have been used to enhance saccharification efficiency by promoting oxidative cleavage of recalcitrant polysaccharides and lignin removal, thereby improving glucose yields while reducing cellulose demand (Sharma et al., 2022, 2023).

Despite these advances, enzyme systems remain costly and sensitive to process conditions, which constrains large-scale applications. To address this, research has focused on cost-reduction strategies such as onsite enzyme production, enzyme immobilisation, and the development of tailored enzyme cocktails adapted to specific biomass feedstocks (Biely et al., 2016; Robescu et al., 2025). Beyond biofuels, enzymatic hydrolysates of pretreated lignocellulosic biomass also serve as precursors for a variety of value-added bioproducts, including xylo-oligosaccharides, organic acids, lignin-derived aromatics, and platform chemicals such as vanillin, syringaldehyde, and phenolic monomers (Manisha, 2017; Sharma et al., 2023; Alvaréz et al., 2024).

After enzymatic saccharification, microbial fermentation converts the resulting soluble sugars into bioethanol, lipids or other target products such as butanol, acetone, and lactic acid (Faraco, 2013; Khunnonkwao et al., 2024). The selection of fermenting microorganisms depends on the composition of the hydrolysate, particularly the relative proportion of hexoses and pentoses, and the content of inhibitors in the fermenting broth. A variety of microorganisms have been investigated for ethanol production, including: yeasts such as *Saccharomyces cerevisiae*, *Scheffersomyces stipites* and *Scheffersomyces shehatae*; bacteria such as *Zymomonas mobilis*, *Klebsiella oxytoca*, and engineered *Escherichia coli* stains; and, *Mucor indicus* and other filamentous fungi from the genera *Penicillium*, *Trichoderma* and *Aspergillus*, which have primarily been evaluated at laboratory or pilot scale. While *S. cerevisiae* remains the industrial standard due to its robustness, inhibitor tolerance, and high ethanol productivity, alternative microorganisms continue to attract research interest for specific traits. These include the ability to ferment pentose sugars such as xylose, or highly efficient ethanol production pathways (Paulova et al., 2015; Saxena et al., 2023; Sharma et al., 2023, Al-Hammadi et al., 2025).

The bioconversion of lignocellulosic biomass represents a promising route for transforming agricultural and industrial residues into value-added products (**Paper II**), supporting the global transition toward a waste-to-wealth circular bioeconomy. Among these residues, cassava by-products such as peels, fibres, and cassava process press water share a composition similar to that of fresh roots. They consist mainly of starch (approximately 90%), of which about 20% is linear amylose and 80% branched amylopectin,

making them a carbohydrate-rich substrates suitable for microbial bioconversion (Hierro-Iglesias et al., 2022).

In this thesis, cassava peels and fibres were selected as representative substrates for bio-processing. In these hydrolysates, the hemicellulosic and cellulosic fractions provided fermentable sugars for lipid and ethanol production, whereas the solid residues (filtrate cake), mainly containing lignin, were not evaluated in this study (**Paper II**; Figure 7).

Figure 7. Conversion of cassava residues in hydrolysates (Adapted from **Paper II**). (a) Pretreatment unit for steam expansion (STEX) of the lignocellulosic biomass at Lund University, Sweden; (b-c) Biomass after STEX; (d) Enzymatic hydrolysis applied for cassava biomass.

4.3 Conversion of cassava peels and fibres into value-added side streams

4.3.1 Oleaginous microorganisms and lipid metabolism

Lipids are indispensable biomolecules in all organisms, serving as structural components of membranes, energy reserves, and regulatory agents (Sandager et al., 2002; Lingwood & Simons, 2010; Eisenberg & Buttner, 2014). In most microorganisms, lipids account for approximately 7–15% of their dry cell mass (Kaneko et al., 1976; Ali & Szabó, 2023). However, oleaginous yeasts possess an exceptional ability to accumulate lipids exceeding 20% of their dry cell mass (Passoth et al., 2023), with some strains reaching levels above 70% under optimised conditions (Ratledge & Wynn, 2002; Ochsenreither et al., 2016).

Oleaginous yeasts efficiently convert a wide range of carbon sources – including sugars from lignocellulosic hydrolysates, organic acids, aromatic compounds derived from lignin and glycerol from biodiesel production –

into lipids that closely resemble vegetable oils, making them promising candidates for biofuels, chemicals, and nutraceuticals (Valdés et al., 2000; Bharathiraja et al., 2017; Blomqvist et al., 2018; Passoth & Sandgren, 2019). Taxonomically, oleaginous yeasts include ascomycetous species such as *Yarrowia lipolytica, Lipomyces starkeyi* and *Blastobotrys adeninivorans*, as well as basidiomycetous species such as *Rhodotorula toruloides, Rhodotorula glutinis, Rhodotorula babjevae, Cutaneotrichosporon curvatum* (syn. *Cryptococcus curvatus*), and *Cutaneotrichosporon oleaginosus* (Sanya et al., 2021; Mota et al., 2022).

Lipid accumulation generally occurs in two metabolic phases: a growth phase, during which nutrients are abundant, and biomass formation predominates; followed by a lipid accumulation phase, triggered by excess carbon and limited nitrogen, phosphorous, or sulphur availability (Granger et al., 1993; Wen et al., 2020; Diaz-Navarrete et al., 2023). Under such nutrient stress, the metabolic flux shifts from cell growth toward the synthesis and storage of triacylglycerols (TAGs) and, in some cases, free fatty acids (Ratledge & Wynn, 2002; Shapaval et al., 2019; Nagaraj et al., 2022).

In oleaginous yeasts such as *R. toruloides*, sugars including glucose and xylose are metabolised through glycolysis and the pentose phosphate pathway (PPP), generating adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) required for fatty acid biosynthesis. Under nitrogen-sufficient conditions, most carbon flux is directed towards biomass formation through the tricarboxylic acid (TCA) cycle, supporting active protein, nucleotide, and cell-wall biosynthesis. When nitrogen becomes limiting, cellular growth is restricted, because nitrogen is essential for amino acids and macromolecule biosynthesis. Under nitrogen limitation, a key regulatory event occurs in the TCA cycle: mitochondrial isocitrate dehydrogenase activity is inhibited due to reduced availability of intracellular adenosine monophosphate (AMP), which is consumed during nitrogen starvation. This inhibition prevents the conversion of isocitrate to α-ketoglutarate, leading to citrate accumulation within the mitochondria. Excess citrate is subsequently exported to the cytosol, where ATP-citrate lyase converts citrate into acetyl-CoA – the main precursor for *de novo* fatty acid biosynthesis (Ratledge, 2014; Fakas, 2017; Passoth et al., 2023). Excess carbon is therefore redirected from energy-generating pathways toward storage metabolism, resulting in the synthesis of

triacylglycerols (TAGs) via the Kennedy pathway and their storage in lipid bodies. Under optimised cultivation conditions, this metabolic shift enables storage lipids to constitute more than half of the total cell mass, positioning *R. toruloides* as a robust and versatile platform for sustainable microbial oil production (Passoth et al., 2023).

Together, these metabolic capabilities make oleaginous yeast such as *R. toruloides* CBS 14, not only efficient lipid producers but also highly adaptable biocatalysts capable of converting low-cost, carbon-rich agro-industrial residues into renewable bio-based products (**Paper II**). Their capacity to thrive under nutrient-limited and stress conditions is highlighted for valorising complex substrates such as cassava processing residues.

4.3.2 *Rhodotorula toruloides* as a promising oleaginous yeast

Among oleaginous yeasts, *R. toruloides* is one of the most studied yeast species, second to the model organism *Yarrowia lipolytica*. This non-conventional basidiomycetous yeast (Sporidiobolaceae family) is remarkable for its ability to accumulate exceptionally high levels of lipids up to 76% of its cell dry weight (Wu et al., 2010; Ageitos et al. 2011; Xue et al., 2018; Shen et al., 2017).

Beyond its exceptional ability for lipid accumulation, *R. toruloides* exhibits remarkable tolerance to inhibitors typically found in lignocellulosic hydrolysates, enabling efficient growth, and synthesis from complex, low-cost substrates (Brandenburg et al., 2021; Fernandes et al., 2023). The fatty acid composition of lipids produced by *R. toruloides* is generally similar to that of conventional vegetable oils, being dominated by oleic, palmitic, and linoleic acids, which supports its broad applicability for both bio-based fuels and oleochemical applications. In addition, certain *R. toruloides* strains have been shown to produce triacylglycerols with physicochemical properties similar to cocoa butter, suggesting potential niche applications in the food and confectionery sectors (Wei et al., 2017; Sun et al., 2023; Wu et al., 2023; Lee et al., 2024).

The combination of high lipid productivity, substrate flexibility, and co-production of valuable metabolites highlights *R. toruloides* as a robust yeast for integrated valorisation of cassava processing residues and its potential for sustainable bioconversion of cassava-derived lignocellulosic feedstocks.

4.3.3 Lipid production by cassava hydrolysates using *R. toruloides*

In the experiment reported in **Paper II**, *R. toruloides* CBS 14 was cultivated using cassava peel and fibre hydrolysates as carbon source to evaluate its ability to convert hemicellulosic sugars into microbial lipids (Figure 8). Hydrolysates were diluted to 75% (*v/v*) and supplemented with 5% (*v/v*) cassava process press water, with or without addition of 2 g/L of ammonium sulphate, as nitrogen source. The addition of cassava press water provided an additional source of nitrogen and organic compounds while promoting valorisation of this liquid residue within the same bioprocess. This contributes to a more integrated and sustainable use of cassava processing streams.

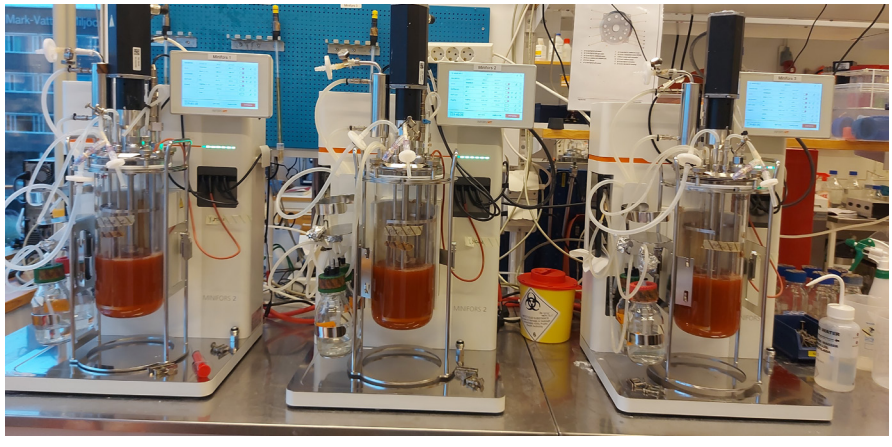

Figure 8. Cultivation of *R. toruloides* CBS 14 in cassava fibre hydrolysate in bioreactors.

In cassava peel hydrolysate, *R. toruloides* CBS 14 performed well with and without nitrogen supplementation (Table 2). The addition of ammonium sulphate slightly increased biomass formation (from 17.1 to 19.3 g/L CDW) and accelerated glucose utilisation, shortening the time required to complete sugar depletion from 168 h to 96 h. Although, lipid content decreased slightly from 34.9% of CDW in the absence of ammonium sulphate to 30.3% of CDW upon ammonium sulphate supplementation, this reduction was not pronounced. Lipid yields, expressed as g lipid per g glucose consumed, ranged from 0.13 to 0.15 g/g in cultivations with and without nitrogen supplementation, respectively. Overall, nitrogen supplementation primarily stimulated growth and metabolic activity without substantially affecting lipid productivity. Importantly, the three-day reduction in fermentation time

represents a meaningful advantage for industrial applications, as faster sugar conversion directly translates into lower energy and operational costs. These outcomes are consistent with well-established metabolic behaviour of oleaginous yeasts, in which available nitrogen supports biomass formation, whereas high C/N conditions favour lipid accumulation (Ratledge 2013; Sitepu et al., 2014; Lopes et al., 2020; Saini et al., 2021; **Paper II**).

A similar cultivation approach using cassava fibre hydrolysate (**Paper II**; Table 2) showed that despite its higher initial glucose concentration (150 g/L), the performance of *R. toruloides* was influenced by the complex composition of the fibre-derived matrix, which includes residual lignocellulosic components and potential inhibitory compounds (Jönsson et al., 2013; Moreno et al., 2022). Without nitrogen supplementation, the yeast reached 16.5 g/L CDW and accumulated 49.6% lipids, resulting in the highest lipid yield (0.19 g/g). When ammonium sulphate was added, lipid content reduced to 23%, and lipid yield fell to 0.02 g/g, despite similar initial glucose levels. Notably, CDW also decreased under nitrogen supplementation, indicating that the reduced lipid accumulation cannot be attributed solely to stimulation of biomass formation. Instead, these results suggest that the fibre hydrolysate imposes additional physiological or inhibitory stress, leading to carbon being channelled towards maintenance energy and carbon dioxide (CO_2) rather than biomass or lipid synthesis. This indicates that, although nitrogen supplementation improved sugar utilisation and shortened fermentation time (from 192 h to 72 h), it simultaneously repressed lipid accumulation, suggesting a clear trade-off between biomass formation and lipid storage.

To investigate whether substrate concentration contributed to the observed inhibitory effects, cultivations were repeated at lower substrate dilution (20% *v/v*). Under these conditions, both growth and lipid accumulation decreased substantially, indicating that nutrient density and carbon availability are key determinants for efficient lipid biosynthesis (Costa et al., 2024). Nevertheless, *R. toruloides* has been shown in previous studies to accumulate substantially higher levels of lipids than those reported in **Paper II** (Nagaraj et al., 2022; Almuhayawi et al., 2023).

Overall, these findings highlight the ability of *R. toruloides* CBS 14 to convert cassava-derived hydrolysates into microbial lipids, demonstrating the importance of optimising nutrient balance, cultivation parameters, and detoxification strategies to improve process efficiency (**Paper II**; **Paper III**).

Table 2. Growth and lipid accumulation of *Rhodotorula toruloides* CBS 14 cultivated in 75% (*v/v*) cassava peel and fibre hydrolysates with and without nitrogen supplementation (2 g/L AS). AS – Ammonium sulphate. CDW – Cell dry weight. Values represent means ± standard deviations (Adapted from **Paper II**).

	75% hydrolysate				**20% hydrolysate**	
	Peels	**Peels +As**	**Fibres**	**Fibres +As**	**Fibres**	**Fibres +As**
Cultivation time (h)	168	96	192	72	96	96
Initial glucose concentration (g/L)	50	50	150	150	50	50
CDW (g/L)	17.14 ± 0.06	19.28 ± 1.27	16.51 ± 2.15	13.52 ± 0.47	8.51 ± 0.60	12.15 ± 0.44
Lipid content (% CDW)	34.94 ± 2.00	30.31 ± 7.68	49.55 ± 1.29	23.14 ± 0.65	10.41 ± 1.15	25.81 ± 5.02
Lipid concentration (g/L)	5.99 ± 0.36	5.89 ± 1.86	8.18 ± 1.02	3.13 ± 0.03	0.88 ± 0.04	3.12 ± 0.51
Lipid yield (g/g glucose)	0.15 ± 0.00	0.13 ± 0.00	0.19 ± 0.00	0.02 ± 0.00	0.07 ± 0.00	0.02 ± 0.00

4.3.4 Yeast oil as potential replacement for vegetable oil

Lipids are mainly constituted of fatty acids, which are classified according to the degree of unsaturation as saturated (SFAs), monounsaturated (MUFAs), or polyunsaturated (PUFAs) (Domínguez et al., 2019). Based on the position of the first double bond from the methyl end, unsaturated fatty acids are further distinguished as ω-3 or ω-6 types, with linoleic (ω-6) and α-linolenic (ω-3) acids being essential since humans cannot synthesise them (Orsavová et al., 2015). Vegetable oils – composed mainly of triglycerides containing diverse fatty acids – are a key dietary source of MUFAs and PUFAs up to a chain length of C18, such as oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, which pay crucial roles in cardiovascular and metabolic health (Jiménez-López et al., 2020). Beyond serving as nutrition compounds, their physicochemical properties make them valuable for multiple food applications, including frying, bakery formulations, and

emulsified products (Gavahian et al., 2019; Barros et al., 2021). Common edible oils include olive-, sunflower-, canola-, soybean-, and palm oil, each differing in fatty acid composition and stability, which influence their functionality and health impact (Nieto & Lorenzo, 2022).

However, the rapidly increasing global demand for vegetable oils, places significant pressure on agricultural land, water resources, and ecosystems, particularly in tropical regions where oilseed expansion has been associated with deforestation, biodiversity loss, and competition with food and feed crops (Meijaard et al., 2020; Ikegwu et al., 2022; Sivadas et al., 2025). These sustainability challenges have intensified interest in alternative lipid sources that dissociate lipid production from arable land use.

In this context, microbial lipids, also referred as single-cell oils (SCOs), have emerged as a promising complement, and in some applications, as a substitute to vegetable oils (Bharataja et al., 2017; Carota et al., 2018). SCOs can be produced by a variety of oleaginous microorganisms, including bacteria, algae, filamentous fungi and yeasts. Among the different SCOs, yeast oils are most similar to vegetable oils, having a similar fatty acid composition, which makes them suitable for several applications (Chattopadhyay & Maiti, 2021; Sivadas et al., 2025). These oils predominantly include palmitic (16:0), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2) (Parsons et al., 2020). Their versatility extends from use as feedstock for biofuels and bio-lubricants to incorporation into nutraceutical and pharmaceutical formulations. Over the past decades, about 113 yeasts species have been reported as lipid-accumulating yeasts, particularly species belonging to genera *Yarrowia*, *Rhodotorula*, *Lipomyces*, *Trichosporon*, *Cryptococcus*, *Candida*, and others. Advances in systems biology and metabolic engineering have further improved understanding of their lipid biosynthesis pathways, paving the way for sustainable microbial oil production from low-cost and renewable substrates (Chattopadhyay & Maiti, 2021; Lei et al., 2024).

In **Paper II**, the fatty acid composition of lipids produced by *Rhodotorula toruloides* CBS 14 from cassava peel and fibre hydrolysates was very similar to that of common vegetable oils. Oleic acid (C18:1, n-9), was the main component, followed by palmitic (C16:00), linoleic (C18:2, n-6), and smaller amounts of stearic (C18:0) and α-linolenic (C18:3, n-3) acids. MUFAs were the most abundant group (about 47–60% of total lipids), while SFAs and PUFAs varied depending on the substrate and nitrogen availability. Adding

nitrogen slightly increased desaturation levels, resulting in more MUFAs while keeping the overall lipid composition balanced. Similar fatty acid patterns have been reported for *R. toruloides* grown on other lignocellulosic hydrolysates (Wang et al., 2012; Fei et al., 2016; Nagaraj et al., 2022, Nagaraj et al., 2025). Overall, *R. toruloides* demonstrated the ability to adjust its fatty acid composition under different nutrient growth conditions and to produce valuable fatty acids such as oleic, linoleic and linolenic acids, making it a promising yeast for sustainable microbial oil production.

4.3.5 *Saccharomyces cerevisiae* as a benchmark microorganism for ethanol production

Saccharomyces cerevisiae is one of the most extensively studied microorganisms and remains the benchmark yeast for industrial ethanol production. It is recognised for its long history of safe use, strong fermentative performance, and ability to adapt to a variety of lignocellulose-based growth substrates (Erdei, 2013; Nandy & Srivastava, 2018). Owing to its high metabolic efficiency and robustness, *S. cerevisiae* dominates large-scale bioethanol manufacturing processes, consistently achieving yields above 90% of the theoretical maximum (Caspeta et al. 2015; Tsegaye et al., 2024). Despite its industrial advantages, *S. cerevisiae* faces several physiological challenges under commercial fermentation conditions (Paulova et al., 2015; Alves et al., 2023; Tsegaye et al., 2024).

Recent advances in strain improvement, including genome shuffling, adaptative evolution, and metabolic engineering, have been instrumental in enhancing *S. cerevisiae's* tolerance to thermal, osmotic, and ethanol-related stress (Caspeta et al., 2015; Topaloğlu et al., 2023). These innovations ensure that *S. cerevisiae* continues to serve as the principal reference microorganism for evaluating the performance of non-conventional yeast in lignocellulosic ethanol producing systems.

4.3.6 Ethanol production by cassava hydrolysates using *Saccharomyces cerevisiae*

In the investigation detailed in **Paper II**, *S. cerevisiae* J672 was cultivated in 75% (*v/v*) cassava peel and fibre hydrolysates supplemented with 5% (*v/v*) cassava process press water, to assess their suitability as substrates for ethanol production and to determine how hydrolysate composition and potential inhibitory compounds influenced fermentation performance.

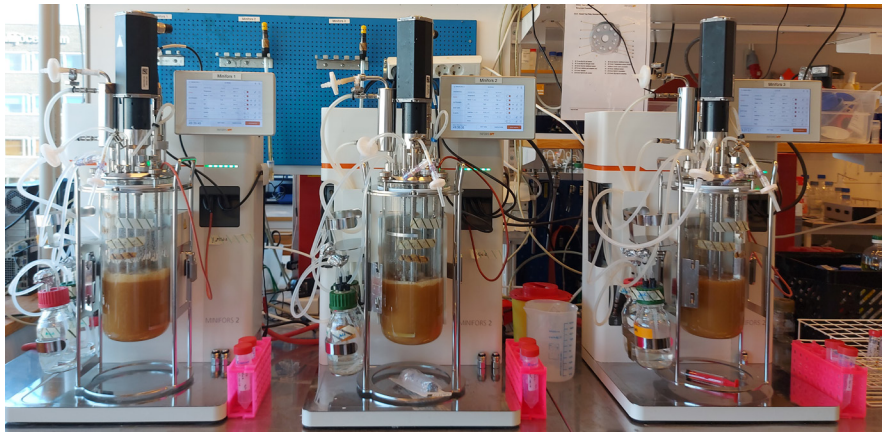

Figure 9. Cultivation of *S. cerevisiae* J672 in cassava fibre hydrolysate in bioreactors.

Fermentations with *S. cerevisiae* J672 in cassava peel and fibre hydrolysates exhibited different fermentation outcomes (Figure 9, Table 3, **Paper II**). In peel hydrolysate, glucose was rapidly consumed and efficiently converted into ethanol, yielding 0.45 ± 0.06 g/g glucose and reaching 23.1 ± 3.2 g/L after 28 h. This indicates that peel hydrolysate supported fast sugar utilisation and efficient ethanol conversion. In contrast, fibre hydrolysate supported higher ethanol titre (48.3 ± 3.3 g/L) but with lower ethanol yield (0.37 ± 0.03 g/g) and incomplete glucose utilisation, accompanied by noticeable glycerol formation. These differences indicate that cassava fibre hydrolysate imposed osmotic and inhibitory stresses, limiting ethanol yield despite high substrate availability.

Such outcomes align with previous reports describing inhibitory effects of furan derivatives, carboxylic acids, and phenolic compounds generated during pretreatment and enzymatic hydrolysis of lignocellulosic feedstocks (van der Pol et al., 2014; Kim et al., 2020; **Paper II**). Similar trends have been reported for other cassava-based substrates, where ethanol yields

strongly depend on feedstock composition, the chosen pretreatment strategy, and detoxification efficiency (Adegbehingbe et al., 2021; Pimpisai et al., 2024; Sokan-Adeaga et al., 2024).

Cassava residues naturally contain cyanogenic glycosides, organic acids, and phenolic compounds that can further inhibit yeast metabolism and ethanol production (Amalia et al., 2021; Nizzy et al., 2022). Additional lignin-derived molecules such as furans, phenolics, and amine-based compounds (e.g., vanillin) generated during enzymatic hydrolysis are also known to interfere with fermentation (Nuwamanya et al., 2012).

Overall, these findings show that cassava residues, particularly peels, represent technically viable lignocellulosic feedstocks for microbial fermentations (Amalia et al., 2021; **Paper II**). However, efficient ethanol production will depend on mitigating inhibitory effects through appropriate detoxification, strain improvement, or process optimisation strategies.

Table 3. Growth and ethanol production of *Saccharomyces cerevisiae* J672 cultivated in 75% (*v/v*) cassava peel and fibre hydrolysates with addition of 5% (*v/v*) cassava process press water. Values represent means ± standard deviations (Adapted from **Paper II**).

	75% hydrolysate	
	Peels	**Fibres**
Cultivation time (h)	28	72
Initial glucose concentration (g/L)	50	150
Final ethanol concentration (g/L)	23.13 ± 3.18	48.29 ± 3.32
Yield ethanol/glucose (g/g)	0.45 ± 0.06	0.37 ± 0.03
Yield CDW/glucose (g/g)	0.13 ± 0.01	0.04 ± 0.00
Yield glycerol/glucose (g/g)	0.05 ± 0.00	0.05 ± 0.00

4.3.7 Estimated potential of cassava residues for microbial lipid and ethanol production in Mozambique

The results presented in **Paper II** demonstrate that pretreatment followed by enzymatic hydrolysis effectively converted cassava peels and fibres into fermentable hydrolysates suitable for microbial production of ethanol and microbial lipids. In Mozambique, annual cassava production is approximately 7.6 million tons of fresh roots (MADER, 2023). Assuming an average moisture content of 50%, this corresponds to about 3.8 million tons of dry matter, of which roughly 15% are peels (~570 000 tons) and 5% are fibres (~190 000 tons) generated as solid processing residues (**Paper I**; **Paper II**).

During pretreatment and enzymatic hydrolysis, water was added to the biomass, resulting in the formation of a wet hydrolysate whose mass exceeded that of the initial dry residue. Under the experimental conditions applied in **Paper II**, 1 kg of dried cassava peels yielded approximately 1.7 kg of wet hydrolysate, while 1 kg of dried cassava fibres yielded approximately 1.3 kg of wet hydrolysate. These hydrolysates were subsequently used as substrates for fermentation (**Paper II**). Final concentrations of ethanol and microbial lipids were quantified in the fermentation broth (g/L), converted to total product mass per cultivation, and subsequently normalised to the mass of dry cassava residue used to generate the hydrolysate. This normalisation allowed product yields to be expressed as percentages on a dry residue basis (*w/w*), which were used for national-scale extrapolation.

Experimental data from **Paper II** showed that fermentation of peel hydrolysate with *S. cerevisiae* resulted in an ethanol yield corresponding to 5.24% (*w/w*) on a dry peel basis, while cultivation with *R. toruloides* led to microbial lipid accumulation equivalent to 1.36% (*w/w*) on a dry peel basis. Similarly, fermentation with cassava fibre hydrolysate resulted in an ethanol yield corresponding to 8.37% (*w/w*) on a dry fibre basis during *S. cerevisiae* cultivation, while *R. toruloides* fermentation led to microbial lipid accumulation equivalent to 1.42% (*w/w*) on a dry fibre basis (**Paper II**). All values represent theoretical yields normalised to the dry mass of cassava residues.

When extrapolated to a national scale, these experimentally determined conversion efficiencies correspond to theoretical annual potentials of approximately 29 900 tons of ethanol and 7 750 tons of microbial lipids from cassava peels, and about 15 900 tons of ethanol and 2 700 tons of microbial lipids from fibres, assuming full residue availability and excluding collection and processing losses. Altogether, cassava processing residues in Mozambique could theoretically generate approximately 45 800 tons of ethanol and 10 450 tons of microbial lipids per year (**Paper II**).

These estimations underscore the considerable, yet largely underexploited, potential of cassava residues as bioresource for a circular and sustainable bioeconomy. Integrating residue valorisation into existing cassava-processing systems could substantially reduce environmental burdens, promote waste-to-value conversion, and enhance local economic opportunities through decentralised bioproduct generation. Such an approach would strengthen cassava-based value chains and align with national strategies for renewable energy, bioindustry and rural development. This provides the conceptual bridge to the next chapter, which explores yeast adaptation and tolerance mechanisms in cyanide-rich cassava processing effluents (**Paper I**; **Paper III**).

5. Cyanide glycosides in cassava

5.1 Cyanide metabolism

Cassava is one of more than 2000 plant species capable of producing cyanogenic glycosides (Vetter, 2000; Møller, 2010; Gleadow & Møller, 2014). The main compounds are linamarin and lotaustralin, synthesised from the amino acids valine and isoleucine respectively, in a molar concentration ratio of 93:7 (Nartey, 1969; Burns et al., 2012). Linamarin is distributed throughout the tuber, with the highest concentration found in the outer cortical layers beneath the periderm, while its content in the leaves can exceed that in the root parenchyma by more than tenfold. The biosynthesis of these compounds occurs primarily in the leaves, from where they are translocated through the phloem to the roots (Burns et al., 2012). Some additional synthesis occurs in the periderm, thus, the elevated cyanogenic potential in the outer tissues (Jørgensen et al., 2005; Nyaika et al., 2024).

In intact cassava tissues, linamarin is stored within vacuoles, spatially separated from its hydrolytic enzyme linamarase, a β-glucosidase enzyme associated with the cell wall and intercellular spaces (White et al., 1998). This spatial compartmentalisation prevents the formation of toxic hydrogen cyanide (HCN) during normal physiological conditions. When plant cells are disrupted by mechanical damage such as cutting, grating, or chewing, the vacuoles rupture, allowing linamarin to encounter linamarase (White et al., 1998; Vetter, 2000). The enzyme linamarase hydrolyses linamarin to produce glucose and acetone cyanohydrin, which then decomposes spontaneously or under the action of α-hydroxynitrile lyase releasing acetone and hydrogen cyanide (White et al., 1998; Burns et al., 2012). Figure 10 illustrates the enzymatic hydrolysis of linamarin and the subsequent release of hydrogen cyanide.

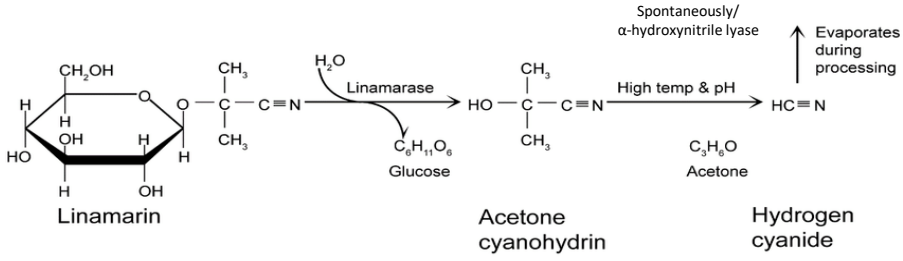

Figure 10. Enzymatic hydrolysis of linamarin leading to the release of hydrogen cyanide (HCN). Linamarin is hydrolysed by linamarase to form glucose and acetone cyanohydrin, which subsequently decomposes, either spontaneously or via α-hydroxynitrile lyase, releasing hydrogen cyanide (Adapted from Kuliahsari et al., 2021).

5.2 Microbial cyanide degradation pathways

Although cyanide is highly toxic to most organisms, it occurs naturally and plays diverse biological roles. Cyanogenic compounds are produced by several organisms, including plants (such as cassava, almonds, and beans), fungi, bacteria, and even some arthropods such as beetles and butterflies (Baxter & Cummings, 2006; Dash et al., 2009). In nature, cyanide functions as a defence compound against predators, a regulator of insect mating behaviour, and in some microorganisms, as a precursor for secondary metabolites such as antibiotics (Martinez & Diaz, 2024). Microorganisms capable of surviving in cyanide-rich environments have evolved metabolic adaptations that allow them not only to tolerate but also transform cyanide into less toxic or assimilated forms through distinct adaptation pathways (Alvillo-Riveira et al., 2021; Berkinbayeva et al., 2025).

Microorganisms capable of degrading cyanide employ a range of biochemical strategies that transform this toxic compound into less harmful or assimilable forms. These mechanisms are generally grouped into oxidative, hydrolytic, reductive, and substitution pathways, depending on the enzymatic reactions involved and the environmental conditions under which they occur (Dash et al., 2009; Luque-Amagro et al., 2018).

In oxidative degradation, cyanide is converted into cyanate (OCN^-) through the action of enzymes such as cyanide monooxygenase or cyanide dioxygenase. Cyanate can then be further hydrolysed to yield ammonia and carbon dioxide, which serve as nitrogen and carbon sources for cellular metabolism. The hydrolytic pathway involves enzymes such as cyanidase or

cyanide hydratase, which directly hydrolyse cyanide to formamide or formic acid, releasing ammonia in the process (Luque-Amagro et al., 2018; Alvillo-Riveira et al., 2021). Under anaerobic or low-oxygen conditions, microorganisms may employ reductive pathways, in which cyanide or metal-cyanide complexes are reduced to methane or other simple compounds. Alternatively, in the substitution (replacement) pathway, cyanide reacts with sulphur-containing compounds to produce thiocyanate, catalysed by rhodanese or related sulfurtransferases. Thiocyanate can subsequently undergo further degradation to yield sulphate and ammonia (Luque-Amagro et al., 2018; Alvillo-Riveira et al., 2021).

These enzymatic processes not only detoxify cyanide but also allow microorganisms to recover essential nutrients for growth. The efficiency and dominance of each pathway depend on environmental factors such as oxygen availability, pH, temperature, and the microbial community composition (Akcil et al., 2003; Mekuto et al., 2016).

5.3 Cassava wastewater valorisation and applications

Cassava processing (**Paper I**) generates considerable amounts of solid and liquid residues (Olukanni & Olatunji, 2018; Oghenejoboh et al., 2021, **Paper II**; **Paper III**). Processing 1 ton of fresh cassava roots into high-quality cassava flour typically produces 250–300 kg of peels, 50–100 kg of fibrous bagasse, and 250–300 kg of wastewater (Ekop et al., 2019; Obonokut et al., 2022). In starch factories, the same quantity of roots generates 20-50 kg of peels, about 600 kg of fibrous pulp, and 12–20 m^3 of cyanide-rich wastewater (Ekop et al., 2019). Although discarded, these by-products contain appreciable levels of organic matter, sugars, and nutrients that could support microbial growth (**Paper II**; **Paper III**). However, the reuse of cassava residues is limited due to their cyanide content, derived from cyanogenic glucosides naturally present in the crop.

During processing, enzymatic degradation of these compounds releases hydrogen cyanide (HCN), contributing to the overall toxicity of cassava wastewater (**Paper I**; **Paper III**). Continuous discharge of such effluents has been associated with soil acidification, reduced fertility, and disruption of microbial communities near processing sites (Igbinosa & Igiehon, 2015; Ajao et al., 2025). Conventional chemical treatments are costly and often ineffective for effluents with high organic loads, whereas biological

processes provide more sustainable and adaptable alternatives (Kandasamy et al., 2015; Maciel et al., 2023; Berkinbayeva et al. 2025).

Cassava wastewater, also known as cassava press water, is a nutrient-rich effluent generated during root pressing (**Paper I**). It contains starch, free sugars (mainly glucose), proteins, lipids, minerals, and cyanogenic compounds, making it both an environmental challenge and a valuable bioresource (De Oliveira Schmidt et al., 2023). Owing to its high organic load and balanced nutrient composition, research has explored its reuse in agriculture and biotechnology (**Paper II**; **Paper III**). When applied in controlled doses, cassava wastewater can function as a biofertilizer and natural pesticide, improving crop productivity and soil fertility, while reducing dependence on mineral fertilisers (Bezerra et al., 2017; Pinto-Zevallos et al., 2018; Costa et al., 2020).

Beyond agricultural use, cassava press water has gained attention as a low-cost substrate for microbial and biotechnological applications. It has been successfully applied in anaerobic digestion for biogas and biohydrogen production (Andreani et al., 2015; Watthier et al., 2019; Achi et al., 2020), achieving a high chemical oxygen demand (COD) removal efficiencies and methane yields when properly pre-treated to reduce cyanide levels (Montoro et al., 2019; Andrade Cruz et al., 2020). Cassava wastewater has additionally been explored as a fermentation medium for microbial metabolite production, supporting the synthesis of carotenoids and fatty acids by *Rhodotorula glutinis* (Ribeiro et al., 2019; **Paper II**) and biosurfactants by *Bacillus subtilis* (Nitschke & Pastore, 2004).

These findings demonstrate that cassava press water, despite its cyanide content, possesses a nutrient composition capable of sustaining microbial growth and metabolite production (**Paper II**; **Paper III**). Notably, several yeast species have been reported to tolerate and adapt to cassava-derived effluents, highlighting their potential role in detoxification and bioconversion processes, aspects further investigated in **Paper III**.

5.4 Microorganisms isolated from the cassava processing chain and their tolerance mechanisms

The presence of cyanide and other inhibitory compounds in wastewater from cassava processing imposes strong selective pressure on the resident microbiota, favouring the persistence of tolerant species (**Paper III**). Yeasts are promising candidates for effluent valorisation and detoxification due to their metabolic versatility, robustness, and stress tolerance (Wang et al., 2018; González et al., 2025). To date, no cyanide-degrading enzymatic pathway has been conclusively demonstrated in yeasts. Shen et al. (2021) reported cyanide reduction during fermentations involving *Saccharomyces cerevisiae*, however, the results are difficult to interpret, as experimental design did not allow a clear distinction between biodegradation and abiotic losses such as volatilisation. Thus, while their findings suggest a potential biodegradative potential, the evidence remains inconclusive. In contrast, non-conventional yeasts belonging to the species *Pichia exigua*, isolated from cassava effluents, have demonstrated cyanide tolerance (Banwo et al., 2023). These findings indicate that cassava-processing environments host yeast communities with adaptative traits of high biotechnological interest (Maciel et al., 2023).

Cyanide is a potent metabolic inhibitor that binds to cytochrome oxidase, blocking the terminal step of the respiratory chain and preventing cellular oxygen uptake (Maciel et al., 2023; Bebarta & Nath, 2025). Several yeasts have evolved strategies to survive in cyanide-rich environments. Reported mechanisms include cyanide-insensitive respiration, in which alternative oxidases bypass the blocked cytochrome pathway (Henry et al., 1974; Ainsworth et al., 1980; Veiga et al., 2003), and surface adsorption or complexation, which decreases the concentration of free cyanide in the medium (Dehghani et al., 2016). These mechanisms maintain redox balance and energy metabolism, enabling yeasts to persist in effluents that would otherwise inhibit their growth.

Selected yeasts isolated from cassava processing units (**Paper I**) were tested to evaluate their behaviour in the presence of cyanide (**Paper III**). The isolates included *Rhodotorula mucilaginosa* J703, *Wickerhamomyces anomalus* J704, *Torulaspora delbrueckii* J705, *Kwoniella heveanensis* J706, *Kazachstania unispora* J707 and *Rhodotorula glutinis* CBS 2890 (reference strain). These yeasts were cultivated in media containing synthetic cyanide (KCN) and in cassava process press water, which naturally contains cyanide.

The experiments did not confirm cyanide tolerance; however, *T. delbrueckii* J705 and *W. anomalus* J704 showed comparatively better performance under cyanide stress, being able to survive and grow in the presence of cyanide, particularly in cassava press water. These observations suggest that some yeasts associated with cassava-processing environments may possess adaptative traits that allow them to withstand toxic compounds such as cyanide.

T. delbrueckii and *W. anomalus* have previously been reported as stress-tolerant yeasts (Passoth et al., 2006; Kemsawasd et al., 2015; Li et al., 2023). *T. delbrueckii* is recognised for its ability to tolerate multiple stress conditions and for its potential relevance in industrial applications at both physiological and biochemical levels (Pacheco et al., 2012; Fernandes et al., 2021). *W. anomalus* has received scientific attention due to its distinctive physiology, potential as biocontrol agent, and its broad metabolic capacity, particularly in the wine industry, where it enhances fermentation performance and contributes to aroma formation (Passoth et al., 2006; Li et al., 2023; Carbonero-Pacheco et al., 2025). These physiological traits may explain their better performance under cyanide stress observed in the present study (**Paper III**).

In a sampling of cassava effluents, a greater yeast diversity was detected, including *W. anomalus*, *Pichia bovicola*, *Candida tropicalis*, *Magnusomyces ingens* and *Saccharomyces cerevisiae*. This suggests temporal variability likely influenced by environmental and processing conditions. Interestingly, several yeasts species identified in these effluents – such as *W. anomalus* and *Pichia* spp. – have also been identified in **Paper I** within the cassava processing chain. Their recurrence across both food products and effluent samples supports the hypothesis that these yeasts constitute a stable community within cassava processing environments, rather than resulting from incidental contamination.

Four representative strains corresponding to the four most frequent yeast species isolated from cassava effluents – *P. bovicola* J709, *C. tropicalis* J710, *M. ingens* J711, and *S. cerevisiae* J712 – were evaluated for cyanide tolerance in liquid culture (**Paper III**). Among them, *P. bovicola* J709 showed the highest tolerance (up to 0.2 g/L KCN), while *M. ingens* performed optimally at 0.04 g/L KCN. These results highlight the potential of native isolates as promising candidates for further characterisation and for

future applications in cyanide-rich cassava effluent bioprocesses, as discussed in **Paper III**.

A detailed assay with the two most cyanide-tolerant yeast isolates revealed distinct physiological responses. *M. ingens* J711 achieved superior growth in cassava press water compared to *P. bovicola* J709, particularly when ammonium sulphate was supplemented. In synthetic media, cyanide concentrations decreased at comparable rates in both inoculated cultures and their corresponding blanks, indicating that volatilisation was the primary mechanism of cyanide loss. In contrast, cyanide levels in cassava press water remained stable throughout cultivation, suggesting that its complex matrix limited evaporation. No cyanide accumulation was detected in the microbial biomass, and residual cyanide measured at time point 0 h was attributed to liquid carryover. Overall, the findings demonstrate that both isolates tolerated cyanide and may have contributed slightly to its reduction, although no conclusive evidence of cyanide metabolism or biodegradation was observed under the tested conditions (**Paper III**).

6. Summary conclusion

This thesis explores three interlinked dimensions of cassava utilisation in Mozambique: (i) food safety within traditional processing systems; (ii) biotechnological valorisation of cassava processing residues, and (iii) isolation of cyanide tolerant yeasts with potential detoxification traits. By combining field-based microbial assessments, bioprocess optimisation, and microbial-physiological studies on yeast tolerance, the results provide new insights into how cassava-based value chains can be evolved towards safer and more sustainable practices.

Cassava is among the most consumed carbohydrate sources in Mozambique, as well as in many other developing tropical countries. Its processing into roasted cassava flour (Mozambican *rale*) supports food security and rural livelihoods, thus its widespread consumption underscores the need to ensure food safety for human health. The results from **Paper I** demonstrated that artisanal *rale* production in southern Mozambique presents minor hygiene challenges but remains microbiologically safe when basic sanitary measures are followed. Although bacterial and fungal contaminants were occasionally detected, overall microbial loads and toxin-producing moulds were low. Fermentation and roasting proved to be the most critical processing steps for cyanide detoxification and microbial inactivation in *rale*. These findings reinforce that strengthening hygiene, proper cooling and drying after roasting, and improved storage practices could further enhance the safety and quality of traditional cassava-based foods, especially *rale*.

Building on these observations, **Paper II** addressed the valorisation of cassava-processing residues – particularly peels, fibres, and process press water – which are often discarded as waste, on arable land. Given that these cassava processing residues remain rich in fermentable sugars and lignocellulosic material (cellulose, hemicellulose, and residual starch), it was essential to study the process residues' potential as a renewable feedstock for microbial lipid and ethanol production through enzymatic hydrolysis, with cassava press water further incorporated as a nutrient-rich supplement during yeast fermentation. The oleaginous yeast *R. toruloides* CBS 14 efficiently converted cassava hydrolysates into microbial lipids, whereas *S. cerevisiae* J672 produced ethanol under similar growth conditions. When extrapolated to Mozambique´s national cassava output, these cassava processing residues

could yield approximately 10 450 tons of microbial lipids and 45 800 tons of ethanol annually. These results demonstrate that cassava residues, traditionally treated as waste, can be transformed into valuable lignocellulosic resources, contributing to circular-bioeconomy strategies and sustainable rural development.

Paper III investigated the cyanide tolerance of yeasts associated with cassava-processing environments, focusing on their potential role in effluent detoxification and bioprocess integration. Cassava process press water, a cyanide-rich by-product of root processing, represents both an environmental challenge and a potential microbial growth medium. Yeasts isolated from cassava-processing chains and effluents were tested in media containing synthetic and natural cyanide sources, i.e., cassava press water. Although none of the isolates demonstrated cyanide biodegradation, several strains – including *T. delbrueckii* J705, *W. anomalus* J704, *P. bovicola* J709, and *M.ingens* J711 – exhibited notable cyanide tolerance, maintaining growth under cyanide stress. The non-conventional yeasts *P. bovicola* J709 and *M. ingens* J711 showed the highest survival rates, with *M. ingens* J711 displaying improved growth in cassava press water supplemented with ammonium sulphate. These findings reveal the presence of native yeast populations capable of withstanding cyanide exposure, suggesting that such strains could contribute to biological treatment and valorisation of cassava effluents.

The three studies comprising this thesis provide complementary insights into cassava´s microbiological safety, residue bioconversion potential, and microbial resilience to cyanide stress. By linking traditional food systems with modern biotechnological approaches, this work contributes to a holistic framework for safer food production and sustainable residue management, advancing the transition towards a circular cassava-based bioeconomy in Mozambique and beyond.

7. Future perspectives

Building on the findings of this thesis, several opportunities emerge for advancing both the scientific understanding and practical applications of cassava-based systems in Mozambique and similar contexts.

From a food safety perspective, future research should focus on developing optimisation protocols within the cassava processing units to enhance hygienic practices for *rale* production. The introduction of low-cost drying facilities and innovative packing technologies could significantly reduce microbial contamination while extending product shelf life. Applying molecular and metagenomic tools would allow a more detailed characterisation of the beneficial microbial consortia involved in cassava fermentation, clarifying their roles in flavour development, detoxification, and spoilage prevention. These insights could support the formulation of starter cultures specifically adapted to local processing conditions, strengthening both food safety and product consistency.

On the bioprocessing front, the valorisation of cassava residues offers promising prospects for producing biofuels and microbial lipids that can be used as ingredients in food and feed. Scaling up the processes tested in this thesis will require optimisation of fermentation conditions and nutrient balance to enhance lipid and ethanol yields. Particular attention should be given to the use of cassava process press water as a liquid feedstock for microbial fermentations. Although this effluent contains cyanide, its nutrient composition makes it a valuable low-cost substrate that could be effectively integrated into fermentation systems after suitable detoxification and process control. A detailed characterisation of its nutrient profile would therefore be crucial to optimise its use, refine supplementation strategies, and ensure consistent microbial performance. Results from this work showed that cyanide tends to evaporate during fermentation and does not bind to yeast biomass. This resulting microbial biomass could be explored as a source of single-cell protein, providing an additional valorisation pathway that connects biofuel production with sustainable feed and food applications within a circular cassava-based bioeconomy.

Regarding cyanide tolerance and effluent management, future studies should investigate the molecular basis of yeast resilience to cyanide exposure using transcriptomic, proteomic, and metabolomic approaches to identify key regulatory pathways and detoxification mechanisms. Understanding

these adaptative responses could enable the selection or engineering of strains capable of partial cyanide detoxification, complementing existing physicochemical biomass treatments and advancing sustainable effluent management strategies.

Finaly, collaborative efforts between academia, industry, and local communities will be necessary to translate these scientific findings into practical solutions that strengthen food security, environmental protection, and socio-economic resilience in developing countries.

References

Abirami, V., Velmurugan, M., Thangamani, C., Natarajan, S. K., Davamani, V., Indu, R. C., & Venkatachalam, S. R. (2025). Cassava intercropping systems for enhanced land productivity and farmer livelihoods: A review. *Plant Science Today*, *12*(3). https://doi.org/10.14719/pst.8409

Abolore, R. S., Jaiswal, S., & Jaiswal, A. K. (2024). Green and sustainable pretreatment methods for cellulose extraction from lignocellulosic biomass and its applications: A review. *Carbohydrate Polymer Technologies and Applications*, *7*, 100396. https://doi.org/10.1016/j.carpta.2023.100396

Achi, C. G., Hassanein, A., & Lansing, S. (2020). Enhanced biogas production of cassava wastewater using zeolite and biochar additives and manure co-digestion. *Energies*, *13*(2), 491. https://doi.org/10.3390/en13020491

Adebayo, W. G. (2023). Cassava production in Africa: A panel analysis of the drivers and trends. *Heliyon*, *9*(9), e19939. https://doi.org/10.1016/j.heliyon.2023.e19939

Adebayo-Oyetoro, A. O., Oyewole, O. B., Obadina, A. O., & Omemu, M. A. (2013). Microbiological safety assessment of fermented cassava flour "*Lafun*" available in Ogun and Oyo States of Nigeria. *International Journal of Food Science*, *2013*, 1–5. https://doi.org/10.1155/2013/845324

Adegbehingbe, K. T., Faparusi, F., & Adeleke, B. S. (2021). Bioethanol production from cassava peels inoculated with *Saccharomyces cerevisiae* and *Zymomonas mobilis*. *Journal of Advances in Microbiology*, 58–67. https://doi.org/10.9734/jamb/2021/v21i930384

Adetan, D. A., Adekoya, L. O., & Aluko, O. B. (2003). Characterisation of some properties of cassava root tubers. *Journal of Food Engineering*, *59*(4), 349–353. https://doi.org/10.1016/S0260-8774(02)00493-4

Adewusi, S. R., & Bradbury, J. H. (1993). Carotenoids in cassava: Comparison of open-column and HPLC methods of analysis. *Journal of the Science of Food and Agriculture*, *62*(4), 375–383. https://doi.org/10.1002/jsfa.2740620411

Adjovi, Y. C. S., Gnonlonfin, B. J. G., Bailly, S., Bailly, J. D., Tadrist, S., Puel, O., Oswald, I. P., & Sanni, A. (2015). Occurrence of mycotoxins in cassava (*Manihot esculenta* Crantz) and its products. *International Journal of Food Safety, Nutrition and Public Health*, *5*(3–4), 217. https://doi.org/10.1504/IJFSNPH.2015.070157

Ageitos, J. M., Vallejo, J. A., Veiga-Crespo, P., & Villa, T. G. (2011). Oily yeasts as oleaginous cell factories. *Applied Microbiology and Biotechnology*, *90*(4), 1219–1227. https://doi.org/10.1007/s00253-011-3200-z

Ainsworth, P. J., Ball, A. J. S., & Tustanoff, E. R. (1980). Cyanide-resistant respiration in yeast: I. Isolation of a cyanide-insensitive NAD(P)H oxidoreductase. *Archives of Biochemistry and Biophysics*, *202*(1), 172–186. https://doi.org/10.1016/0003-9861(80)90420-8

Ajao, A. T., Awe, S., Aborisade, W. T., Jimoh, F. A., & Ajao, F. J. (2025). Bioremediation of soil contaminated with cassava mill effluents - A structural, functional, and metagenomics approach. *Next Research*, *2*(3), 100439. https://doi.org/10.1016/j.nexres.2025.100439

Akcil, A., Karahan, A. G., Ciftci, H., & Sagdic, O. (2003). Biological treatment of cyanide by natural isolated bacteria (*Pseudomonas* sp.). *Minerals Engineering*, *16*(7), 643–649. https://doi.org/10.1016/S0892-6875(03)00101-8

Akpoghelie, P. O., Owheruo, J. O., Edo, G. I., Yousif, E., Zainulabdeen, K., Jikah, A. N., Mohammed, A. A., Ndudi, W., Nwachukwu, S. C., Opiti, R. A., Ainyanbhor, I. E., Onyibe, P. N., Ugbune, U., Ezekiel, G. O., Ekokotu, H. A., Oghroro, E. E. A., Ekpekpo, L. D., Isoje, E. F., Igbuku, U. A., Agbo, J. J. (2025). The benefits and processing technologies of gari, a famous indigenous food of Nigeria. Discover Food, *5*(1), 91. https://doi.org/10.1007/s44187-025-00370-1

Alcocer-García, H., Ramírez-Márquez, C., & Ponce-Ortega, J. M. (2025). Circular and decentralized refineries for a sustainable energy transition. *Chemical Engineering and Processing*, *217*, 110479. https://doi.org/10.1016/j.cep.2025.110479

Al-Hammadi, M., Anadol, G., Martín-García, F. J., Moreno-García, J., Keskin Gündoğdu, T., & Güngörmüşler, M. (2025). Scaling bioethanol for the future: The commercialization potential of extremophiles and non-conventional microorganisms. *Frontiers in Energy Research*, *13*. https://doi.org/10.3389/fenrg.2025.1565273

Ali, O., & Szabó, A. (2023). Review of eukaryote cellular membrane lipid composition, with special attention to the fatty acids. *International Journal of Molecular Sciences*, *24*(21), 15693. https://doi.org/10.3390/ijms242115693

Almuhayawi, M. S., Hassan, E. A., Almasaudi, S., Zabermawi, N., Azhar, E. I., Najjar, A., Alkuwaity, K., Abujamel, T. S., Alamri, T., & Harakeh, S. (2023). Biodiesel production through *Rhodotorula toruloides* lipids and utilization of de-oiled biomass for Congo red removal. *Sustainability*, *15*(18), 13412. https://doi.org/10.3390/su151813412

Álvarez, C., Duque, A., Sánchez-Monedero, A., González, E. J., González-Miquel, M., & Cañadas, R. (2024). Exploring recent advances in lignocellulosic biomass waste delignification through the combined use of eutectic solvents and intensification techniques. *Processes*, *12*(11), 2514. https://doi.org/10.3390/pr12112514

Alves, W. R., da Silva, T. A., Zandoná Filho, A., & Pereira Ramos, L. (2023). Lactic acid production from steam-exploded sugarcane bagasse using *Bacillus coagulans* DSM2314. *Fermentation*, *9*(9), 789. https://doi.org/10.3390/fermentation9090789

Alvillo-Rivera, A., Garrido-Hoyos, S., Buitrón, G., Thangarasu-Sarasvathi, P., & Rosano-Ortega, G. (2021). Biological treatment for the degradation of cyanide: A review. *Journal of Materials Research and Technology*, *12*, 1418–1433. https://doi.org/10.1016/j.jmrt.2021.03.030

Amalia, A. V., Fibriana, F., Widiatningrum, T., & Hardianti, R. D. (2021). Bioconversion and valorization of cassava-based industrial wastes to bioethanol gel and its potential application as a clean cooking fuel. *Biocatalysis and Agricultural Biotechnology*, *35*, 102093. https://doi.org/10.1016/j.bcab.2021.102093

Amelework, A. B., Bairu, M. W., Maema, O., Venter, S. L., & Laing, M. (2021). Adoption and promotion of resilient crops for climate risk mitigation and import substitution: A case analysis of cassava for South African agriculture. *Frontiers in Sustainable Food Systems*, *5*, 617783. https://doi.org/10.3389/fsufs.2021.617783

Andrade Cruz, I., Andrade, L. R. S., Jesus, A. A. de, Vasconcelos, B. R. de, Bharagava, R. N., Bilal, M., Figueiredo, R. T., Souza, R. L. de, & Romanholo Ferreira, L. F. (2022). Potential of eggshell waste derived calcium for sustainable production of biogas from cassava wastewater. *Journal of Environmental Management*, *321*, 116000. https://doi.org/10.1016/j.jenvman.2022.116000

Andrade, L. R. S., Felisardo, R. J. A., Cruz, I. A., Bilal, M., Iqbal, H. M. N., Mulla, S. I., Bharagava, R. N., Souza, R. L. de, Azevedo, L. C. B., & Ferreira, L. F. R. (2022). Integrated biorefinery and life cycle assessment of cassava processing residue–From production to sustainable evaluation. *Plants*, *11*(24), 3577. https://doi.org/10.3390/plants11243577

Andreani, C. L. & Torres, Douglas & Schultz, L. & Carvalho, Karina & Gomes, Simone. (2015). Hydrogen production from cassava processing wastewater in an anaerobic fixed bed reactor with bamboo as a support material. *Engenharia Agricola*. *35*, 578–587. https://doi.org/10.1590/1809-4430-Eng.Agríc.v35n3p578-587/2015

Anikwe, M. A. N., & Ikenganyia, E. E. (2018). Ecophysiology and production principles of cassava (*Manihot* species) in Southeastern Nigeria. In V. Waisundara (Ed.), *Cassava*. IntechOpen. https://doi.org/10.5772/intechopen.70828

Anyanwu, C. N., Ibeto, C. N., Ezeoha, S. L., & Ogbuagu, N. J. (2015). Sustainability of cassava (*Manihot esculenta* Crantz) as industrial feedstock, energy and food crop in Nigeria. *Renewable Energy*, *81*, 745–752. https://doi.org/10.1016/j.renene.2015.03.075

Banwo, K., Tosin Ojetunde, J., & Falade, T. (2023). Probiotic and cyanide degrading potentials of *Pediococcus pentosaceus* and *Pichia exigua* isolated from cassava products effluent. *Food Biotechnology*, *37*(1), 1–24. https://doi.org/10.1080/08905436.2022.2163252

Barros, J. C., Munekata, P. E. S., de Carvalho, F. A. L., Domínguez, R., Trindade, M. A., Pateiro, M., & Lorenzo, J. M. (2021). Healthy beef burgers: Effect of animal fat replacement by algal and wheat germ oil emulsions. *Meat Science*, *173*, 108396. https://doi.org/10.1016/j.meatsci.2020.108396

Baxter, J., & Cummings, S. P. (2006). The current and future applications of microorganism in the bioremediation of cyanide contamination. *Antonie van Leeuwenhoek*, *90*(1), 1–17. https://doi.org/10.1007/s10482-006-9057-y

Bebarta, V. S., & Nath, A. K. (2025). Redirecting Intermediary Metabolism to Counteract Cyanide Poisoning. *FASEB Journal*, *39*(12), e70709. https://doi.org/10.1096/fj.202400230RR

Berkinbayeva, A., Kenzhaliyev, B., Smailov, K., Aimagambetov, A., Kamenov, B., Saulebekkyzy, S., Tolegenova, N., & Putri, P. S. R. (2025). An overview of biological cyanide elimination from tailing wastewater as a promising tool for sustainable utilization. *Water Research X*, *29*, 100400. https://doi.org/10.1016/j.wroa.2025.100400

Bezerra, M. G. S., Silva, G. G. C. da, Difante, G. dos S., Emerenciano Neto, J. V., Oliveira, E. M. M., & Oliveira, L. E. C. de. (2017). Cassava wastewater as organic fertilizer in 'Marandu' grass pasture. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *21*(6), 404–409. https://doi.org/10.1590/1807-1929/agriambi.v21n6p404-409

Bharathiraja, B., Sridharan, S., Sowmya, V., Yuvaraj, D., & Praveenkumar, R. (2017). Microbial oil – A plausible alternate resource for food and fuel application. *Bioresource Technology*, *233*, 423–432. https://doi.org/10.1016/j.biortech.2017.03.006

Biely, P., Singh, S., & Puchart, V. (2016). Towards enzymatic breakdown of complex plant xylan structures: State of the art. *Biotechnology Advances*, *34*(7), 1260–1274. https://doi.org/10.1016/j.biotechadv.2016.09.001

Blomqvist, J., Pickova, J., Tilami, S. K., Sampels, S., Mikkelsen, N., Brandenburg, J., Sandgren, M., & Passoth, V. (2018). Oleaginous yeast as a component in fish feed. *Scientific Reports*, *8*(1), 15945. https://doi.org/10.1038/s41598-018-34232-x

Borku, A. W., Tora, T. T., & Masha, M. (2025). Cassava in focus: A comprehensive literature review, its production, processing landscape, and multi-dimensional benefits to society. *Food Chemistry Advances*, *7*, 100945. https://doi.org/10.1016/j.focha.2025.100945

Bradbury, J. H. & Holloway, W. D. (1988). *Chemistry of tropical root crops: Significance for nutrition and agriculture in the Pacific*. (Monograph No. 6, pp. 76–104). Australian Centre for International Agricultural Research.

Brandenburg, J., Blomqvist, J., Shapaval, V., Kohler, A., Sampels, S., Sandgren, M., & Passoth, V. (2021). Oleaginous yeasts respond differently to carbon sources present in lignocellulose hydrolysate. *Biotechnology for Biofuels*, *14*(1), 124. https://doi.org/10.1186/s13068-021-01974-2

Bratley, K., & Meyer-Cirkel, A. (2025). *Understanding agricultural output in Mozambique - Using remote sensing to initiate a discussion on development*. International Monetary Fund.

Burns, A. E., Bradbury, J. H., Cavagnaro, T. R., & Gleadow, R. M. (2012). Total cyanide content of cassava food products in Australia. *Journal of Food Composition and Analysis*, *25*(1), 79–82. https://doi.org/10.1016/j.jfca.2011.06.005

Cambaza, E. (2023). Mozambique: Country Profile. *Encyclopedia*, *3*(1), 143–167. https://doi.org/10.3390/encyclopedia3010011

Carbonero-Pacheco, J., García-Jiménez, Á., García-García, J. C., Santos-Dueñas, I. M., García-Martínez, T., Moreno, J., Moreno-García, J., & Mauricio, J. C. (2025). Use of *Wickerhamomyces anomalus* strains from biologically aged wines to improve the sensorial profile of young white wines. *Applied Sciences*, *15*(3), 1546. https://doi.org/10.3390/app15031546

Carota, E., Crognale, S., D'Annibale, A., & Petruccioli, M. (2018). Bioconversion of agro-industrial waste into microbial oils by filamentous fungi. *Process Safety and Environmental Protection*, *117*, 143–151. https://doi.org/10.1016/j.psep.2018.04.022

Caspeta, L., Castillo, T., & Nielsen, J. (2015). Modifying yeast tolerance to inhibitory conditions of ethanol production processes. *Frontiers in bioengineering and biotechnology*, *3*, 184. https://doi.org/10.3389/fbioe.2015.00184

Charles, A. L., Chang, Y. H., Ko, W. C., Sriroth, K., & Huang, T. C. (2004). Some physical and chemical properties of starch isolates of cassava genotypes. *Starch-Stärke*, *56*(9), 413-418. https://doi.org/10.1002/star.200300226

Chattopadhyay, A., & Maiti, M. K. (2021). Lipid production by oleaginous yeasts. *Advances in applied microbiology*, *116*, 1–98. https://doi.org/10.1016/bs.aambs.2021.03.003

Chiona, M., Ntawuruhunga, P., Benesi, I. R. M., Matumba, L., & Moyo, C. C. (2014). Aflatoxins contamination in processed cassava in Malawi and Zambia. *African Journal of Food, Agriculture, Nutrition and Development*, *14*(3). https://www.ajol.info/index.php/ajfand/article/view/104795

Chiwona-Karltun, L., Nyirenda, D., Mwansa, C. N., Kongor, J. E., Brimer, L., Haggblade, S., & Afoakwa, E. O. (2015). Farmer preference, utilization, and biochemical composition of improved cassava (*Manihot esculenta* Crantz) Varieties in Southeastern Africa. *Economic Botany*, *69*(1), 42–56. https://doi.org/10.1007/s12231-015-9298-7

Costa, C., & Delgado, C. (2019). *The Cassava Value Chain in Mozambique*. World Bank, Washington, DC. https://doi.org/10.1596/31754

Costa, G. d. S., Martinez-Burgos, W. J., dos Reis, G. A., Puche, Y. P., Vega, F. R., Rodrigues, C., Serra, J. L., Campos, S. d. M., & Soccol, C. R. (2024). Advances in biomass and microbial lipids production: Trends and prospects. *Processes*, *12*(12), 2903. https://doi.org/10.3390/pr12122903

Cumbana, A., Mirione, E., Cliff, J., & Bradbury, J. H. (2007). Reduction of cyanide content of cassava flour in Mozambique by the wetting method. *Food Chemistry*, *101*(3), 894–897. https://doi.org/10.1016/j.foodchem.2006.02.062

Dash, R. R., Gaur, A., & Balomajumder, C. (2009). Cyanide in industrial wastewaters and its removal: A review on biotreatment. *Journal of Hazardous Materials*, *163*(1), 1–11. https://doi.org/10.1016/j.jhazmat.2008.06.051

De Oliveira Schmidt, V. K., de Vasconscelos, G. M. D., Vicente, R., de Souza Carvalho, J., Della-Flora, I. K., Degang, L., de Oliveira, D., & de Andrade, C. J. (2023). Cassava wastewater valorization for the production of biosurfactants: Surfactin, rhamnolipids, and mannosileritritol lipids. *World Journal of Microbiology & Biotechnology*, *39*(2), 65. https://doi.org/10.1007/s11274-022-03510-2

Dehghani, R., Moosavi, G., Takhtfiroozeh, S. M., & Rashedi, G. (2016). Investigation of the removal of cyanide from aqueous solutions using biomass *Saccharomyces cerevisiae*. *Desalination and Water Treatment*, *57*(56), 27349–27354. https://doi.org/10.1080/19443994.2016.1172262

Devi, A., Singh, A., Bajar, S., Pant, D., & Din, Z. U. (2021). Ethanol from lignocellulosic biomass: An in-depth analysis of pre-treatment methods, fermentation approaches and detoxification processes. *Journal of Environmental Chemical Engineering*, *9*(5), 105798. https://doi.org/10.1016/j.jece.2021.105798

Dias P. (2012). *Analysis of incentives and disincentives for cassava in Mozambique* (Technical notes series, MAFAP). Food and Agriculture Organization of the United Nations.

Díaz-Navarrete, P., Marileo, L., Madrid, H., Belezaca-Pinargote, C., & Dantagnan, P. (2023). Lipid production from native oleaginous yeasts isolated from Southern Chilean soil cultivated in industrial vinasse residues. *Microorganisms*, *11*(10), 2516. https://doi.org/10.3390/microorganisms1110251

Djeni, N. T., Bouatenin, K. M. J.-P., Assohoun, N. M.C., Toka, D. M., Menan, E. H., Dousset, X., & Dje, K. M. (2015). Biochemical and microbial characterization of cassava inocula from the three main *attieke* production zones in Côte d'Ivoire. *Food Control*, *50*, 133–140. https://doi.org/10.1016/j.foodcont.2014.08.046

Dominguez-Torres, C., & Briceno-Garmendia, C. (2011). *Mozambique's Infrastructure: A Continental Perspective*. World Bank. https://doi.org/10.1596/1813-9450-5885

Donovan, C., Haggblade, S., Salegua, V. A., Cuambe, C., Mudema, J., & Tomo, A. (2011). *Cassava Commercialization in Mozambique* (IDWP). https://doi.org/10.22004/ag.econ.120744

Dos Anjos, I. V., Coelho, N., Duarte, H., Proença, D. N., Duarte, M. F., Barros, R., Raposo, S., Gonçalves, S., Romano, A., & Medronho, B. (2025). From lignocellulosic residues to protein sources: insights into biomass pre-treatments and conversion. *Polymers*, *17*(16), 2251. https://doi.org/10.3390/polym17162251

Eisenberg, T., & Büttner, S. (2014). Lipids and cell death in yeast. *FEMS yeast research*, *14*(1), 179–197. https://doi.org/10.1111/1567-1364.12105

Ekop, I., Simonyan K.J., & Evwierhoma E.T. (2019). Utilization of Cassava Wastes for Value Added Products: An Overview. *International Journal of Scientific Engineering and Science*, *3*(1):31–39.

El-Sharkawy, M. A. (2004). Cassava biology and physiology. *Plant Molecular Biology*, *56*(4), 481–501. https://doi.org/10.1007/s11103-005-2270-7

Erdei, B. (2013). *Development of integrated cellulose- and starch-based ethanol production and process design for improved xylose conversion*. [Doctoral Thesis (compilation), Division of Chemical Engineering, Department of Chemical Engineering, Lund University].

Fakas, S. (2017). Lipid biosynthesis in yeasts: A comparison of the lipid biosynthetic pathway between the model nonoleaginous yeast *Saccharomyces cerevisiae* and the model oleaginous yeast *Yarrowia lipolytica*. *Engineering in Life Sciences*, *17*(3), 292–302. https://doi.org/10.1002/elsc.201600040

Food and Agriculture Organization of the United Nations & International Fund for Agricultural Development. (2000) The World Cassava Economy: Facts, Trends and Outlook. Food and Agriculture Organisation of the United Nations.

Food and Agriculture Organization of the United Nations (2023). Indicators Definitions. CountrySTAT. https://www.fao.org/in-action/countrystat/

Food and Agriculture Organization of the United Nations. (2025) FAOSTAT. http://www.fao.org/faostat/en/#hom

Faraco, V. (Ed.). (2013). *Lignocellulose conversion: Enzymatic and microbial tools for bioethanol production*. Springer. https://doi.org/10.1007/978-3-642-37861-4

Fei, Q., O'Brien, M., Nelson, R., Chen, X., Lowell, A., & Dowe, N. (2016). Enhanced lipid production by *Rhodosporidium toruloides* using different fed-batch feeding strategies with lignocellulosic hydrolysate as the sole carbon source. *Biotechnology for Biofuels*, *9*(1), 130. https://doi.org/10.1186/s13068-016-0542-x

Fernandes, M. A., Mota, M. N., Faria, N. T., & Sá-Correia, I. (2023). An evolved strain of the oleaginous yeast *Rhodotorula toruloides*, multi-tolerant to the major inhibitors present in lignocellulosic hydrolysates, exhibits an altered cell envelope. *Journal of fungi*, *9*(11), 1073. https://doi.org/10.3390/jof9111073

Fernandes, T., Silva-Sousa, F., Pereira, F., Rito, T., Soares, P., Franco-Duarte, R., & Sousa, M. J. (2021). Biotechnological importance of *Torulaspora delbrueckii*: From the obscurity to the spotlight. *Journal of Fungi (Basel, Switzerland)*, *7*(9), 712. https://doi.org/10.3390/jof7090712

Flibert, G., Some, K., Kabore, D., Sawadogo-Lingani, H., Traore, Y., & Savadogo, A. (2016). Origins, production, and utilization of cassava in Burkina Faso, a contribution of a neglected crop to household food security. *Food Science & Nutrition*, *5*. https://doi.org/10.1002/fsn3.408

Forkum, A. T., Wung, A. E., Kelese, M. T., Ndum, C. M., Lontum, A., Kamga, E. B., Nsaikila, M. N., & Okwen, P. M. (2025). Safety of cassava and cassava-based products: A systematic review. *Frontiers in Sustainable Food Systems*, *9*. https://doi.org/10.3389/fsufs.2025.1497609

Gacheru, P., Abong, G., Okoth, M., Lamuka, P., Shibairo, S., & Katama, C. (2015). Cyanogenic content, aflatoxin level and quality of dried cassava chips and flour sold in Nairobi and coastal regions of Kenya. *Current Research in Nutrition and Food Science Journal*, *3*(3), 197–206. https://doi.org/10.12944/CRNFSJ.3.3.03

Galbe, M., & Wallberg, O. (2019). Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. *Biotechnology for Biofuels*, *12*(1), 294. https://doi.org/10.1186/s13068-019-1634-1

Gallego-García, M., Moreno, A. D., Manzanares, P., Negro, M. J., & Duque, A. (2023). Recent advances on physical technologies for the pretreatment of food waste and lignocellulosic residues. *Bioresource Technology*, *369*, 128397. https://doi.org/10.1016/j.biortech.2022.128397

Gavahian, M., Peng, H. J., & Chu, Y. H. (2019). Efficacy of cold plasma in producing *Salmonella*-free duck eggs: Effects on physical characteristics, lipid oxidation, and fatty acid profile. *Journal of food science and technology*, *56*(12), 5271–5281. https://doi.org/10.1007/s13197-019-03996-z

Gil, L. & Buitrago, A. J. A. (2002). La Yuca en la Alimentacion Animal: In B. Ospina, H. Ceballos (Eds.), *La Yuca en el Tercer Milenio Sistemas Modernos de Produccion, Procesamiento, Utilizacion y Comercialización* (pp. 527-569). Centro Internacional de Agricultura Tropical (CIAT). https://www.scirp.org/reference/referencespapers?referenceid=472645

Gleadow, R. M., & Møller, B. L. (2014). Cyanogenic glycosides: Synthesis, physiology, and phenotypic plasticity. *Annual Review of Plant Biology*, *65*, 155–185. https://doi.org/10.1146/annurev-arplant-050213-040027

González, J., Villarreal-Huerta, D., Rosas-Paz, M., & Segal-Kischinevzky, C. (2025). Biotechnological applications of yeasts under extreme conditions. In P. Buzzini, B. Turchetti, & A. Yurkov (Eds.), *Extremophilic Yeasts* (pp. 459–501). Springer Nature. https://doi.org/10.1007/978-3-031-97453-3_11

Granger, L. M., Perlot, P., Goma, G., & Pareilleux, A. (1993). Effect of various nutrient limitations on fatty acid production by *Rhodotorula glutinis*. *Applied Microbiology and Biotechnology*, *38*(6), 784–789. https://doi.org/10.1007/bf00167145

Guerriero, G., Hausman, J.-F., Strauss, J., Ertan, H., & Siddiqui, K. S. (2016). Lignocellulosic biomass: Biosynthesis, degradation, and industrial utilization. *Engineering in Life Sciences*, *16*(1), 1–16. https://doi.org/10.1002/elsc.201400196

Halake, N. H., & Chinthapalli, B. (2020). Fermentation of Traditional African Cassava Based Foods: Microorganisms role in nutritional and safety value. *Journal of Experimental Agriculture International*, 56–65. https://doi.org/10.9734/jeai/2020/v42i930587

Henry, M. le F., Hamaide-Deplus, M. C., & Nyns, E. J. (1974). Cyanide-insensitive respiration of *Candida lipolytica*. *Antonie van Leeuwenhoek*, *40*(1), 79–91. https://doi.org/10.1007/BF00394556

Hierro-Iglesias, C., Chimphango, A., Thornley, P., & Fernández-Castané, A. (2022). Opportunities for the development of cassava waste biorefineries for the production of polyhydroxyalkanoates in Sub-Saharan Africa. *Biomass and Bioenergy*, *166*, 106600. https://doi.org/10.1016/j.biombioe.2022.106600

Hossain, M. D., Yan, Q., Zhou, Z., Zhang, X., Wittayakun, S., Napasirth, V., Napasirth, P., Lukuyu, B. A., & Tan, Z. (2025). Cassava as a feedstuff for ruminant feeding system in Belt and Road countries: Innovations, benefits and challenges. *Journal of Agriculture and Food Research*, *21*, 101874. https://doi.org/10.1016/j.jafr.2025.101874

Howeler, R. H. (2000). Cassava agronomy research in Asia: Has it benefited cassava farmers? In In R. H. Howeler & S. L. Tan (Eds.), *Cassava's potential in Asia in the 21st century: Present situation and future research and development needs Proceedings of the sixth regional workshop, held in Ho Chi Minh City, Vietnam, Feb. 21–25, 2000* (pp. 345–382). Centro Internacional de Agricultura Tropical (CIAT), Cassava Office for Asia. https://hdl.handle.net/10568/80328

Hudson, J. F., & Ogunsua, A. O. (1974). Lipids of cassava tubers (*Manihot esculenta* Crantz). *Journal of the science of food and agriculture*, *25*(12), 1503–1508. https://doi.org/10.1002/jsfa.2740251210

Igbinosa, E.O., & Igiehon, O.N. (2015) The impact of cassava effluent on the microbial and physicochemical characteristics on soil dynamics and structure. *Jordan J Biol Sci 8*(2): 107-112. https://doi.org/10.12816/0027556

Ikegwu, T. M., Ezegbe, C. C., Odo, E. N., Okolo, C. A., Mba, J. C., Agu, H. O. (2022). Processing of oilseeds in the tropics: Prospects and challenges. In *Oilseed crops—uses, biology and production*. IntechOpen. https://doi.org/10.5772/intechopen.106247

Immanuel, S. Jaganathan, D., Prakash, P., & Sivakumar, P. S. (2024). Cassava for Food Security, Poverty Reduction and Climate Resilience: A Review. *Indian Journal of Ecology*. *51*(1), 21–31, https://doi.org/10.55362/IJE/2024/4191

Isimah, M. O., Chukwurah, G. O., Okeke, F. O., Nwankwo, C. F., & Nnoli, C. I. (2023). Awareness of the effects of small-scale cassava mill effluent on the environment in Ika North East local government area, Delta State, Nigeria. *Environmental Monitoring and Assessment*, *195*(12), 1431. https://doi.org/10.1007/s10661-023-12026-7

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., Lorenzo, J. M., Barba, F. J., Prieto, M. A., & Simal-Gandara, J. (2020). Bioactive compounds and quality of extra virgin olive oil. *Foods*, *9*(8), 1014. https://doi.org/10.3390/foods9081014

Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource technology*, *199*, 103–112. https://doi.org/10.1016/j.biortech.2015.10.009

Jönsson, L. J., Alriksson, B., & Nilvebrant, N.-O. (2013). Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnology for Biofuels*, *6*(1). https://doi.org/10.1186/1754-6834-6-16

Jørgensen, K., Bak, S., Busk, P. K., Sørensen, C., Olsen, C. E., Puonti-Kaerlas, J., & Møller, B. L. (2005). Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of the biosynthesis by RNA interference technology. *Plant Physiology*, *139*(1), 363-374. https://doi.org/10.1104/pp.105.065904

Kandasamy, S., Dananjeyan, B., Krishnamurthy, K., & Benckiser, G. (2015). Aerobic cyanide degradation by bacterial isolates from cassava factory wastewater. *Brazilian Journal of Microbiology*, *46*(3), 659–666. https://doi.org/10.1590/S1517-838246320130516

Kaneko, H., Hosohara, M., Tanaka, M., & Itoh, T. (1976). Lipid composition of 30 species of yeast. *Lipids*, *11*(12), 837–844. https://doi.org/10.1007/BF02532989

Kemsawasd, V., Viana, T., Ardö, Y., & Arneborg, N. (2015). Influence of nitrogen sources on growth and fermentation performance of different wine yeast species during alcoholic fermentation. *Applied microbiology and biotechnology*, *99*(23), 10191–10207. https://doi.org/10.1007/s00253-015-6835-3

Khieu, B., Chhay, T., Ogle, R. B., & Preston, T. R. (2005). Research on the use of cassava leaves for livestock feeding in Cambodia. In *Proceeding of the regional workshop on "The use of cassava roots and leaves for on-farm animal feeding"* (pp. 17-19, Hue, Vietnam).

Khunnonkwao, P., Thitiprasert, S., Jaiaue, P., Khumrangsee, K., Cheirsilp, B., & Thongchul, N. (2024). The outlooks and key challenges in renewable biomass feedstock utilization for value-added platform chemical via bioprocesses. *Heliyon*, *10*(10), e30830. https://doi.org/10.1016/j.heliyon.2024.e30830

Kim, H., Lee, S., Ahn, Y., Lee, J., & Won, W. (2020). Sustainable production of bioplastics from lignocellulosic biomass: technoeconomic analysis and life-cycle assessment. *ACS Sustainable Chemistry & Engineering*, *8*(33), 12419–12429. https://doi.org/10.1021/acssuschemeng.0c02872

Kolawole, P. O., Agbetoye, L., & Ogunlowo, S. A. (2010). Sustaining World Food Security with Improved Cassava Processing Technology: The Nigeria Experience. *Sustainability*, *2*(12), 3681–3694. https://doi.org/10.3390/su2123681

Konno, K. (2011). Plant latex and other exudates as plant defense systems: Roles of various defense chemicals and proteins contained therein. *Phytochemistry*, *72*(13), 1510–1530. https://doi.org/10.1016/j.phytochem.2011.02.016

Kosoe, E. A., & Ogwu, M. C. (2024). Sustainable cassava processing: Processes, techniques, wastes, and waste streams. In *Sustainable Cassava* (pp. 257–272). Elsevier. https://doi.org/10.1016/B978-0-443-21747-0.00011-4

Kostinek, M., Specht, I., Edward, V. A., Schillinger, U., Hertel, C., Holzapfel, W. H., & Franz, C. M. A. P. (2005). Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of *gari*, a traditional African food. *Systematic and Applied Microbiology*, *28*(6), 527–540. https://doi.org/10.1016/j.syapm.2005.03.001

Kouamé, A. K., Djéni, T. N., N'Guessan, F. K., & Dje, M. K. (2013). Postprocessing microflora of commercial *attieke* (a fermented cassava product) produced in the south of Côte d'Ivoire. *Letters in Applied Microbiology*, *56*(1), 44–50. https://doi.org/10.1111/lam.12014

Kuliahsari, D. E., Sari, I. N. I., & Estiasih, T. (2021). Cyanide detoxification methods in food: A review. *IOP Conference Series. Earth and Environmental Science*, *733*(1), 12099. https://doi.org/10.1088/1755-1315/733/1/012099

Lateef, A., & Ojo, M. O. (2016). Public health issues in the processing of cassava (*Manihot esculenta*) for the production of *lafun* and the application of hazard analysis control measures. *Quality Assurance and Safety of Crops & Foods*, *8*(1), 165-177.

Lee, Y., Lee, L., Zhang, L., & Zhou, Q. (2024). Association between fatty acid intake and age-related macular degeneration: a meta-analysis. *Frontiers in nutrition*, *11*, 1403987. https://doi.org/10.3389/fnut.2024.1403987

Lei, Y., Wang, X., Sun, S., He, B., Sun, W., Wang, K., Chen, Z., Guo, Z., & Li, Z. (2024). A review of lipid accumulation by oleaginous yeasts: Culture mode. *Science of The Total Environment*, *919*, 170385. https://doi.org/10.1016/j.scitotenv.2024.170385

Li, Y., Jiang, G., Long, H., Liao, Y., Wu, L., Huang, W., & Liu, X. (2023). Contribution of trehalose to ethanol stress tolerance of *Wickerhamomyces anomalus*. *BMC Microbiology*, *23*(1), 239. https://doi.org/10.1186/s12866-023-02982-y

Lingwood, D., & Simons, K. (2010). Lipid rafts as a membrane-organizing principle. *Science*, *327*(5961), 46–50. https://doi.org/10.1126/science.1174621

Lopes, H. J. S., Bonturi, N., Kerkhoven, E. J., Miranda, E. A., & Lahtvee, P.-J. (2020). C/N ratio and carbon source-dependent lipid production profiling in *Rhodotorula toruloides*. *Applied Microbiology and Biotechnology*, *104*(6), 2639–2649. https://doi.org/10.1007/s00253-020-10386-5

Luque-Almagro, V. M., Cabello, P., Sáez, L. P., Olaya-Abril, A., Moreno-Vivián, C., & Roldán, M. D. (2018). Exploring anaerobic environments for cyanide and cyano-derivatives microbial degradation. *Applied Microbiology and Biotechnology*, *102*(3), 1067–1074. https://doi.org/10.1007/s00253-017-8678-6

Maciel, A. C., da Silva Pena, R., do Nascimento, L. D., de Oliveira, T. A., Chagas-Junior, G. C. A., & Lopes, A. S. (2023). Health exposure risks and bioremediation of cyanide in cassava processing effluents: An overview. *Journal of Water Process Engineering*, *55*, 104079. https://doi.org/10.1016/j.jwpe.2023.104079

Ministério da Agricultura e Desenvolvimento Rural (MADER). (2023). *Inquérito agrário integrado (IAI 2023)*. https://www.agricultura.gov.mz/wp-content/uploads/2024/09/Inquerito-Agrario-Integrado-IAI-2023.pdf

Manisha, Yadav, S. K. (2017). Technological advances and applications of hydrolytic enzymes for valorization of lignocellulosic biomass. *Bioresource Technology*, *245*, 1727–1739. https://doi.org/10.1016/j.biortech.2017.05.066

Marc, R. A. (2022). Implications of mycotoxins in food safety. In *Mycotoxins and food safety-recent advances*. IntechOpen. https://doi.org/10.5772/intechopen.102495

Martinez, M., & Diaz, I. (2024). Plant cyanogenic-derived metabolites and herbivore counter-defences. *Plants*, *13*(9), 1239. https://doi.org/10.3390/plants13091239

Matusse, C., Lucamba, Z., Bila, J., Macuamule, C., Sampaio, A., Afonso, S., Venâncio, A., & Rodrigues, P. (2024). Aflatoxin contamination of various staple foods from Angola and Mozambique. *Toxins*, *16*(12), 516. https://doi.org/10.3390/toxins16120516

McKey, D., Cavagnaro, T. R., Cliff, J., & Gleadow, R. (2010). Chemical ecology in coupled human and natural systems: People, manioc, multitrophic interactions and global change. *Chemoecology*, *20*(2), 109–133. https://doi.org/10.1007/s00049-010-0047-1

Meijaard, E., Brooks, T. M., Carlson, K. M., Slade, E. M., Garcia-Ulloa, J., Gaveau, D. L. A., Lee, J. S. H., Santika, T., Juffe-Bignoli, D., Struebig, M. J., Wich, S. A., Ancrenaz, M., Koh, L. P., Zamira, N., Abrams, J. F., Prins, H. H. T., Sendashonga, C. N., Murdiyarso, D., Furumo, P. R., Sheil, D. (2020). The environmental impacts of palm oil in context. *Nature Plants*, *6*(12), 1418–1426. https://doi.org/10.1038/s41477-020-00813-w

Mekuto, L., Ntwampe, S. K. O., & Akcil, A. (2016). An integrated biological approach for treatment of cyanidation wastewater. *The Science of the Total Environment*, *571*, 711–720. https://doi.org/10.1016/j.scitotenv.2016.07.040

Møller, B. L. (2010). Functional diversifications of cyanogenic glucosides. *Current Opinion in Plant Biology*, *13*(3), 337–346. https://doi.org/10.1016/j.pbi.2010.01.009

Montagnac, J. A., Davis, C. R., & Tanumihardjo, S. A. (2009). Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comprehensive Reviews in Food Science and Food Safety*, *8*(3), 181–194. https://doi.org/10.1111/j.1541-4337.2009.00077.x

Montoro, S. B., Lucas, J., Santos, D. F. L., & Costa, M. S. S. M. (2019). Anaerobic co-digestion of sweet potato and dairy cattle manure: A technical and economic evaluation for energy and biofertilizer production. *Journal of Cleaner Production*, *226*, 1082–1091. https://doi.org/10.1016/j.jclepro.2019.04.148

Moreno, A. D., González-Fernández, C., & Tomás-Pejó, E. (2022). Insights into cell robustness against lignocellulosic inhibitors and insoluble solids in bioethanol production processes. *Scientific Reports*, *12*(1), 557. https://doi.org/10.1038/s41598-021-04554-4

Morgan, N. K., & Choct, M. (2016). Cassava: Nutrient composition and nutritive value in poultry diets. *Animal Nutrition*, *2*(4), 253–261. https://doi.org/10.1016/j.aninu.2016.08.010

Mota, M. N., Múgica, P., & Sá-Correia, I. (2022). Exploring yeast diversity to produce lipid-based biofuels from agro-forestry and industrial organic residues. *Journal of Fungi*, *8*(7), 687. https://doi.org/10.3390/jof8070687

Nadia, H. S., Paul, K., Cornelius, M. W., & Elijah, M. A. (2021). Phenotypic evaluation of cassava genotypes (*Manihot esculenta*) under moisture stress. *African Journal of Agricultural Research*, *17*(6), 836–843. https://doi.org/10.5897/AJAR2020.14747

Nagaraj, Y. N., Blomqvist, J., Sampels, S., Pickova, J., Sandgren, M., Gajdoš, P., Čertík, M., & Passoth, V. (2025). Supercritical carbon dioxide extraction of lipids and carotenoids from *Rhodotorula toruloides* CBS 14 in comparison with conventional extraction methods. *Biotechnology for Biofuels and Bioproducts*, *18*(1), 35. https://doi.org/10.1186/s13068-025-02632-7

Nagaraj, Y. N., Burkina, V., Okmane, L., Blomqvist, J., Rapoport, A., Sandgren, M., Pickova, J., Sampels, S., & Passoth, V. (2022). identification, quantification and kinetic study of carotenoids and lipids in *Rhodotorula toruloides* CBS 14 cultivated on wheat straw hydrolysate. *Fermentation*, *8*(7), 300. https://doi.org/10.3390/fermentation8070300

Nandy, S. K., & Srivastava, R. K. (2018). A review on sustainable yeast biotechnological processes and applications. *Microbiological research*, *207*, 83–90. https://doi.org/10.1016/j.micres.2017.11.013

Nartey, F. (1969). Studies on cassava, *Manihot utilissima*. II. Biosynthesis of Asparagine-^{14}C from ^{14}C-labelled hydrogen cyanide and its relations with cyanogenesis. *Physiologia Plantarum*, *22*(5), 1085–1096. https://doi.org/10.1111/j.1399-3054.1969.tb07470.x

Ngiki, Y. U., Igwebuike, J. U., & Moruppa, S. M. (2014). Utilisation of cassava products for poultry feeding: a review. *The International Journal of Science and Technoledge*, *2*(6), 48.

Nhassico, D., Muquingue, H., Cliff, J., Cumbana, A., & Bradbury, J. H. (2008). Rising African cassava production, diseases due to high cyanide intake and control measures. *Journal of the Science of Food and Agriculture*, *88*(12), 2043–2049. https://doi.org/10.1002/jsfa.3337

Nieto, G., & Lorenzo, J. M. (2022). Plant source: Vegetable oils. In *Food Lipids* (pp. 69–85). Elsevier. https://doi.org/10.1016/B978-0-12-823371-9.00011-3

Ning, P., Yang, G., Hu, L., Sun, J., Shi, L., Zhou, Y., Wang, Z., & Yang, J. (2021). Recent advances in the valorization of plant biomass. *Biotechnology for Biofuels*, *14*(1), 102. https://doi.org/10.1186/s13068-021-01949-3

Nitschke, M., & Pastore, G. M. (2004). Biosurfactant production by *Bacillus subtilis* using cassava-processing effluent. *Applied biochemistry and biotechnology*, *112*(3), 163–172. https://doi.org/10.1385/abab:112:3:163

Nizzy, A. M., & Kannan, S. (2022). A review on the conversion of cassava wastes into value-added products towards a sustainable environment. *Environmental Science and Pollution Research International*, *29*(46), 69223–69240. https://doi.org/10.1007/s11356-022-22500-3

National Research Council (NRC) (1992). *Applications of biotechnology to traditional fermented foods: Report of an ad hoc panel of the Board on Science and Technology for International Development* (pp.12, Cassava Processing in Africa). National Academies Press. https://www.ncbi.nlm.nih.gov/books/NBK234676/

Nuwamanya, E., Chiwona-Karltun, L., Kawuki, R. S., & Baguma, Y. (2012). Bio-ethanol production from non-food parts of cassava (*Manihot esculenta* Crantz). *Ambio*, *41*(3), 262–270. https://doi.org/10.1007/s13280-011-0183-z

Nweke, F. I. (2004). *New Challenges in the Cassava Transformation in Nigeria and Ghana* (Vol.118) International Food Policy Research Institute.

Nyaika, J., Abayomi, L., Parmar, A., & Coast, O. (2024). Cyanide in cassava: Understanding the drivers, impacts of climate variability, and strategies for food security. *Food and Energy Security*, *13*(4), e573. https://doi.org/10.1002/fes3.573

Obadina, A. O., Oyewole, O. B., Sanni, L. O., Tomlins, K. I., & Westby, A. (2008). Identification of hazards and critical control points (CCP) for cassava *fufu* processing in South-West Nigeria. *Food Control*, *19*(1), 22–26. https://doi.org/10.1016/j.foodcont.2007.01.002

Obafemi, Y. D., Oranusi, S. U., Ajanaku, K. O., Akinduti, P. A., Leech, J., & Cotter, P. D. (2022). African fermented foods: Overview, emerging benefits, and novel approaches to microbiome profiling. *NPJ Science of Food*, *6*(1), 15. https://doi.org/10.1038/s41538-022-00130-w

Obonukut, M., Alabi, S., & Jock, A. (2022). Biochar from Cassava Waste: A paradigm shift from waste to wealth. In *Biochar—productive technologies, properties and applications*. IntechOpen. https://doi.org/10.5772/intechopen.105965

Ochsenreither, K., Glück, C., Stressler, T., Fischer, L., & Syldatk, C. (2016). Production strategies and applications of microbial single cell oils. *Frontiers in Microbiology*, *7*, 1539. https://doi.org/10.3389/fmicb.2016.01539

Ogbonna, J. C., Nakajima, M., & Neves, M. A. das. (2025). Characterization and emulsifying ability of cassava peels solubilized using hydrothermal treatments. *Polymers*, *17*(4), 496. https://doi.org/10.3390/polym17040496

Oghenejoboh, K. M., Orugba, H. O., Oghenejoboh, U. M., & Agarry, S. E. (2021). Value added cassava waste management and environmental sustainability in Nigeria: A review. *Environmental Challenges*, *4*, 100127. https://doi.org/10.1016/j.envc.2021.100127

Ojo, A. O. (2023). An overview of lignocellulose and its biotechnological importance in high-value product production. *Fermentation*, *9*(11), 990. https://doi.org/10.3390/fermentation9110990

Okigbo, B. N. (1980). Nutritional implications of projects giving high priority to the production of staples of low nutritive quality: the case for cassava (*Manihot esculenta* Crantz) in the humid tropics of West Africa. *Food and Nutrition Bulletin*, 2 (4), 1-10. https://doi.org/10.1177/156482658000200404

Okolieuwa, C. C., Nnamchi, C. I., Imumolen, C., & Ikwunne, T. (2025). exploring the impact of bioethanol and cassava feedstock on the future of industrial applications. *American Journal of Applied Sciences and Engineering*, *6*(1), 10–27. 10.5281/zenodo.15189233

Okolo, E., & Makanjuola, A. T. (2021). Microbial evaluation of *garri* sold within Ahmadu Bello University main campus, Samaru-Zaria, Kaduna State. *Science World Journal*, *16*(3), 259-265.

Olaniyan, S. A., Hussein, J. B., Oke, M. O., Akinwande, B. A., Workneh, T. S., Ayodele, M., & Adeyemi, I. A. (2025). Assessment of the physicochemical characteristics of by-products of cassava processing and their effects on biodiversity. *Environmental Monitoring and Assessment*, *197*(5), 533. https://doi.org/10.1007/s10661-025-13951-5

Olukanni, D. O., & Olatunji, T. O. (2018). Cassava waste management and biogas generation potential in selected local government areas in Ogun State, Nigeria. *Recycling*, *3*(4), 58. https://doi.org/10.3390/recycling3040058

Omojokun, J. (2013). Regulation and enforcement of legislation on food safety in Nigeria. In *Mycotoxin and Food Safety in Developing Countries*. IntechOpen. https://doi.org/10.5772/54423

Ono, L. T., & Taniwaki, M. H. (2021). Fungi and mycotoxins in cassava (*Manihot esculenta* Crantz) and its products. *Brazilian Journal of Food Technology*, *24*, e2020240. https://doi.org/10.1590/1981-6723.24020

Oresegun, A., Fagbenro, O. A., Ilona, P., & Bernard, E. (2016). Nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed. *Cogent Food & Agriculture*, *2*(1), 1147323. https://doi.org/10.1080/23311932.2016.1147323

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences*, *16*(6), 12871–12890. https://doi.org/10.3390/ijms160612871

Pacheco, A., Santos, J., Chaves, S., Almeida, J., Leão, C., & Sousa, M. J. (2012). The emerging role of the yeast *Torulaspora delbrueckii* in bread and wine production: Using genetic manipulation to study molecular basis of physiological responses. In *Structure and Function of Food Engineering* (pp. 340–370). IntechOpen. https://doi.org/10.5772/46024

Parsons, S., Raikova, S., & Chuck, C. J. (2020). The viability and desirability of replacing palm oil. *Nature Sustainability*, *3*(6), 412–418. https://doi.org/10.1038/s41893-020-0487-8

Passoth, V., & Sandgren, M. (2019). Biofuel production from straw hydrolysates: Current achievements and perspectives. *Applied Microbiology and Biotechnology*, *103*(13), 5105–5116. https://doi.org/10.1007/s00253-019-09863-3

Passoth, V., Brandenburg, J., Chmielarz, M., Martín-Hernández, G. C., Nagaraj, Y., Müller, B., & Blomqvist, J. (2023). Oleaginous yeasts for biochemicals, biofuels and food from lignocellulose-hydrolysate and crude glycerol. *Yeast*, *40*(8), 290–302. https://doi.org/10.1002/yea.3838

Passoth, V., Fredlund, E., Druvefors, U. Ä., & Schnürer, J. (2006). Biotechnology, physiology and genetics of the yeast *Pichia anomala*. *FEMS Yeast Research*, *6*(1), 3–13. https://doi.org/10.1111/j.1567-1364.2005.00004.x

Paulova, L., Patakova, P., Branska, B., Rychtera, M., & Melzoch, K. (2015). Lignocellulosic ethanol: Technology design and its impact on process efficiency. *Biotechnology Advances*, *33*(6, Part 2), 1091–1107. https://doi.org/10.1016/j.biotechadv.2014.12.002

Periyasamy, S., Beula Isabel, J., Kavitha, S., Karthik, V., Mohamed, B. A., Gizaw, D. G., Sivashanmugam, P., & Aminabhavi, T. M. (2023). Recent advances in consolidated bioprocessing for conversion of lignocellulosic biomass into bioethanol – A review. *Chemical Engineering Journal, 453*. https://doi.org/10.1016/j.cej.2022.139783

Pimpisai, T., Maneerattanarungroj, C., Kingkaew, E., & Ochaikul, D. (2024). Bioethanol production from cassava starch using co-culture of saccharolytic molds with *Saccharomyces cerevisiae* TISTR 5088. *Science Asia, 50*(4), 1. https://doi.org/10.2306/scienceasia1513-1874.2024.071

Pinto Zevallos, D. M., Pereira Querol, M., & Ambrogi, B. G. (2018). Cassava wastewater as a natural pesticide: Current knowledge and challenges for broader utilisation. *Annals of Applied Biology*, *173*(3), 191–201. https://doi.org/10.1111/aab.12464

Pinto-Zevallos, D. M., Pareja, M., & Ambrogi, B. G. (2016). Current knowledge and future research perspectives on cassava (*Manihot esculenta* Crantz) chemical defenses: An agroecological view. *Phytochemistry*, *130*, 10–21. https://doi.org/10.1016/j.phytochem.2016.05.013

Pitt, J. I. & Hocking, A. D. (2022). Fungi and Food Spoilage (4th ed.). Springer.

Rahman, S. (2025). The utilization of cassava leaves as a functional food based on local wisdom. In *Advances in functional foods: New perspectives and sustainable practices*. IntechOpen. https://doi.org/10.5772/intechopen.1012543

Ratledge C. (2014). The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: a reappraisal and unsolved problems. *Biotechnology letters*, *36*(8), 1557–1568. https://doi.org/10.1007/s10529-014-1532-3

Ratledge, C. (2013). Microbial oils: an introductory overview of current status and future prospects. *Oléagineux Corps Gras Lipides*, *20*(6), D602. https://doi.org/10.1051/ocl/2013029

Ratledge, C., & Wynn, J. P. (2002). The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Advances in Applied Microbiology*, *51* (1), 45-44. https://doi.org/10.1016/S0065-2164(02)51000-5

Rawel, H. M. & Kroll, J. (2003). Die Bedeutung von Cassava (*Manihot esculenta,* Crantz) als Hauptnahrungsmittel in tropischen Ländern. *Deutsche Lebensmittel-Rundschau 99*,102–10.

Ray, R. C., & Sivakumar, P. S. (2009). Traditional and novel fermented foods and beverages from tropical root and tuber crops: review. *International Journal of Food Science & Technology*, *44*(6), 1073–1087. https://doi.org/10.1111/j.1365-2621.2009.01933.x

Ribeiro, J. E. S., Sant'Ana, A. M. da S., Martini, M., Sorce, C., Andreucci, A., Melo, D. J. N. de, & Silva, F. L. H. da. (2019). *Rhodotorula glutinis* cultivation on cassava wastewater for carotenoids and fatty acids generation. *Biocatalysis and Agricultural Biotechnology*, *22*, 101419. https://doi.org/10.1016/j.bcab.2019.101419

Robescu, M. S., & Bavaro, T. (2025). A comprehensive guide to enzyme immobilization: All you need to know. *Molecules*, *30*(4), 939. https://doi.org/10.3390/molecules30040939

Roscoe, V., Lombaert, G. A., Huzel, V., Neumann, G., Melietio, J., Kitchen, D., Kotello, S., Krakalovich, T., Trelka, R., & Scott, P. M. (2008). Mycotoxins in breakfast cereals from the Canadian retail market: A 3-year survey. *Food Additives & Contaminants: Part A*, *25*(3), 347–355. https://doi.org/10.1080/02652030701551826

Saini, R. K., Prasad, P., Sreedhar, R. V., Akhilender Naidu, K., Shang, X., & Keum, Y.-S. (2021). Omega−3 polyunsaturated fatty acids (PUFAs): Emerging plant and microbial sources, oxidative stability, bioavailability, and health benefits—A Review. *Antioxidants*, *10*(10), 1627. https://doi.org/10.3390/antiox10101627

Salvador, E. M., Steenkamp, V., & McCrindle, C. M. E. (2014). Production, consumption and nutritional value of cassava (*Manihot esculenta* Crantz) in Mozambique: An overview. *Journal of Agricultural Biotechnology and Sustainable Development*, *6*(3), 29–38. https://doi.org/10.5897/JABSD2014.0224

Sandager, L., Gustavsson, M. H., Ståhl, U., Dahlqvist, A., Wiberg, E., Banas, A., Lenman, M., Ronne, H., & Stymne, S. (2002). Storage lipid synthesis is non-essential in yeast. *The Journal of biological chemistry*, *277*(8), 6478–6482. https://doi.org/10.1074/jbc.M109109200

Sant'Ana Júnior, D. B., Kelbert, M., Hermes de Araújo, P. H., & de Andrade, C. J. (2025). Physical pretreatments of lignocellulosic biomass for fermentable sugar production. *Sustainable Chemistry*, *6*(2), 13. https://doi.org/10.3390/suschem6020013

Sanya, D. R. A., Onésime, D., Passoth, V., Maiti, M. K., Chattopadhyay, A., & Khot, M. B. (2021). Yeasts of the *Blastobotrys* genus are promising platform for lipid-based fuels and oleochemicals production. *Applied Microbiology and Biotechnology*, *105*(12), 4879–4897. https://doi.org/10.1007/s00253-021-11354-3

Saxena, A., Hussain, A., Parveen, F., & Ashfaque, M. (2023). Current status of metabolic engineering of microorganisms for bioethanol production by effective utilization of pentose sugars of lignocellulosic biomass. *Microbiological Research*, *276*, 127478. https://doi.org/10.1016/j.micres.2023.127478

Scaria, S. S., Balasubramanian, B., Meyyazhagan, A., Gangwar, J., Jaison, J. P., Kurian, J. T., Pushparaj, K., Pappuswamy, M., Park, S., & Joseph, K. S. (2024). Cassava (*Manihot esculenta* Crantz)—A potential source of phytochemicals, food, and nutrition—An updated review. *eFood*, *5*(1), e127. https://doi.org/10.1002/efd2.127

Shapaval, V., Brandenburg, J., Blomqvist, J., Tafintseva, V., Passoth, V., Sandgren, M., & Kohler, A. (2019). Biochemical profiling, prediction of total lipid content and fatty acid profile in oleaginous yeasts by FTIR spectroscopy. *Biotechnology for Biofuels*, *12*(1), 140. https://doi.org/10.1186/s13068-019-1481-0

Sharma, S., Tsai, M.-L., Sharma, V., Sun, P.-P., Nargotra, P., Bajaj, B. K., Chen, C.-W., & Dong, C.-D. (2023). Environment friendly pretreatment approaches for the bioconversion of lignocellulosic biomass into biofuels and value-added products. *Environments*, *10*(1), 6. https://doi.org/10.3390/environments10010006

Sharma, V., Tsai, M.-L., Nargotra, P., Chen, C.-W., Kuo, C.-H., Sun, P.-P., & Dong, C.-D. (2022). Agro-Industrial food waste as a low-cost substrate for sustainable production of industrial enzymes: A critical review. *Catalysts*, *12*(11), 1373. https://doi.org/10.3390/catal12111373

Shen, H., Zhang, X., Gong, Z., Wang, Y., Yu, X., Yang, X., & Zhao, Z. K. (2017). Compositional profiles of *Rhodosporidium toruloides* cells under nutrient limitation. *Applied Microbiology and Biotechnology*, *101*(9), 3801–3809. https://doi.org/10.1007/s00253-017-8157-0

Shen, T., Wu, Q., & Xu, Y. (2021). Biodegradation of cyanide with *Saccharomyces cerevisiae* in Baijiu fermentation. *Food Control*, *127*, 108107. https://doi.org/10.1016/j.foodcont.2021.108107

Shigaki, T. (2016). Cassava: The nature and uses. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds), *Encyclopedia of Food and Health* (pp. 687–693). Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00124-0

Shukla, A., Kumar, D., Girdhar, M., Kumar, A., Goyal, A., Malik, T., & Mohan, A. (2023). Strategies of pretreatment of feedstocks for optimized bioethanol production: Distinct and integrated approaches. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 44. https://doi.org/10.1186/s13068-023-02295-2

Sitepu, I. R., Garay, L. A., Sestric, R., Levin, D., Block, D. E., German, J. B., & Boundy-Mills, K. L. (2014). Oleaginous yeasts for biodiesel: current and future trends in biology and production. *Biotechnology advances*, *32*(7), 1336–1360. https://doi.org/10.1016/j.biotechadv.2014.08.003

Sivadas, D., Shokri, S., Cattaruzza, A., & Chuck, C. J. (2025). Oleogels produced using single cell oils from the oleaginous yeast *Metschnikowia pulcherrima* as potential fat replacers. *Food Hydrocolloids*, *172*. https://doi.org/10.1016/j.foodhyd.2025.111921

Sokan-Adeaga, A. A., Ana, G. R. E. E., Olorunnisola, A. O., Sokan-Adeaga, M. A., Roy, H., Reza, M. S., & Islam, Md. S. (2024). Ethanol production from cassava peels using *Saccharomyces cerevisiae* via ethanologenic fermentation process. *Arab Gulf Journal of Scientific Research*, *42*(4), 1664–1684. https://doi.org/10.1108/AGJSR-06-2023-0264

Sun, H., Gao, Z., Zhang, L., Wang, X., Gao, M., & Wang, Q. (2023). A comprehensive review on microbial lipid production from wastes: Research updates and tendencies. *Environmental Science and Pollution Research*, *30*(33), 79654–79675. https://doi.org/10.1007/s11356-023-28123-6

Tang, C., Cavka, A., Bui, M., & Jönsson, L. J. (2024). Comparison of simultaneous saccharification and fermentation with LPMO-supported hybrid hydrolysis and fermentation. *Frontiers in Bioengineering and Biotechnology*, *12*, 1419723. https://doi.org/10.3389/fbioe.2024.1419723

Tewe, O. O., & Lutaladio, N. (2004). *Cassava for livestock feed in sub-Saharan Africa*. Food and Agriculture Organization of the United Nations.

Tivana, L. D. (2012). *Cassava processing: Safety and protein fortification*. [Doctoral Thesis (compilation), Department of Food Technology, Engineering and Nutrition, Lund University].

Topaloğlu, A., Esen, Ö., Turanlı-Yıldız, B., Arslan, M., & Çakar, Z. P. (2023). From *Saccharomyces cerevisiae* to ethanol: Unlocking the power of evolutionary engineering in metabolic engineering applications. *Journal of Fungi*, *9*(10), 984. https://doi.org/10.3390/jof9100984

Tran, T. T. A., Le, T. K. P., Mai, T. P., Nguyen, D. Q., Tran, T. T. A., Le, T. K. P., Mai, T. P., & Nguyen, D. Q. (2019). Bioethanol production from lignocellulosic biomass. In *Alcohol fuels—Current technologies and future prospect*. IntechOpen. https://doi.org/10.5772/intechopen.86437

Tsegaye, K. N., Alemnew, M., & Berhane, N. (2024). *Saccharomyces cerevisiae* for lignocellulosic ethanol production: A look at key attributes and genome shuffling. *Frontiers in Bioengineering and Biotechnology*, *12*, 1466644. https://doi.org/10.3389/fbioe.2024.1466644

Valdés, G., Mendonça, R. T., & Aggelis, G. (2020). Lignocellulosic biomass as a substrate for oleaginous microorganisms: A Review. *Applied Sciences*, *10*(21), 7698. https://doi.org/10.3390/app10217698

van der Pol, E. C., Bakker, R. R., Baets, P., & Eggink, G. (2014). By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio)chemicals and fuels. *Applied Microbiology and Biotechnology*, *98*(23), 9579–9593. https://doi.org/10.1007/s00253-014-6158-9

Vasconcelos, A. T., Twiddy, D. R., Westby, A., & Reilly, P. J. A. (1990). Detoxification of cassava during gari preparation. *International Journal of Food Science and Technology*, *25*(2), 198–203. https://doi.org/10.1111/j.1365-2621.1990.tb01074.x

Veiga, A., Arrabaça, J. D., & Loureiro-Dias, M. C. (2003). Cyanide-resistant respiration, a very frequent metabolic pathway in yeasts. *FEMS Yeast Research*, *3*(3), 239–245. https://doi.org/10.1016/S1567-1356(03)00036-9

Vetter J. (2000). Plant cyanogenic glycosides. *Toxicon*, *38*(1), 11–36. https://doi.org/10.1016/s0041-0101(99)00128-2

Wang, Q., Guo, F.-J., Rong, Y.-J., & Chi, Z.-M. (2012). Lipid production from hydrolysate of cassava starch by *Rhodosporidium toruloides* 21167 for biodiesel making. *Renewable Energy*, *46*, 164–168. https://doi.org/10.1016/j.renene.2012.03.002

Wang, Y., Qiu, L., & Hu, M. (2018). Application of yeast in the wastewater treatment. *E3S Web of Conferences*, *53*, 04025. https://doi.org/10.1051/e3sconf/20185304025

Watthier, E., Andreani, C. L., Torres, D. G. B., Kuczman, O., Tavares, M. H. F., Lopes, D. D., & Gomes, S. D. (2019). Cassava wastewater treatment in fixed-bed reactors: Organic matter removal and biogas production. *Frontiers in Sustainable Food Systems*, *3*. https://doi.org/10.3389/fsufs.2019.00006

Wei, Y., Siewers, V., & Nielsen, J. (2017). Cocoa butter-like lipid production ability of non-oleaginous and oleaginous yeasts under nitrogen-limited culture conditions. *Applied Microbiology and Biotechnology*, *101*(9), 3577–3585. https://doi.org/10.1007/s00253-017-8126-7

Wen, Z., Zhang, S., Odoh, C. K., Jin, M., & Zhao, Z. K. (2020). *Rhodosporidium toruloides*—A potential red yeast chassis for lipids and beyond. *FEMS Yeast Research*, *20*(5). https://doi.org/10.1093/femsyr/foaa038

White, W. L. B., Arias-Garzon, D. I., McMahon, J. M., & Sayre, R. T. (1998). Cyanogenesis in Cassava: The role of hydroxynitrile lyase in root cyanide production. *Plant Physiology*, *116*(4), 1219–1225. https://doi.org/10.1104/pp.116.4.1219

Wi, S. G., Cho, E. J., Lee, D.-S., Lee, S. J., Lee, Y. J., & Bae, H.-J. (2015). Lignocellulose conversion for biofuel: A new pretreatment greatly improves downstream biocatalytic hydrolysis of various lignocellulosic materials. *Biotechnology for Biofuels*, *8*(1), 228. https://doi.org/10.1186/s13068-015-0419-4

Woźniak, A., Kuligowski, K., Świerczek, L., & Cenian, A. (2025). Review of lignocellulosic biomass pretreatment using physical, thermal and chemical methods for higher yields in bioethanol production. *Sustainability*, *17*(1), 287. https://doi.org/10.3390/su17010287

Wu, C.-C., Honda, K., & Kazuhito, F. (2023). Current advances in alteration of fatty acid profile in *Rhodotorula toruloides*: A mini-review. *World Journal of Microbiology and Biotechnology*, *39*(9), 234. https://doi.org/10.1007/s11274-023-03595-3

Wu, S., Hu, C., Jin, G., Zhao, X., & Zhao, Z. K. (2010). Phosphate-limitation mediated lipid production by *Rhodosporidium toruloides*. *Bioresource Technology*, *101*(15), 6124–6129. https://doi.org/10.1016/j.biortech.2010.02.111

Xue, S. J., Chi, Z., Zhang, Y., Li, Y. F., Liu, G. L., Jiang, H., Hu, Z., & Chi, Z. M. (2018). Fatty acids from oleaginous yeasts and yeast-like fungi and their potential applications. *Critical reviews in biotechnology*, *38*(7), 1049–1060. https://doi.org/10.1080/07388551.2018.1428167

Yusuf, A. B., Abdulrauf, M. S., Shehu, A., Shehu, F., Ibrahim, G. A., & Samaila, U. (2024). Chemical and biological analysis of processed cassava *garri* sold in some selected local government areas in Kebbi State: A Food Safety Perspective. *Scholars Bulletin*, *10*(10), 254–266. https://doi.org/10.36348/sb.2024.v10i10.002

Zainuddin, I. M., Fathoni, A., Sudarmonowati, E., Beeching, J. R., Gruissem, W., & Vanderschuren, H. (2018). Cassava post-harvest physiological deterioration: From triggers to symptoms. *Postharvest Biology and Technology*, *142*, 115–123. https://doi.org/10.1016/j.postharvbio.2017.09.0048

Zhang, M., Xie, L., Yin, Z., Khanal, S. K., & Zhou, Q. (2016). Biorefinery approach for cassava-based industrial wastes: Current status and opportunities. *Bioresource Technology*, *215*, 50–62. https://doi.org/10.1016/j.biortech.2016.04.026

Zhou, A., & Thomson, E. (2009). The development of biofuels in Asia. *Applied Energy*, *86*(1), 11–20.

Zhu, N., Jin, H., Kong, X., Zhu, Y., Ye, X., Xi, Y., Du, J., Li, B., Lou, M., & Shah, G. M. (2020). Improving the fermentable sugar yields of wheat straw by high-temperature pre-hydrolysis with thermophilic enzymes of *Malbranchea cinnamomea*. *Microbial Cell Factories*, *19*(1), 149. https://doi.org/10.1186/s12934-020-01408-y

Ziegler-Devin, I., Chrusciel, L., & Brosse, N. (2021). Steam explosion pretreatment of lignocellulosic biomass: A mini-review of theorical and experimental approaches. *Frontiers in chemistry*, *9*, 705358. https://doi.org/10.3389/fchem.2021.705358

Zoghlami, A., & Paës, G. (2019). Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. *Frontiers in Chemistry*, *7*. https://doi.org/10.3389/fchem.2019.00874

Zvinavashe, E., Elbersen, H. W., Slingerland, M., Kolijn, S., & Sanders, J. P. M. (2011). Cassava for food and energy: Exploring potential benefits of processing of cassava into cassava flour and bioenergy at farmstead and community levels in rural Mozambique. *Biofuels, Bioproducts and Biorefining*, *5*(2), 151–164. https://doi.org/10.1002/bbb.272

Popular science summary

Cassava is one of the most important staple crops in Mozambique, ensuring food security and providing income for rural households. Its roots are traditionally processed into cassava roasted flour, locally known as *rale*, which is widely consumed as food across the whole country. However, handling practices during this process can influence both product quality and food safety, and the process itself generates large amounts of solid and liquid wastes that are seldom reused.

This thesis explores how cassava can be used more efficiently and safely by linking food production, waste stream valorisation, and environmental protection within the same system. The first part examined the microbial safety of *rale* produced in smallholder processing units in Southern Mozambique. Although occasional microbial contaminants were detected, *rale* met food safety standards, and roasting proved to be the key step for reducing both microbes and the naturally-occurring cyanide compounds found in fresh cassava.

The second part demonstrated that cassava residues – such as peels, fibres, and processing cassava press water – can be converted into valuable products instead of being discarded. Using selected yeasts strains, these residues were efficiently transformed into useful products such as microbial oils and ethanol, showing potential for biofuel or other bioproduct applications.

Finally, yeast strains isolated from cassava effluents displayed remarkable tolerance to cyanide, suggesting their potential role in natural detoxification processes.

Overall, this study connects traditional cassava processing with modern biotechnology to support safer food production and reduce waste. Cassava is not only a staple food for Mozambicans – it also shows a strong potential as a renewable resource capable of contributing to a more sustainable, circular, bioeconomy for the country.

Populärvetenskaplig sammanfattning

Kassava är en av de viktigaste basgrödorna i Moçambique och spelar en avgörande roll för livsmedelssäkerhet och inkomster för landsbygdsbefolkningen. Rötterna hos kassava bearbetas traditionellt till rostat kassavamjöl, kallat *rale* Moçambique, som är mycket konsumerat i landet. Bearbetning av kassavarötter till *rale* genererar dock stora mängder fasta och flytande restprodukter som sällan återanvänds, och hanteringsmetoderna kan påverka både produktkvalitet och livsmedelssäkerhet.

I denna avhandling undersöker vi hur kassava kan användas mer effektivt och säkert genom att koppla samman livsmedelsproduktion, restvärdesförädling och miljöskydd i ett och samma system. Den första delen av avhandlingen utvärderas den mikrobiologiska säkerheten hos *rale* från småskaliga produktionsenheter i södra Moçambique. Trots att vissa mikrobiella föroreningar påträffades uppfyllde *rale* livsmedelssäkerhetskraven, och rostningen visade sig vara det avgörande steget för att minska både mängden mikrober och de naturligt förekommande cyanidföreningar som finns i färsk kassava.

Den andra delen visade att kassavarestprodukter – såsom skal, fibrer och pressvatten – kan omvandlas till värdefulla produkter i stället för att kastas. Med hjälp av utvalda jäststammar kunde dessa restprodukter effektivt omvandlas till användbara produkter såsom mikrobiella oljor och etanol, med potential för produktion av bioenergi eller för användning inom andra biotekniska tillämpningar.

Slutligen uppvisade jäststammar isolerade från restvätskeströmmar från kassava processning en anmärkningsvärd tolerans mot cyanid, vilket tyder på en möjlig användning av dessa inom naturliga avgiftningsprocesser.

Sammanfattningsvis kopplar denna studie samman traditionell kassavabearbetning med modern bioteknik för att stödja en säkrare livsmedelsproduktion och minskad mängd avfall vid kassavaprocessning. Kassava är inte bara en basföda i Moçambique – den har också en stor potential som en förnybar resurs som kan bidra till en mer hållbar och cirkulär bioekonomi i landet.

Resumo de divulgação científica popular

A mandioca é uma das principais culturas alimentares, em Moçambique, crucial para a segurança alimentar e geração de rendimento para muitas famílias rurais. As suas raízes são tradicionalmente processadas em farinha torrada, localmente conhecida como *rale*, amplamente consumida em todo o país. No entanto, este processamento gera grandes quantidades de resíduos sólidos e líquidos que raramente são reaproveitados, ainda que as práticas de manuseamento possam influenciar a qualidade e a segurança do produto final.

Esta tese investiga como a mandioca pode ser utilizada de forma mais eficiente e segura, ligando a produção de alimentos, a valorização de resíduos e a protecção ambiental num único sistema.

A primeira parte avaliou a segurança microbiológica do *rale* produzido em unidades de processamento familiares, no sul de Moçambique. Embora alguns contaminantes microbianos tenham sido identificados, o *rale* cumpriu os padrões de segurança alimentar, e a etapa de torrefação revelou-se essencial para reduzir tanto os microrganismos como os compostos cianogénicos naturalmente presentes na mandioca fresca.

A segunda parte da pesquisa demonstrou que os resíduos da mandioca – como cascas, fibras e água prensada – podem ser convertidos em produtos de valor acrescentado, em vez de serem descartados. Usando estirpes seleccionadas de leveduras, estes resíduos foram transformados eficientemente em produtos úteis, como óleos microbianos e etanol, com potencial para biocombustíveis ou outras aplicações biotecnológicas.

Finalmente, estirpes de leveduras isoladas de efluentes de processamento de mandioca mostraram notável tolerância ao cianeto, sugerindo o seu possível papel em processos naturais de detoxificação.

Em geral, este estudo conecta o processamento tradicional da mandioca com abordagens modernas da biotecnologia, demonstrando que a segurança alimentar e a valorização de resíduos podem coexistir num quadro sustentável. A mandioca não é apenas um alimento básico para os moçambicanos – ela também possui grande potencial como recurso renovável capaz de apoiar a transição do país para uma bioeconomia circular baseada em resíduos.

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Microbial Contamination and Food Safety Aspects of Cassava Roasted Flour ("Rale") in Mozambique

Andreia Massamby [1,2,3], Su-lin L. Leong [1], Bettina Müller [1], Lucas Tivana [2,3], Volkmar Passoth [1], Custódia Macuamule [3,4] and Mats Sandgren [1,*]

[1] Department of Molecular Sciences, Uppsala BioCentrum, Swedish University of Agricultural Sciences, P.O. Box 7051, 750 07 Uppsala, Sweden; andreia.massamby@slu.se (A.M.); su-lin.leong@slu.se (S.-l.L.L.); bettina.muller@slu.se (B.M.); volkmar.passoth@slu.se (V.P.)
[2] Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, Maputo P.O. Box 257, Mozambique; lucastivana@yahoo.co.uk
[3] Centre of Excellence in Agri-Food Systems and Nutrition, Eduardo Mondlane University, Maputo P.O. Box 257, Mozambique
[4] Faculty of Veterinary Sciences, Eduardo Mondlane University, Maputo P.O. Box 257, Mozambique; custodia.macuamule@uem.mz
* Correspondence: mats.sandgren@slu.se

Abstract: Cassava is an important staple food that contributes to the food security of small-scale Mozambican farmers. In southern Mozambique, cassava roots are usually processed into cassava roasted flour, locally known as "rale". The handling and processing practices connected to "rale" production may introduce microbial contamination. We assessed the microbial contamination of "rale" processed in local farmers' associations and consumed either locally or sold in rural markets. Microbial sampling was carried out both during the warmer rainy and cooler dry seasons, and microorganisms of relevance for food safety and fermentation were enumerated. The results revealed variation in terms of microbial diversity in all stages of cassava root processing. In samples collected in the warmer rainy season, molds, lactic acid bacteria, general aerobic bacteria and *Bacillus* spp. were isolated, whereas in samples collected in the cooler dry season, other groups of microorganisms such as yeasts and *Staphylococcus aureus* were present. *Wickerhamomyces anomalus*, *Rhodotorula mucilaginosa*, *Pichia exigua*, *Meyerozyma caribbica* and *Torulaspora delbrueckii* were the most frequent yeast species found within the cassava processing stages. Aflatoxin-producing molds were observed infrequently in this study, and only at low counts, thus, the risk for aflatoxin contamination appears to be low. The results obtained from the Illumina 16S rRNA gene sequencing can be considered a complementary technique to the plating methods relied on in this study. From a food quality and safety point of view, this staple food does not appear to pose a high risk for foodborne disease.

Keywords: cassava roasted flour; "rale"; food quality; food safety; microbial contamination; microbial diversity; Mozambique

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1. Introduction

Cassava (*Manihot esculenta Crantz*) is an important staple food in most tropical regions worldwide and represents a source of nourishment especially in Africa, Asia, and South America [1,2]. Africa is considered the continent with the largest cassava production, where this crop is cultivated in around 40 countries. Nigeria is the largest producer, harvesting more than 59 Mt of fresh cassava roots annually [3–6]. In Mozambique, cassava is ranked as the most important staple food, followed by maize [7]. At least 97% of small-scale

Mozambican farmers select cassava as a main production crop, due to its ability to grow in different ranges of climate and altitudes, and its tolerance to a wide variety of soils, diseases and drought when compared to other agricultural crops [2,8,9].

This subsistence crop is important for food security at several levels, both for government and rural families—it has potential to increase farmers' incomes, and reduce rural and urban poverty levels [10–12]. It is considered an attractive and low-risk crop for African farmers, being produced with family labor using simple hand instruments [13]. Cassava also holds a great promise for feeding Africa's growing population, as a readily available and cheap staple food for low–income rural households [12].

Producing nutritional and safe food products from cassava is a challenge for many reasons. Fresh cassava roots have a very short shelf-life of 1–3 days after harvest, limiting food security [8,14]. After harvesting, fresh cassava roots deteriorate rapidly due to a complex biochemical and physiological process, known as postharvest physiological deterioration, PPD. The rapid PDD reduces both the shelf life and quality attributes of cassava roots [15]. Furthermore, despite its nutritional value, cassava contains antinutrients such as phytates, tannins (phenolics), oxalates, nitrates/nitrites, and saponins. These compounds can be toxic and hinder the absorption of certain nutrients [13,16,17]. Some bitter cassava varieties contain high levels of toxic cyanogenic compounds (cyanogenic glycosides) in edible parts. These cassava varieties can represent a source of intoxication for consumers if not prepared properly [13]. The use of adequate processing techniques can reduce both the antinutrients and the cyanogenic glycoside levels in cassava varieties, resulting in better nutrient quality, higher levels of vitamins, especially the B group, essential amino acids, and improvement in protein digestibility [13,18].

In Africa, the processing techniques for dried fermented cassava products such as cassava flour, often lead to low quality products since they are not usually protected during the drying process from environmental contamination including the action of animals and pests. As a result, fouling products, exposure to microorganisms, mycotoxin formation and contamination by pathogens can be observed [1]. Records of aflatoxin contamination by *Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus* have previously been reported in cassava processed products such as cassava roasted fermented flour from Malawi and Zambia [19]. On the other hand, another study in Tanzania concluded that samples of cassava flour collected immediately after the drying process did not show any aflatoxin contamination [20]. Likewise, contamination of stored cassava flour by *A. flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor racemosus* and *Fusarium oxysporum*, has also been reported in Nigeria [21].

In southern Mozambique, cassava is commonly processed to produce cassava roasted flour, also known as "rale", a traditional food consumed by families. Most of the "rale" production in the country is handmade, using simple tools and non-motorized equipment, by rural producers for their own consumption or sale. The industrial production of "rale" in Mozambique is limited. However, there are some cassava processing associations that benefit from the use of specialized and more advanced equipment. Their products are destined for sale in small, open-air or rural markets in the regions, including in larger cities. The cassava roasted flour processing chain starts with the reception of the cassava roots, followed by washing, peeling, chopping, pressing, sieving, and finally, roasting of the final product. In the southern region of Mozambique, there are 18 identified cassava processing farmers associations which produce "rale", of which 5 are in the Gaza province and 13 in the Inhambane province. The "rale" produced in these associations is intended for consumption by the association's members and for sale in the local markets.

The processing of cassava roots to "rale" appears to be simple; however, it is necessary that hygienic aspects are controlled at all stages to avoid contamination by microorganisms

that can compromise the quality of the final product and its safety for consumers [22]. Few studies have been carried out to deeply evaluate the microbiological safety and quality of this traditional fermented cassava product. The present study aimed at surveying relevant microbes as indicators of hygienic quality existing (a) within the whole chain of processing cassava in the main processing unit (production unit called "*Unit J*" in this study); (b) in "rale" produced in different cassava processing units; and (c) in "rale" sold in rural markets. Our results show how the microbial contamination can affect the food quality and safety of the cassava derivatives produced, consumed and sold in southern Mozambique based on microbiological and molecular approaches.

2. Materials and Methods

2.1. Description of Study Area

The cassava sampling took place in the Gaza (24°54′02.7″ S; 33°57′37.5″ E) and the Inhambane (24°39′06.0″ S; 34°36′27.0″ E) provinces, in the southern part of Mozambique and at two different occasions: November 2020 and August 2021. From a climatic point of view, what differentiates the two climate seasons in Mozambique is the amount of rain that falls during these seasons. Mozambique has two seasons: a rainy season which normally lasts from November to April, and a dry season between May and October.

The temperature remains relatively stable throughout the year with differences of just a few degrees between the seasons and between day and night. Maximum temperatures vary between 24 °C and 30 °C along the south coast, with the hottest months between December and February. The same happens with minimum temperatures ranging between 14 °C and 22 °C, with June and July having the coldest temperatures. The annual precipitation varies from 800 mm to 900 mm per year [23].

2.2. Sampling Procedure for Assessment of Indicator Microbes for Hygiene Quality

2.2.1. Steps of Casava Processing Within *Unit J* and Sampling

The microbial contamination at different stages of processing cassava (from roots to cassava roasted flour) was assessed in the main cassava processing association unit, hereafter called *Unit J*. This unit is considered a model for cassava processing, including the application of food and hygiene practices during the "rale" processing.

The cassava processing started with manual cassava harvesting at cassava farmers' fields of *Unit J*. After harvest, the roots were transported to the association unit by animal-drawn carts or trucks. Upon arrival, cassava tubers were discharged directly onto a plastic sheet or net on the ground, where the association members proceeded with the peeling process. This was carried out outdoors and manually, without any washing or sanitizing step included. The peeled cassava roots were washed once by hand in big plastic basins containing tap water mixed with sodium hypochlorite to remove the impurities and soil residues. The washed and sanitized cassava roots were placed in clean plastic basins and thereafter, 4 stages of processing were followed (Figure 1).

The first stage of cassava processing is called "*Chopping*". This was performed using a gasoline-powered chopper. The chopped cassava mass was placed into clean raffia bags. After chopping the roots, the "*Pressing*" stage was carried out using a manually driven mechanical press for no more than 24 h. The raffia bags with chopped cassava mass were placed into the pressing machine. From this point, the press water was released, and the pressing stage was finished. The cassava pressed mass was taken out from the raffia bags and placed in big metal containers for sampling, and the press water was collected and stored in clean plastic bottles.

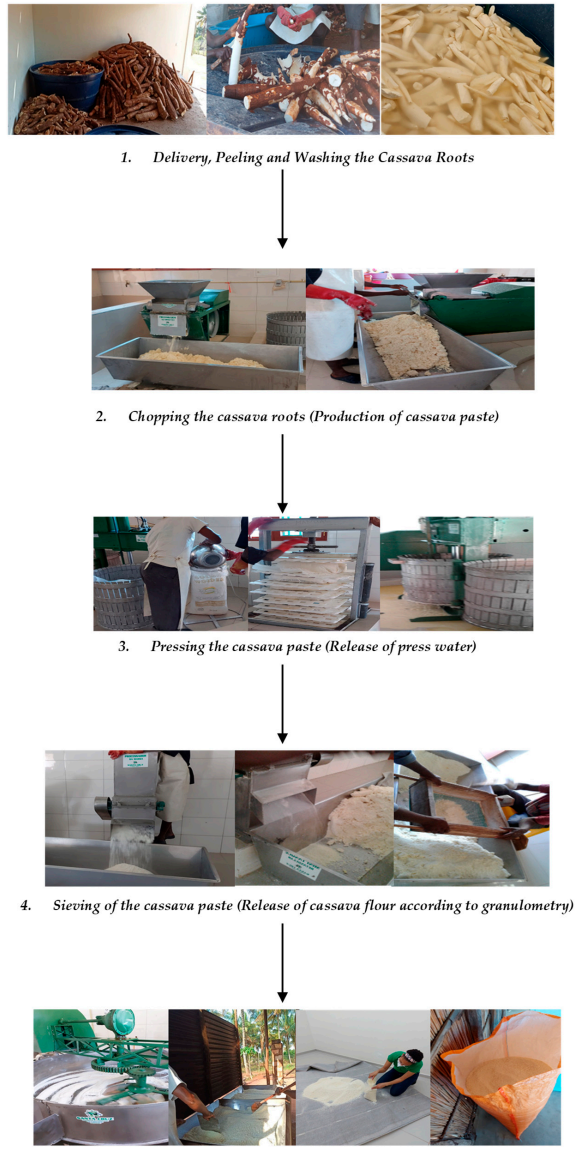

Figure 1. Stages of processing cassava within *Unit J*.

After pressing, the cassava mass was introduced into a gasoline-powered grater for disaggregation and reduction of agglomerates. This stage is called *"Grating"*. The *"Sieving"* process consisted of the screening of the cassava mass in a polyethylene net with wooden borders to separate the small homogenous particles from non-grated pieces and fibrous material.

Finally, the "*Roasting*" of the resulting processed cassava mass was either carried out outdoors (November 2020) or indoors (August 2021). This was due to the season and the installation of a new roasting machine at *Unit J*. The drying and cooling of "rale" was carried out on top of a tarpaulin on the floor of a closed storage room for approximately 7–15 days to reduce as much as possible the humidity left in the product. After cooling, the "rale" was placed in raffia bags, and when necessary, it was packed in plastic bags of 1 kg each for further distribution.

Approximately 500 g of casava material was randomly collected in triplicates for microbial assessment at each stage of the processing procedure, respectively: *Chopping*, *Pressing* (including the *Press water*), *Sieving* and *Roasting*, both during rainy and dry seasons.

2.2.2. Sampling Procedure for Assessment of Indicator Microbes for Hygiene Quality in "Rale" Produced in Different Cassava Processing Units

To assess the microbial contamination in "rale" processed and stored in cassava processing units, six cassava processing units located in Gaza and Inhambane provinces, including the reference *Unit J*, were selected for sampling collection, and these cassava processing units are hereafter called *Units V*, *W*, *P*, *Z*, and *C*. From each season, approximately 500 g of stored cassava roasted flour was collected in triplicate at each processing unit. The cassava processing units were selected considering the following criteria: having a considerable cassava processing activity and having been trained to keep good hygiene practices during cassava processing according to Mozambican's Agricultural Authorities.

2.2.3. Sampling Procedure for Assessment of Indicator Microbes for Hygiene Quality in "Rale" Sold in Different Rural Markets

The study of the microbial contamination of "rale" sold in rural markets in Mozambique included a total of five different markets located along the National Road Number 1 (EN1), hereafter called Markets *AM*, *GB*, *MK*, *ES*, and *MC*. The EN1 connects the Southern part of the country to the Centre and Northern regions of Mozambique. The markets were selected considering easy access and history/or tradition of selling "rale". In each market, three vendors were randomly selected, and approximately 500 g of the "rale" was collected in triplicates, comprising a total of 15 unique "rale" vendors.

The samples collected at different stages of processing cassava at *Unit J* (Section 2.2.1) and the "rale" collected both in cassava processing units (Section 2.2.2) and rural markets (Section 2.2.3) were stored in sterile plastic bags and kept frozen (−20 °C) until transportation to the Department of Molecular Sciences, Swedish University of Agricultural Sciences (Uppsala) for microbial and molecular analyses.

2.3. Culture-Based Analyses of Indicator Microbes for Hygiene Quality

All chemicals and culture media were obtained from Merck KGaA, Darmstadt, Germany; Sigma-Aldrich Inc., St. Louis, MO, USA and Oxoid Ltd., Basingstoke, Hampshire, UK. About 25 g of cassava sample from each triplicate was aseptically transferred to Erlenmeyer flasks containing 225 mL with sterile peptone water (0.1% peptone, *w*/*v*) to dilute the samples. The casava samples were then homogenized for 120 s at normal speed using a Stomacher 400 Laboratory blender (Seward Medical, London, UK). These samples were serially diluted and poured or spread plated on relevant selection media for enumeration of viable counts as specified by the manufacturer to isolate the desired microbes. The following groups of microorganisms were screened for:

(a) Yeasts and Molds

Yeasts and Molds were isolated by surface plating in triplicates on Dichloran Rose-Bengal Agar plates supplemented with 0.1 g/L Chloramphenicol to inhibit bacterial growth [24,25]. The yeast plates were placed in an incubator at 25°C for 72 h. After incubation, approx. 50 yeasts colonies representative of various colony morphologies present were transferred to Yeast Peptone Dextrose Agar (20 g/L peptone, 10 g/L Yeast extract, 20 g/L glucose, 20 g/L agar) supplemented with 0.1 g/L Chloramphenicol, and incubated at 25 °C for 2–3 days. Mold plates were incubated at 25 °C for 7 days. After incubation, approx. 30 molds representing all observed colony morphologies were transferred and cultivated on Malt Extract Agar at 25°C for 3–7 days for identification.

(b) Lactic Acid Bacteria and Bacterial Indicators of Hygienic Quality

Lactic Acid Bacteria (LAB) were quantified on De Man Rogosa Sharpe Agar supplemented with 0.1 g/L Delvocide (active compound, natamycin; Gist-Brocades B.V., Delft, The Netherlands) to inhibit fungal growth. Plates were incubated anaerobically using a GasPackTM EZ system (Becton Dickinson; Sparks, MD, USA) at 30 °C for 48 h [26,27].

Total bacterial Counts (TBC) were enumerated by the pour plate method using Tryptone Glucose Yeast Extract Agar supplemented with 0.1 g/L Delvocide to suppress fungal growth. Plates were incubated at 30 °C for 3 days [25,28].

Enterobacteriaceae were quantified on Violet Red Bile Agar (VRBG) by pour plating. Plates were incubated at 37 °C for 24 h [29]. Presumptive *Escherichia coli* was enumerated by an additional set of VRBG Agar plates incubated at 44 °C for 24 h [30,31].

(c) Enumeration of *Bacillus cereus*, *Bacillus* spp., *Staphylococcus aureus* and *Escherichia coli*

To enumerate *B. cereus* and other aerobic spore formers, the serial dilution tubes were heated for 13 min. in a water bath at 80 °C before plating. *B. cereus* was enumerated by surface plating on Mannitol Egg-yolk Polymyxin Agar (MYPA) followed by incubation at 37 °C for 24 h. All large, rounded colonies, pink in color and surrounded by a precipitation zone were enumerated as presumptive *B. cereus* [32,33].

Bacillus spp. counts were quantified on Reinforced Clostridial Agar incubated aerobically at 37 °C for 24 h.

S. aureus was quantified on Baird-Parker Agar with egg-yolk tellurite by surface plating followed by incubation at 37 °C for 48 h. Gray-black colonies with haloes were enumerated as presumptive *S. aureus* [34,35].

Presumptive colonies of *E. coli*, *B. cereus* and *S. aureus* were confirmed using PCR as described by [36]. Microbial counts were expressed as log10 mean (n = 3) cfu/g of cassava solid sample or cfu/mL of cassava press water sample.

All microbial analyses described above were performed for samples collected within the whole chain of cassava processing at *Unit J*, whereas for "rale" (the finished product) collected both in different markets and different cassava processing units, we focused on enumeration of yeasts, molds, *S. aureus*, TBC, *Enterobacteriaceae* and *B. cereus*.

2.4. Yeast and Mold Identification

Preliminary identifications of purified representative isolates as described in Section 2.3–a), were based on macro and micro-morphology. Isolates that appeared to have similar macro and micro-morphology were grouped and given codes for further steps. These representative yeast and mold isolates were grown in Yeast Extract Peptone Dextrose broth (20 g/L peptone, 10 g/L Yeast extract, 20 g/L glucose) for DNA extraction and sequence-based species identification.

PCR analysis was performed for yeast isolates by selecting single colonies as templates. Material from a single yeast colony was boiled in 20 µL 0.02 M NaOH for 5 min,

and 2 µL of the resulting suspension was used as PCR template with primers NL1/NL4 to amplify the D1/D2 region of the 26S rRNA gene [37,38]. For molds, DNA was extracted using the method described by [39]. The following genes were selected for identification by partial amplification and sequencing: translation elongation factor 1 α for presumptive *Fusarium* spp. with primers EF1/EF2 [40]; β-tubulin gene in *Penicillium* subgenus *Penicillium* spp. with primers bt2a/bt2b [41]; and rDNA internal transcribed spacer in all other species with primers ITS1F/ITS4 [42]. Amplicons were sequenced at Macrogen, Amsterdam, The Netherlands, and all representative isolates were identified by sequence search and comparison against the NCBI database (Nucleotide Blast, Core nucleotide database "https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlaSearch&LINK_LOC=blasthome (accessed on 25 June 2023)".

2.5. Culture-Independent Analysis of Bacterial Community by Illumina Amplicon Sequencing

16S rRNA gene sequencing was applied as a culture-independent method to gain an overview of bacterial diversity. DNA was extracted using a Quick-DNA Fungal/Bacterial Microprep Kit (Zymo Research™, Freiburg, Germany) including an additional bead-beating step: briefly, 20 ± 10 mg sample was weighed in ZR BashingBead™ Lysis tubes (Freiburg, Germany) and placed on ice. A total of 750 µL of BashingBead™ Buffer (Freiburg, Germany) was added in the sample directly to the tube and capped tightly. The tube was placed in a FastPrep Instrument (MPBiomedicals™, Freiburg, Germany) for 40 s at speed setting 6.0 and centrifuged at 10,000× *g* for 10 min at room temperature (20 °C). Then, 400 µL of the obtained supernatant was transferred into a new microcentrifuge tube and treated as described in the manufacturer's protocol. The DNA concentration was approximated using Qubit© fluorometer dsDNA protocol (Invitrogen –Thermo Fisher Scientific, Dreieich, Germany). DNA was purified in triplicates for all samples, and some replicates had to be diluted before PCR to overcome inhibitory effects. PCR amplification, purification, and barcoding/preparation of libraries for Illumina Sequencing were performed using the protocol modified by [43].

16S rRNA gene amplicon libraries were constructed as triplicates using two consecutive PCR procedures. The first PCR targeted and amplified the V4 region of bacteria, using the primers 515F (ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGBCAGCMGCCGCGAA) and 805R (AGACGTGTGCTCTTCCGATCTGGACTACHVGGGTWTCTAAT), and it attaches adaptors to the amplicons [43]. The reaction mixture contained 2× Phusion High-Fidelity DNA Polymerase/dNTP mix (Thermo Fischer Scientific, Hudson, NH, USA), 10 µM of each primer, and approx. 5–10 ng DNA template in a final volume of 25 µL. The condition for amplification was as follows: initial denaturing at 98 °C for 30 s, 30 cycles of 10 s at 98 °C, 30 s at 60 °C, 4 s at 72 °C, and a final extension at 72 °C for 2 min. The PCR products were checked for size and quality by electrophoresis.

Amplicons were purified using Agencourt AMPure XP (Becker Coulter, Brea, CA, USA), using a magnetic particle/DNA volume ratio of 0.8:1. In the second PCR, Illumina-compatible barcodes were added to the amplicons [44]. The PCR reaction contained 10 µL purified amplicon from the first step, 2× Phusion High-Fidelity DNA Polymerase/dNTP mix and 10 µM each of the primers 5′-AATGATACGGCGACCACCGAGATCTACACX8ACACTCTTTCCCTACACGACG-3′ and 5′-CAAGCAGAAGACGGCATACGAGATX8GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′, where X8 in the primer sequence represented a specific Illumina-compatible barcode (Eurofins– Genomics). The total volume was 25 µL. The following conditions were used for the second PCR step: initial denaturing at 98 °C for 30 s, 8 cycles of 10 s at 98 °C, 30 s at 62 °C, 5 s at 72 °C, and a final extension at 72 °C for 2 min. The PCR products were checked by electrophoresis and purified using

Agencourt AMPure XP. The PCR products were then each diluted to a DNA concentration of approx. 24 nM and pooled together.

Pair-end sequencing was performed on the MiSeq platform (Illumina, Inc., San Diego, CA, USA) at ScilifeLab, National Genomics Infrastructure (Stockholm, Sweden). Amplicon sequence variants, abundancies and taxonomic affiliation were determined using the package dada2 (version 1.6.0) [45] in R (version 3.4.0), which is implemented on the SLUBI computing cluster in Uppsala (running on CentOS Linux release 7.1.1503; module handling by Modules based on Lua: Version 6.0.1 "https://www.slubi.se/ (accessed on 16 October 2023)". For further details see [44].

2.6. Statistical Analysis

Statistical analysis was carried out using R in the RStudio (2024.09.1) environment [46]. The normality function from the *dlookr* version 0.6.3 package [47] was used to retrieve the results of the *Shapiro* test and therefore assess whether the collected data fulfil the assumptions for the ANOVA test. The *Bartlett's* test was performed using an inbuilt function from the R software (version 4.3.2) to assess other ANOVA assumptions (homogeneity of variances). Since the assumptions were not satisfied, the *Kruskal–Wallis's* test was used as a non-parametric alternative to ANOVA. The *Dunn* test was performed as a post-hoc test to derive pairwise multiple comparisons among significant groups of microorganisms in *Cassava Processing Stages*, *Cassava Processing Units* and *Rural Markets*. The *p*-values for multiple comparisons were adjusted using the Bonferroni method. All results were considered significant at $p < 0.05$. In general, the *gtsummary* package version 1.7.2 [48] was used to compute both the descriptive statistics and inferential statistics. The *rstatix* package version 0.7.2 [49] was used to derive both the *Kruskal–Wallis's* and *Dunn* tests.

For Illumina 16S rRNA gene sequencing results, all abundances below the cut-off value of 0.5% were removed from considerations.

3. Results

3.1. Microbes as Indicators of Hygienic Quality Within Unit J

Table 1 describes microbes indicative of hygienic quality isolated from cassava samples collected in different stages of cassava processing within *Unit J* during the rainy and dry seasons. Molds, Lactic Acid Bacteria (LAB), Aerobic bacteria (Total Aerobic Bacteria Counts, TBC) and *Bacillus* spp. were observed in the cassava samples from the rainy season, and the presence of yeasts and *S. aureus* were found in cassava samples collected during the dry season.

The counts of microorganisms in the processing chain of cassava differ significantly between each stage of processing cassava tubers to cassava roasted flour ("rale") in both rainy and dry seasons, as well as when combining all seasons (Table 1). The dry season generally reported higher counts of microorganisms in the cassava processing chain compared to the rainy season. On both occasions, the counts of *Enterobacteriaceae*, presence of *E. coli*, and *B. cereus* were below the detection limit (Table 1).

Specifically, in the rainy season, the levels of contamination of cassava samples by yeasts and *S. aureus* were below the detection limit in all processing stages. Mold counts were generally low, with the roasting process having the highest counts (mean 1.33 log cfu/g) followed by chopping and pressing stages (mean 0.33 log cfu/g). LAB, TBC and *Bacillus* spp. were only detected during the chopping, pressing, and sieving stages, with counts ranging from 5.54 to 6.81 log cfu/g for LAB, 5.32 to 6.53 log cfu/g for TBC, and 3.04 to 3.33 log cfu/g for *Bacillus* spp. (Table 1).

In contrast, during the dry season, the presence of yeasts and *S. aureus* were ob-served in the stages of chopping, pressing, and sieving. The pressing stage recorded the highest

cfu in both groups of microorganisms (4.21 log cfu/g for yeasts and 5.73 log cfu/g for *S. aureus*, respectively). In this season, molds, LAB, and TBC were also present during all cassava processing stages. The highest mold count was observed in the roasting process (2.72 log cfu/g), while the lowest values were reported during the sieving stage (*bdl*). For LAB and TBC, the highest cfu counts were observed in the pressing stage (6.50 log cfu/g for LAB and 6.22 log cfu/g for TBC), and the lowest values were found for the press water collected during the pressing stage (*bdl* for LAB and 2.23 log cfu/mL for TBC). The presence of *Bacillus* spp. in this season was below the limit of detection (Table 1). When combining all seasons of study, significant differences were observed within cassava processing stages for LAB, TBC and *Bacillus* spp.

Table 1. Enumeration (log cfu/g) of different microbial groups isolated within *Unit J* during rainy, dry and both seasons. Values are presented as mean ± standard deviation (n = 3 for each season and n = 6 for both seasons). Different superscript letters represent significant differences ($p < 0.05$).

Microbes	Cassava Processing Stages					
	Chopping [1]	*Pressing* [1]	*Press Water* [1]	*Sieving* [1]	*Roasting* [1]	*p-Value* [2]
Rainy season (November 2020)						
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Molds	0.33 ± 0.58	0.33 ± 0.58	0.10 ± 0.17	▪	1.33 ± 1.59	0.52
LAB	5.54 ± 0.13 ^{a}	6.81 ± 0.90 ^{a}	*bdl*	5.71 ± 0.34 ^{a}	*bdl*	0.011 *
S. aureus	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
TBC	5.32 ± 0.08 ^{a}	6.17 ± 0.10 ^{a}	*bdl*	6.53 ± 0.4 ^{a}	*bdl*	0.009 **
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Pres. of *E. coli*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus spp.	3.04 ± 0.87 ^{a}	3.33 ± 1.15 ^{a}	*bdl*	3.05 ± 0.40 ^{a}	*bdl*	0.026 *
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Dry season (August 2021)						
Yeast	4.13 ± 0.34 ^{a}	4.21 ± 0.05 ^{a}	2.84 ± 0.79 ^{a}	4.02 ± 0.18 ^{a}	*bdl*	0.026 *
Molds	0.67 ± 1.15	0.40 ± 0.17	0.58 ± 0.51	*bdl*	2.72 ± 0.13	0.05
LAB	4.52 ± 0.31 ^{a}	6.50 ± 0.65 ^{a}	*bdl*	*bdl*	3.35 ± 0.60 ^{a}	0.011 *
S. aureus	5.61 ± 0.12 ^{a}	5.73 ± 0.05 ^{a}	*bdl*	5.50 ± 0.56 ^{a}	*bdl*	0.024 *
TBC	5.90 ± 0.21 ^{ab}	6.22 ± 0.27 ^{a}	2.23 ± 0.11 ^{b}	5.67 ± 0.30 ^{ab}	3.25 ± 1.08 ^{ab}	0.017 *
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Pres. of *E. coli*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus spp.	3.04 ± 0.87 ^{a}	3.33 ± 1.15 ^{a}	*bdl*	3.05 ± 0.40 ^{a}	*bdl*	0.028 *
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
All seasons (Rainy and Dry)						
Yeast	2.91 ± 1.35	2.96 ± 1.38	2.27 ± 0.80	2.86 ± 1.28	1.85 ± 0.16	0.81
Molds	0.33 ± 0.82	0.20 ± 0.25	1.41 ± 1.44	*bdl*	0.37 ± 0.43	0.1
LAB	5.03 ± 0.60 ^{ab}	6.65 ± 0.72 ^{a}	2.36 ± 0.40 ^{b}	6.10 ± 0.47 ^{a}	2.67 ± 0.83 ^{b}	<0.001 ***
S. aureus	3.80 ± 1.98	3.87 ± 2.04	*bdl*	3.75 ± 1.95	*bdl*	0.09
TBC	5.61 ± 0.35 ^{ab}	6.19 ± 0.18 ^{a}	2.11 ± 0.14 ^{b}	6.10 ± 0.57 ^{a}	2.62 ± 0.97 ^{b}	<0.001 ***
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Pres. of *E. coli*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus spp.	3.04 ± 0.78 ^{a}	3.33 ± 1.03 ^{a}	1.85 ± 0.16 ^{b}	3.05 ± 0.36 ^{a}	*bdl*	<0.001 ***
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*

Abbreviations: *bdl*—below the detection limit; *na*—not applicable; LAB—lactic acid bacteria; TBC—total bacterial count; ▪—less than two colonies/plate (10^{-1}); cfu— colony forming units; [1] Mean (SD); [2]* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In the rainy season, molds of the genus *Penicillium* (*P. ochrochloron*, *P. primulinum*, *P. citreonigrum*) were isolated from chopping, pressing and press water samples, whereas the roasting stage was more contaminated by *Alternaria infectoria*, *Cladosporium sphaerospermum* and *A. flavus*. No yeast contamination was observed in all cassava samples assessed in this season (Table 2).

Table 2. Molds and yeasts (% of isolates) identified in cassava samples from *Unit J* during rainy and dry seasons.

	Rainy Season (November 2020)		Dry Season (August 2021)	
	Mold Isolates	**Yeast Isolates**	**Mold Isolates**	**Yeast Isolates**
Chopping	*Penicillium ochrochloron* (100%)	-	*Curvularia* sp. (50%) *Rhizopus stolonifer* (50%)	*Wickerhamomyces anomalus* (71%) *Rhodotorula mucilaginosa* (14%) *Pichia exigua* (7.5%) *Rhodotorula alborubescens* (7.5%)
Pressing	*Penicillium primulinum* (50%) *Penicillium citreonigrum* (50%)	-	*Pestalotiopsis* sp. (16.7%) *Rhizopus stolonifer* (16.7%) *Pitomyces sacchari* (16.7%) *Aspergillus fumigatus* (16.7%) *Penicillium griseofulvum* (16.7%) *Didymella* sp. (16.5%)	*Wickerhamomyces anomalus* (31%) *Pichia exigua* (19%) *Rhodotorula mucilaginosa* (19%) *Meyerozyma caribbica* (12.5%) *Torulaspora delbrueckii* (12.5%) *Candida orthopsilosis* (6%)
Press water	*Penicillium olsonii* (100%)		*Penicillium restrictum* (100%)	*Rhodotorula babjevae* (50%) *Meyerozyma caribbica* (50%)
Sieving	-	-	-	*Wickerhamomyces anomalus* (37.5%) *Rhodotorula mucilaginosa* (25%) *Naganishia diffluens* (12.5%) *Kwoniella heavenis* (6.3%) *Kazachstania unispora* (12.4%) *Candida orthopsilosis* (6.3%)
Roasting	*Alternaria infectoria* (33.3%) *Cladosporium sphaerospermum* (33.3%) *Aspergillus flavus* (33.3%)	-	*Stagonosporopsis* sp. (25%) *Cladosporium sphaerospermum* (12.5%) *Cladosporium cladosporioides* (12.5%) *Cladosporium oxysporum* (12.5%) *Rhizopus stolonifer* (12.5%) *Arthinium* sp. (12.5%) *Dothideales* sp. (12.5%)	

The contamination of the cassava samples by both molds and yeasts was higher during the dry season than the rainy season. Samples were contaminated by *Aspergillus fumigatus*, *Penicillium griseofulvum* and *Rhizopus stolonifer* at the pressing stage, and *Cladosporium cladosporioides*, *Cladosporium oxysporum* and *R. stolonifer* at the roasting stage. In contrast, the chopping, pressing and sieving stages were found to have the highest diversity of yeasts. *Wickerhamomyces anomalus*, *Rhodotorula mucilaginosa*, *Pichia exigua* and *Meyerozyma caribbica* were the most frequent yeast species found in these cassava processing stages. *Rhodotorula babjevae*, a red oleaginous yeast, was also observed in the press water samples.

All samples from the sieving stage were free from contamination by molds in both surveyed seasons (Table 2). Certain microbial isolates could not be identified in the present study due to the lack of reference sequences in the NCBI database (Nucleotide Blast, Core nucleotide database) "https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&

PAGE_TYPE=BlastSearch&LINK_LOC=blasthome (accessed on 15 January 2022)". However, identified isolates presented here were deemed sufficient to give an indication of the types of species present (Table 2).

Illumina 16S rRNA gene sequencing analysis of the bacterial microbiota revealed high prevalence of LAB and Lactobacillales in all samples for the two seasons of the study. The LAB affiliates to the genera *Fructobacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Weissella* (Figure 2). This included samples from the roasting stage during the rainy season where LAB counts were below the detection limit (Table 1). Amplicons representing Cyanobacteria were also abundantly found in the samples. Regarding Gram-negative bacteria, in both seasons, bacteria from the order Rickettsiales, and the genera *Pseudomonas* and *Klebsiella* were observed at somewhat greater abundance in samples collected at the roasting stage. Bacterial species of *Aeromonas* and *Escherichia/Shigella* were also found in samples collected in the roasting stage within the first sampling period.

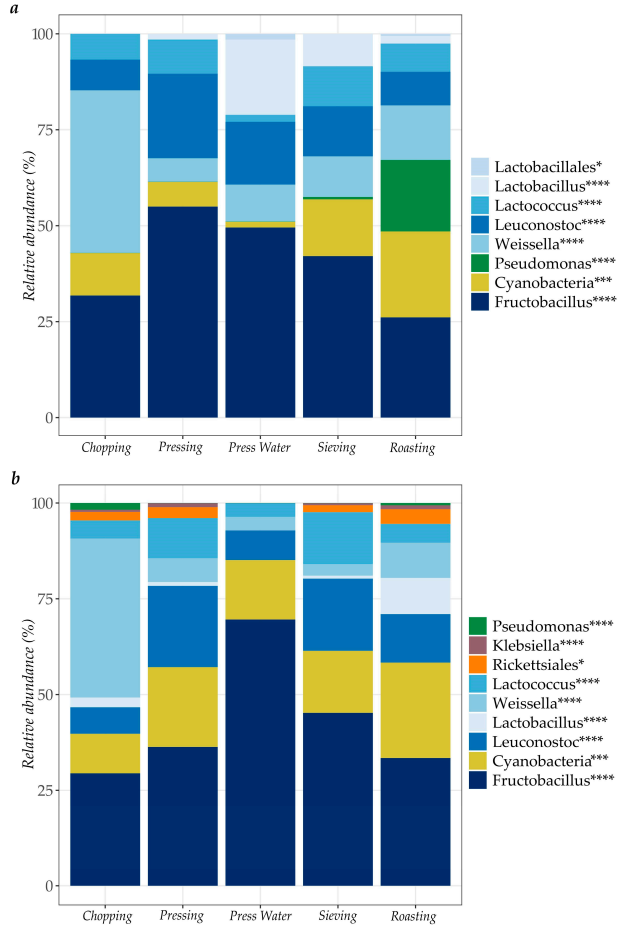

Figure 2. Relative abundance of bacteria at order *, class ***, and genus **** levels in cassava samples (n = 3) collected in different stages of processing within *Unit J* during: (**a**) the rainy season (November 2020) and (**b**) the dry season (August 2021).

3.2. Microbes as Indicators of Hygienic Quality in "Rale" Sampled from Cassava Processing Units

Counts of microorganisms from the final cassava product ("rale") differ significantly between the six main cassava processing units located in Gaza and Inhambane provinces, Mozambique, in both the rainy and dry seasons (Table 3). However, no significant difference in terms of microbial contamination was found when combining the two seasons. On all occasions, yeasts and *Enterobacteriaceae* were always found to be below the limit of detection.

Table 3. Enumeration (log cfu/g) of different microbial groups isolated in "rale" processed in six cassava processing units during rainy, dry and both seasons. Values are presented as mean ± standard deviation (n = 3 for each season and n = 6 for both seasons). Different superscript letters represent significant differences ($p < 0.05$).

Microbes	Cassava Processing Units						
	V [1]	***W*** [1]	***P*** [1]	***Z*** [1]	***C*** [1]	***J*** [1]	***p-Value*** [2]
Rainy season (November 2020)							
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Molds	0.36 ± 0.32	0.20 ± 0.35	1.33 ± 0.17	3.47 ± 2.29	1.53 ± 2.66	0.26 ± 0.24	0.1
S. aureus	*bdl*	2.33 ± 0.58	*bdl*	*bdl*	*bdl*	*bdl*	0.42
TBC	7.30 ± 4.59	*bdl*	*bdl*	*bdl*	3.33 ± 2.31	6.67 ± 3.21	0.054
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Dry season (August 2021)							
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Molds	3.90 ± 0.53 [a]	1.22 ± 1.10 [a]	1.26 ± 0.12 [a]	*bdl*	0.89 ± 0.25 [a]	*bdl*	0.028 *
S. aureus	*bdl*	2.00 ± 0.01	*bdl*	*bdl*	*bdl*	*bdl*	0.42
TBC	1.83 ± 0.30	1.41 ± 0.17	1.75 ± 0.62	1.60 ± 0.11	1.67 ± 0.57	1.34 ± 0.57	0.66
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	2.33 ± 0.58	*bdl*	0.42
All seasons (Rainy and Dry)							
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Molds	2.13 ± 1.98	0.71 ± 0.92	1.29 ± 0.14	2.73 ± 1.66	1.21 ± 1.72	1.13 ± 0.97	0.15
S. aureus	*bdl*	2.17 ± 0.41	*bdl*	*bdl*	*bdl*	*bdl*	0.068
TBC	4.56 ± 4.18	1.70 ± 0.34	1.88 ± 0.41	1.80 ± 0.23	2.34 ± 1.86	4.17 ± 3.43	0.38
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	2.17 ± 0.41	*bdl*	0.42

Abbreviations: *bdl*—below the detection limit; *na*—not applicable; LAB—lactic acid bacteria; TBC—total bacterial count; cfu—colony forming units; [1] Mean (SD); [2] * $p < 0.05$.

During the rainy season, molds appeared to dominate most samples collected from all cassava processing units, with counts varying from 0.20 log cfu/g (*Unit W*) to 3.47 log cfu/g (*Unit Z*). TBC could only be quantified in samples collected in *Unit C* (3.33 log cfu/g), *Unit J* (6.67 log cfu/g) and *Unit V* (7.30 log cfu/g), while *S. aureus* was only found in *Unit W* (2.33 log cfu/g). *B. cereus* was not detected in any of the samples.

In the dry season, TBCs were more frequently isolated than other groups of tested microorganisms, varying from 1.34 log cfu/g (*Unit J*) to 1.83 log cfu/g (*Unit V*). Molds were observed in four out of the six associations included in this study, ranging from 0.89 log cfu/g (*Unit C*) to 3.90 log cfu/g (*Unit V*). The highest counts of *B. cereus* were reported at *Unit C* (2.33 log cfu/g). Similar to the rainy season, *S. aureus* was reported only at *Unit W* (2 log cfu/g), while in other units the counts for this microbe were below the level of detection (Table 3).

The highest diversity of molds in "rale" samples collected at the six cassava processing units was observed in the dry season (Table 4). *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., and *Alternaria* spp. were reported in both seasons.

Table 4. Molds (% of isolates) identified in "rale" processed in six cassava processing units during rainy and dry seasons.

		Rainy Season (November 2020)	Dry Season (August 2021)
		Mold isolates	
Units	*V*	**n.i.*	*Aspergillus ruber* (29%) *Pithomyces sacchari* (43%) *Chaetomium globosum* (28%)
	W	*Penicillium purpureum* (17%) *Alternaria* sp. (17%) *Aspergillus flavus* (17%) *Aspergillus niger* (17%) *Aureobasidium pullulans* (17%) *Cladosporium cladosporioides* (15%)	*Pithomyces sacchari* (44%) *Aspergillus penicillioides* (11%) *Aspergillus chevalieri* (22%) *Fusarium solani* (11%) *Pleurotus ostreatus* (12%)
	P	*Penicillium citreonigrum* (50%) *Fusarium oxysporum* (50%)	*Penicillium citrinum* (7%) *Neopestalotiopsis egyptiaca* (7%) *Chaetomium globosum* (5%) *Pithomyces sacchari* (51%) *Alternaria alternata* (7%) *Pithomyces maydicus* (7%) *Paraphaeosphaeria michotii* (7%) *Aspergillus nidulans* (7%)
	Z	*Penicillium citreonigrum* (50%) *Penicillium ruber* (50%)	-
	C	*Penicillium ruber* (67%) *Trichoderma* sp. (33%)	*Phoma pereupyrena* (10%) *Pithomyces sacchari* (60%) *Aspergillus calidoustus* (10%) *Talaromyces* sp. (10%) *Aspergillus* sp. (10%)
	J	*Aspergillus niger* (14%) *Alternaria infectoria* (14%) *Cladosporium sphaerospermum* (14%) *Penicillium primulinum* (14%) *Penicillium citreonigrum* (16%) *Penicillium ochrochloron* (14%) *Epicoccum* sp. (14%)	-

Abbreviations: **n.i.*—not identified.

Relative abundance of bacterial groups in "rale" samples collected in six different cassava processing units is shown in Figure 3. LAB (*Fructobacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Weisiella*) and the Cyanobacteria class were the most dominant in samples collected both in dry and rainy seasons, and in all cassava processing units. Other genera present included *Klebsiella*, *Escherichia/Shigella*, *Cloacibacterium* and *Staphylococcus*, as well as the order Rickettsiales and family *Neisseriaceae*.

The Gram-negative genera *Pseudoxanthomonas* and *Pseudomonas* were also occasionally present in "rale" samples, and *Unit Z* was found to have the highest diversity of bacterial microbiota.

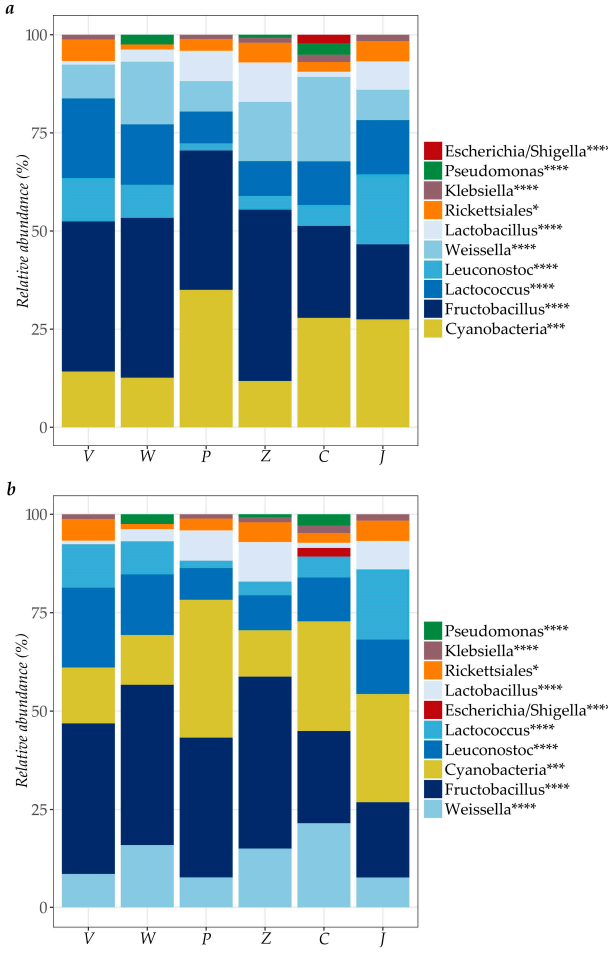

Figure 3. Relative abundance of bacteria at order *, class ***, and genus **** levels in "rale" samples (n = 3) collected in six different cassava processing units during (**a**) the rainy season (November 2020) and (**b**) the dry season (August 2021).

3.3. Microbes as Indicators of Hygienic Quality of "Rale" Collected in Rural Markets

Table 5 describes the enumeration of various microbes in "rale" samples collected in five rural markets, where "rale" is traditionally sold. The diversity of microorganisms varied between the two seasons of study within the five assessed markets. No significant differences in microbial diversity were found between the rural markets during the rainy season, whereas significant differences were found during the dry season and when combining the microbe counts from both seasons.

In the rainy season, *S. aureus* was only confirmed from one market (*MK*) at 2.01 log cfu/g. The same scenario was observed for TBC, which were only reported in one market (*GB*) at 2.05 log cfu/g. The counts for yeasts, *Enterobacteriaceae* and *B. cereus* were below the detection level. Average mold counts were very low, as reflected by a few colonies on occasional plates (Table 5).

Table 5. Enumeration (log cfu/g) of different microbial groups isolated in "rale" sold in five rural markets during rainy, dry and both seasons. Values are presented as mean ± standard deviation (n = 3 for each season and n = 6 for both seasons). Different superscript letters represent significant differences ($p < 0.05$).

Microbes	Rural Markets					
	AM [1]	*GB* [1]	*MK* [1]	*ES* [1]	*MC* [1]	*p-Value* [2]
			Rainy season (November 2020)			
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Molds	*bdl*	▪	*bdl*	▪	▪	*na*
S. aureus	*bdl*	*bdl*	2.01 ± 0.01	*bdl*	*bdl*	0.071
TBC	*bdl*	2.05 ± 0.09	*bdl*	*bdl*	*bdl*	0.41
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
			Dry season (August 2021)			
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	4.55 ± 0.55 [a]	0.008 **
Molds	*bdl*	▪	0.26 ± 0.24 [a]	▪	2.32 ± 0.36 [a]	0.016 *
S. aureus	*bdl*	*bdl*	*bdl*	*bdl*	2.16 ± 0.28	0.41
TBC	2.46 ± 0.05 [a]	2.54 ± 0.06 [a]	1.71 ± 0.20 [a]	2.06 ± 0.28 [a]	1.86 ± 0.29 [a]	0.023 *
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus cereus	2.01 ± 0.00 [a]	*bdl*	*bdl*	*bdl*	2.33 ± 0.58 [a]	0.050 *
			All seasons (rainy and dry)			
Yeast	*bdl*	*bdl*	*bdl*	*bdl*	3.27 ± 1.44	0.012 *
Molds	*bdl*	▪	0.13 ± 0.21 [a]	▪	1.16 ± 1.29 [a]	0.045 *
S. aureus	*bdl*	*bdl*	2.00 ± 0.01	*bdl*	2.08 ± 0.19	0.24
TBC	2.23 ± 0.26 [ab]	2.30 ± 0.28 [a]	1.85 ± 0.20 [b]	2.03 ± 0.18 [ab]	1.93 ± 0.20 [ab]	0.023 *
Enterobacteriaceae	*bdl*	*bdl*	*bdl*	*bdl*	*bdl*	*na*
Bacillus cereus	*bdl*	*bdl*	*bdl*	*bdl*	2.17 ± 0.41	0.063

Abbreviations: *bdl*—below the detection limit; *na*—not applicable; LAB—lactic acid bacteria; TBC—total bacterial count; ▪—less than two colonies/plate (10^{-1}); cfu— colony forming units; [1] Mean (SD); [2] * $p < 0.05$; ** $p < 0.01$.

In contrast, in the dry season, the highest counts for yeasts (4.55 log cfu/g), molds (2.32 log cfu/g), and *S. aureus* (2.16 log cfu/g) were observed in samples belonging to the *MC* market. All market samples were positive for TBC, with values ranging from 1.71 log cfu/g (*MK*) to 2.54 log cfu/g (*GB*). Market *MC* had the highest counts for *B. cereus* (2.33 log cfu/g) followed by market *AM* (2.01 log cfu/g). In this season, all market samples were found to be negative for *Enterobacteriaceae*. When combining both seasons of study, significant differences of microbial contamination were observed among rural markets for molds and TBC (Table 5).

Molds identified in "rale" samples collected in rural markets during the rainy season belonged to the genera *Fusarium*, *Rhizopus* and *Talaromyces* (Table 6). In the dry season, *Pithomyces*, *Aspergillus*, *Talaromyces*, and *Trematosphaeia* were identified. The *MK* market samples had somewhat higher mold counts and much greater species diversity during the dry season compared to the rainy season.

The composition of the bacterial community displayed as relative abundance from "rale" samples collected in rural markets are presented in Figure 4. During both seasons, bacteria from the class Cyanobacteria, and the genera *Fructobacillus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* and the *Weisiella* genus were most frequent in all surveyed markets. Regarding the Gram-negative community, the genus *Klebsiella* and *Acinetobacter*, the family *Neisseriaceae*, and the order Rickettsiales were also dominant in samples from both seasons. The samples from markets *AM* and *ES* reported the highest relative abundance of bacterial community within the rainy season. In contrast, during the dry season, "rale" sold in the *GB*, *MK* and *MC* markets showed high prevalence of bacterial populations, complementing the results found with the plating method (Table 5).

Table 6. Molds (% of isolates) identified in "rale" samples collected in five rural markets during rainy and dry seasons.

		Rainy Season (November 2020)	Dry Season (August 2021)
		Mold Isolates	
Markets	*AM*	*Talaromyces amestolkiae* (100%)	*bdl*
	GB	*Fusarium petroliphilum* (100%)	*Talaromyces* sp. (100%)
	MK	*bdl*	*Pithomyces sacchari* (80%) *Aspergillus shendaweii* (20%)
	ES	*bdl*	*Pithomyces sacchari* (100%)
	MC	*Fusarium solani* (50%) *Rhizopus oryzae* (50%)	*Pithomyces sacchari* (25%) *Aspergillus calidoustus* (25%) *Trematosphaeria grisea* (25%) *Pithomyces chartarum* (25%)

Abbreviations: *bdl*—below the detection limit.

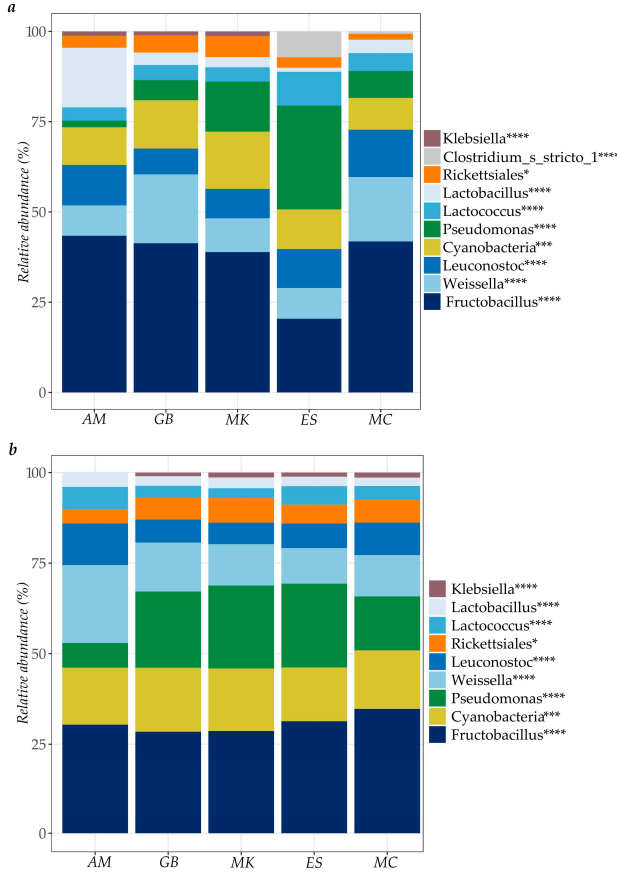

Figure 4. Relative abundance of bacteria at order *, class ***, and genus **** levels in "rale" samples ($n = 3$) collected in five different rural markets during (**a**) the rainy season (November 2020) and (**b**) the dry season (August 2021).

4. Discussion

4.1. Microbes as Indicators of Hygienic Quality Within Unit J

During the processing chain to produce cassava roasted flour ("rale"), the highest counts of molds and bacteria were found precisely after chopping. This can be explained by the high level of humidity that is found in cassava roots [50]. Cassava roots consist of, on average, 70% moisture, which requires prompt processing after harvesting to increase the shelf life of the cassava root products [22]. The rainy season is the most challenging season for Mozambican cassava farmers, as the processing and storage of cassava and its derivatives are more susceptible to mold contamination due to high temperatures and relative humidity ($\pm$ 29 °C $\pm$ 75% HR) at the locations of the associations included in the study. High TBCs were reported by [51] in "rale" samples and they correlated their findings with the contamination of samples by bacteria both from the cassava processors (when handling the flour) and from the environment.

S. aureus was isolated during the dry season at various stages of cassava processing (Table 1). According to [52], the presence of *S. aureus* in processed foods, or on food processing equipment, is generally an indication of inadequate sanitation or handling [52]. There are many records of severe food poisoning outbreaks caused by this microorganism. *S. aureus* can contaminate food processes when handling with bare hands; their presence in cassava samples might be related to direct contact or air-droplet mechanisms such as coughing or sneezing by "rale" processors. Foods contaminated by *S. aureus*, *Bacillus* spp., *Shigella* sp., and *Enterobacter* sp. have been connected to food infections and intoxication leading to different forms of diarrhea diseases among other complications, especially in young children, the elderly and the immunocompromised [51,53,54]. Fortunately, the production of "rale" includes heat treatment during roasting at approx. 110 °C, which is sufficient to eliminate these microbes (as well as natural microbiota such as LAB), though spores of *Bacillus* sp. may survive. In this context, it is noteworthy that *S. aureus* in certain samples from the dry season (Table 1) approaches levels of $>10^6$ cfu/g which indicate a risk for the production of a heat-stable toxin which would still be present in the roasted product [54,55].

Recontamination of samples after roasting may occur if ideal storage conditions are not put in place [51]. As the water content is reduced during roasting, this favors in particular the presence of molds (Table 1), which thrive at low water activity. Additionally, the roasting process in the rainy season samples took place outdoors using less advanced roasters, while for the subsequent dry season, a new roasting machine had been installed in *Unit J*. Rainy season samples were at greater risk for contamination by soil residues, the surrounding environment and sweat, as well as the lack of awareness of hygiene practices when carrying out this activity.

Various mold species were isolated from cassava processing samples (Table 2), including *Penicillium* and *Aspergillus* species, which have been previously reported from cassava derivatives in Benin [56] and Ghana [57]. Some of these molds may cause physical–chemical damage in the product and even potentially affect human and animal health, via the production of aflatoxins and other mycotoxins [58–61]. Fifteen samples of cassava flour in Brazil were investigated by [62] and this study concluded that 80% of the samples were contaminated by *A. niger*, *A. fumigatus* and *Penicillium* species. Contamination of cassava samples by *Penicillium* spp., *Aspergillus* spp., genera *Rhizopus* and *Cladosporium*, and yeasts were reported by [63] in Brazil. In our study, only low colony counts of potentially toxigenic molds such as *A. flavus* were detected in "rale" samples. This would seem to indicate that the risk for aflatoxin contamination is fairly low [64] in the "rale" produced and processed at *Unit J* using the current technology. Likewise, although citreoviridin may have hypothetically been produced by *P. citreonigrum*, the counts were low (Table 2) and

the occurrence of this toxin in foods is not regulated, despite its past role in causing *beriberi* from contaminated rice [65]. A pre-study we conducted in February 2020 in Gaza and Inhambane provinces showed similar trends regarding yeasts, molds, LAB, *Enterobacteriaceae*, TBC and *Bacillus* spp. in samples from the cassava processing chain, suggesting that the results from subsequent seasons (November 2020 and August 2021) were not unusual. *Penicillium* sp., *Aspergillus* sp., *Aureobasidium* sp. and *Cladosporium* sp. were also isolated in the pre-study.

4.2. Microbes as Indicators of Hygienic Quality in "Rale" Sampled from Cassava Processing Units

Regarding the study performed in the cassava processing units, our findings show concordance with the work carried out by [66,67] in Brazil, where they observed high records of mold in cassava flour in at least 67 to 75% of the samples. Studies carried out by [68] in Nigeria reported mold counts ranging from 3.55 to 5.99 log cfu/g, which represents higher contaminations of samples compared to the units of the current study (Table 3). The maximum allowable level for molds in cassava flour is 10^3 cfu/g or 3 log cfu/g [64], meaning that the counts observed in the "rale" processed in the majority of units meet the limits; this is probably assisted by the roasting process which reduces the initial mold load. However, *Units V* and *Z* were slightly over the limit. These units are smaller, processing less volumes than the other units, and therefore, the sampled "rale" was either not fresh or properly stored, contributing to the very high microbial counts.

B. cereus was isolated at low levels from a few units. This can be related to the occurrence of this bacteria in soil, and, from there, its contamination of cereals, tubers and vegetables [69]. *S. aureus* was reported in the most rudimentary unit (*Unit W*) that relies on very old machines and less advanced infrastructure to produce, process and store cassava derivatives. Consequently, our study strongly suggests that reducing contamination from handling is a challenge when using old machinery and infrastructure. The contamination of samples by both molds and *E. coli* reduces the conformity of the cassava product to microbial quality and safety regulations [64].

The microbes identified in the present study are aligned with those obtained by [68], in which they reported the presence of *Aspergillus* spp., *Penicillium* spp., *Fusarium*, *Alternaria* spp., *Cladosporium* sp. and *Rhizopus* sp. in cassava roasted flour samples. Most species listed in Table 2 are non-toxigenic, however, the list does include *A. flavus*, a producer of aflatoxin; *P. citrinum*, a producer of citrinin; *A. niger*, producer of ochratoxin A and fumonisins; and *F. oxysporum*, where a few strains produce fumonisin [65,70]. These toxins are among those whose occurrence in certain foods is regulated in the European Union [71]. Other species producing non-regulated mycotoxins include *P. citreonigrum* (citreoviridin, discussed in Section 4.1) and *A. alternata* (alternariols, tenuazonic acid) [65]. Despite the presence of these species, the risk for mycotoxin production is deemed to be low, because the mold counts were fairly low in all samples (<10^4 cfu/g or 4 log cfu/g), and in particular, *A. flavus* was present at <3 cfu/g or 0.5 log cfu/g. The toxigenic molds are only likely to pose a risk if the "rale" is stored for long periods with high humidity, which would permit mold growth and toxin production.

4.3. Microbes as Indicators of Hygienic Quality in "Rale" Collected in Rural Markets

In general, the market samples revealed lower counts and less diversity of microorganisms compared to the cassava processing unit samples. Furthermore, mycotoxigenic mold species were not observed in the market samples. The presence of molds, yeasts and *S. aureus* in certain market samples might be related with variations in personal hygiene and food safety consciousness, e.g., using clean containers with covers to store the "rale" that is sold in markets as a way to reduce the direct and indirect contaminations by air-borne

droplets and molds and yeast in dust, soil, and air [51]. High loads of bacteria, *Staphylococcus* spp. and coliforms were reported by [72] in dried chips and cassava flour samples in Kenya. These findings were correlated with excessive personnel handling and insufficient hygiene applied during post-harvest processing, handling, and marketing. The same study confirmed the occurrence of high mold counts in cassava flour because of storage practices of the products, meaning that it is very difficult to follow the hygiene-safety regulations outside the processing unit.

The "rale" sold in rural markets of Gaza and Inhambane is usually home-made or mainly produced in artisanal processing units that do not rely on proper processing equipment/technology. This tradition of processing cassava hypothetically contributes to fluctuating hygienic quality in the final product. The goal of standardizing small–scale processing products is a challenge. For instance, in their review, the authors [15] mention that the desirable attributes of cassava flour differ across ethnicities and regions, and emphasize that varying quality of the products among processors and even between batches from the same processor hinders commercialization of locally produced cassava products.

The risk for contamination of "rale" sold in rural markets by molds can be associated with lack of awareness and improper handling by the vendors. Open containers used by vendors to stock the cassava flour in the markets means that they are constantly exposed to air, which permits mold spores and other microbes to contaminate the product [51]. Proper training on good practices especially good hygiene, as well as equipping farmers, processors and retailers with more hygienic equipment and methods, could be a strategy to reduce microbial loads on the cassava products available in the market, leading to improved quality and safety [72,73].

4.4. Culture-Independent Analysis of Bacterial Community

LAB were the dominant microbes (present at >50%) found at all cassava processing stages and in the final "rale" product collected from all of the included processing units (Figures 2–4). These are the primary fermentation organisms during cassava wet-processing and therefore key members of cassava natural microbiota [74]. This is reflected in the relatively high abundance of amplicons from DNA of lactic acid bacteria during processing and in all subsequent samples taken thereafter. The sequencing method applied in this study does not distinguish between DNA from live and dead bacteria, meaning that some of the DNA could be carried over from bacteria present in earlier stages of processing but which are non-viable.

Enterobacteriaceae (*Klebsiella*, *E. coli*/*Shigella*) were detected at low relative abundance in many samples during processing (<2% relative abundance, Figure 2), in "rale" collected from six units (<15% relative abundance, Figure 3) and in "rale" from markets (<5% relative abundance, Figure 4). These genera are indicators of poor hygiene linked to fecal contamination of, for example, processing water or from handlers [75]. However, *Enterobacteriaceae* were not detected during plating of any samples (detection limit < 33 cfu/g or 1.52 log cfu/g). Despite some potential loss of viability among Gram-negative bacteria during freeze-storage of the samples, the overall picture is that this group was unlikely to be present at levels posing a health risk.

Pseudomonas, a commonly abundant environmental and spoilage bacteria [76], was found to be present at low relative abundance during processing (<20% relative abundance, Figure 2) and in "rale" collected from six processing units (<5% relative abundance, Figure 3), but it was increased in abundance in "rale" samples collected at rural markets (50–80% relative abundance, Figure 4). This increase is due to aerobic storage and handling of the "rale" prior to being sold at the markets.

Thus, the results obtained from the Illumina 16S rRNA gene sequencing are in accordance with the trends observed by the plating method, and can be considered a complementary technique to the plating methods applied in this and previous studies e.g., [51,64].

5. Conclusions

This study revealed differences in terms of the microbial contamination within the processing chain of cassava roots to cassava roasted flour produced at processing *Unit J* in the surveyed seasons. Lack of hygiene practices during the processing chain of cassava by the processors might lead to high levels of microbial contamination, compromising the final quality of the product and the food safety for the consumers. Hence, it is important to maintain high quality and safety process standards in place at *Unit J*.

High mold counts in cassava roasted flour indicates anomalies during storage of the cassava product "rale", at the production facilities and/or the markets. The low counts of microorganisms found in "rale" collected both in cassava processing units as well as in the rural markets suggests an acceptable quality of the product for human consumption. To acquire a better overview of microbial hygiene within the markets, frequent sampling would be necessary. However, our study gives indications that the hygiene is reasonable during both the dry and rainy seasons.

The results obtained within this study did not point towards any risks for aflatoxin contamination in cassava samples. The inclusion of more processing units that rely on rudimentary equipment for processing cassava would broaden our understanding of possible variations in microbiota during cassava processing. However, it is still important to maintain and reinforce basic hygiene and sanitary practices to improve quality and safety of the cassava derivatives in southern Mozambique.

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References

1. Adjovi, Y.C.S.; Gnonlonfin, B.J.G.; Bailly, S.; Bailly, J.D.; Tadrist, S.; Puel, O.; Oswald, I.P.; Sanni, A. Occurrence of Mycotoxins in Cassava (Manihot Esculenta Crantz) and Its Products. *Int. J. Food Saf. Nutr. Public Health* **2015**, *5*, 217. [CrossRef]
2. Adebayo, W.G. Cassava Production in Africa: A Panel Analysis of the Drivers and Trends. *Heliyon* **2023**, *9*, e19939. [CrossRef] [PubMed]
3. Guira, F.; Some, K.; Kabore, D.; Sawadogo-Lingani, H.; Traore, Y.; Savadogo, A. Origins, Production, and Utilization of Cassava in Burkina Faso, a Contribution of a Neglected Crop to Household Food Security. *Food Sci. Nutr.* **2017**, *5*, 415–423. [CrossRef] [PubMed]
4. Cock, J.H.; Connor, D.J. Cassava. In *Crop Physiology Case Histories for Major Crops*; Elsevier: London, UK, 2021; pp. 588–633.
5. Ikuemonisan, E.; Mafimisebi, T.; Ajibefun, I.; Adenegan, K. Cassava production in Nigeria: Trends, instability and decomposi-tion analysis (1970–2018). *Heliyon* **2020**, *6*, e05089. [CrossRef]
6. Srivastava, A.K.; Ewert, F.; Akinwumiju, A.S.; Zeng, W.; Ceglar, A.; Ezui, K.S.; Adelodun, A.; Adebayo, A.; Sobamowo, J.; Singh, M.; et al. Cassava yield gap—A model-based assessment in Nigeria. *Front. Sust. Food Syst.* **2023**, *6*, 10587. [CrossRef]
7. McSween, S.; Walker, T.S.; Salegua, V.A.; Pitoro, R. *Economic Impact on Food Security of Varietal Tolerance to Cassava Brown Streak Disease in Coastal Mozambique*; Food Security Collaborative Working Papers 55863; Department of Agricultural, Food, and Resource Economics: East Lansing, MI, USA, 2006.
8. Tivana, L.D. Cassava Processing: Safety and Protein Fortification. Doctoral Thesis, Lund University, Lund, Sweden, 2012.
9. Morgan, N.K.; Choct, M. Cassava: Nutrient Composition and Nutritive Value in Poultry Diets. *Anim. Nutr.* **2016**, *2*, 253–261. [CrossRef]
10. Amelework, A.B.; Bairu, M.W.; Maema, O.; Venter, S.L.; Laing, M. Adoption and Promotion of Resilient Crops for Climate Risk Mitigation and Import Substitution: A Case Analysis of Cassava for South African Agriculture. *Front. Sustain. Food Syst.* **2021**, *5*, 617783. [CrossRef]
11. Manganyi, B.; Lubinga, M.H.; Zondo, B.; Tempia, N. Factors Influencing Cassava Sales and Income Generation among Cassava Producers in South Africa. *Sustainability* **2023**, *15*, 14366. [CrossRef]
12. FAO; IFAD—Food and Agriculture Organization of The United Nations and International Fund For Agricultural Development. A Review of Cassava in Africa with Country Case Studies on Nigeria, Ghana, the United Republic of Tanzania, Uganda and Benin. *Proc. Valid. Forum Glob. Cassava Dev. Strategy* **2005**, *2*, 66.
13. Salvador, E.M.; Steenkamp, V.; McCrindle, C.M.E. Production, Consumption and Nutritional Value of Cassava (Manihot Esculenta, Crantz) in Mozambique: An Overview. *J. Agric. Biotech. Sustain. Dev.* **2014**, *6*, 29–38. [CrossRef]
14. Frediansyah, A. Microbial Fermentation as Means of Improving Cassava Production in Indonesia. In *Cassava*; IntechOpen: London, UK, 2017.
15. Udoro, E.O.; Anyasi, T.A.; Jideani, A.I.O. Process-Induced Modifications on Quality Attributes of Cassava (Manihot Esculenta Crantz) Flour. *Processes* **2021**, *9*, 1891. [CrossRef]
16. Montagnac, J.A.; Davis, C.R.; Tanumihardjo, S.A. Nutritional Value of Cassava for Use as a Staple Food and Recent Advances for Improvement. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 181–194. [CrossRef]
17. Tsige, T.; Basa, B.; Herago, T. Medicinal, Nutritional and Anti-Nutritional Properties of Cassava (Manihot esculenta): A Review. *Acad. J. Nutr.* **2019**, *8*, 34–46.
18. Cardoso, A.P.; Mirione, E.; Ernesto, M.; Massaza, F.; Cliff, J.; Rezaul Haque, M.; Bradbury, J.H. Processing of Cassava Roots to Remove Cyanogens. *J. Food Compos. Anal.* **2005**, *18*, 451–460. [CrossRef]
19. Chiona, M.; Ntawuruhunga, P.; Benesi, I.R.M.; Matumba, L.; Moyo, C.C. Aflatoxins Contamination in Processed Cassava in Malawi and Zambia. *Afr. J. Food Agric. Nutr. Dev.* **2014**, *14*, 8809–8820. [CrossRef]
20. Muzanila, Y.C.; Brennan, J.G.; King, R.D. Residual Cyanogens, Chemical Composition and Aflatoxins in Cassava Flour from Tanzanian Villages. *Food Chem.* **2000**, *70*, 45–49. [CrossRef]
21. Kolawole, O.M.; Adeyemi, B.J.; Kayode, R.M.O.; Ajibola, T.B. The drying effect of colour light frequencies on the nutrient and microbial composition of cassava. *Afr. J. Agric. Res.* **2009**, *4*, 171–177.
22. Jackson, J.; Chiwona-Karltun, L.; Gordon, A. Food Safety and Quality Considerations for Cassava, a Major Staple Containing a Natural Toxicant. In *Food Safety and Quality Systems in Developing Countries*; Elsevier: London, UK, 2020; pp. 343–366.
23. Guia Viagem. Mozambique Climate. Available online: https://www.guiaviagem.org/mocambique-clima/#google_vignette (accessed on 23 August 2023).
24. King Jr, A.D.; Hocking, A.D.; Pitt, J.I. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. *Appl. Environ. Microbiol.* **1979**, *37*, 959–964. [CrossRef]
25. Beuchat, L.R. Media for detecting and enumerating yeasts and moulds. *Int. J. Food Microbiol.* **1992**, *17*, 145–158. [CrossRef]
26. De Man, J.D.; Rogosa, D.; Sharpe, M.E. A medium for the cultivation of lactobacilli. *J. Appl. Microbiol.* **1960**, *23*, 130–135. [CrossRef]
27. Schillinger, U.; Holzapfel, W. Chapter 8. Culture Media for Lactic Acid Bacteria. In *Progress in Handbook of Culture Media For Food and Water Microbiology*; Royal Society of Chemistry: London, UK, 2011.

28. Yarrow, D. Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts*; Elsevier: Amsterdam, The Netherlands, 1998; pp. 77–100.
29. Mercuri, A.J.; Cox, N.A. Coliforms and Enterobacteriaceae isolates from selected foods. *J. Food Prot.* **1979**, *42*, 712–714. [CrossRef] [PubMed]
30. Roth, L.A.; Stiles, M.E.; Clegg LF, L. Reliability of selective media for the enumeration and estimation of Escherichia coli. *Can. Inst. Food Sci. Technol. J.* **1973**, *6*, 230–234. [CrossRef]
31. Coulin, P.; Farah, Z.; Assanvo, J.; Spillmann, H.; Puhan, Z. Characterisation of the microflora of attiéké, a fermented cassava product, during traditional small-scale preparation. *Int. J. Food Microbiol.* **2006**, *106*, 131–136. [CrossRef] [PubMed]
32. Mossel DA, A.; Koopman, M.J.; Jongerius, E. Enumeration of Bacillus cereus in foods. *Appl. Microbiol.* **1967**, *15*, 650–653. [CrossRef]
33. Kim, H.U.; Goepfert, J.M. Enumeration and Identification of Bacillus cereus in Foods: I. 24-Hour Presumptive Test Medium. *Appl. Microbiol.* **1971**, *22*, 581–587. [CrossRef]
34. Zangerl, P.; Asperger, H. Media used in the detection and enumeration of Staphylococcus aureus. *Prog. Ind. Microbiol.* **2003**, *37*, 91–110.
35. Baird-Parker, A.C. The performance of an egg yolk-tellurite medium in practical use. *J. Appl. Bacteriol.* **1962**, *25*, 441–444. [CrossRef]
36. Wang, R.-F.; Cao, W.-W.; Cerniglia, C.E. A Universal Protocol for PCR Detection of 13 Species of Foodborne Pathogens in Foods. *J. Appl. Microbiol.* **1997**, *83*, 727–736. [CrossRef]
37. Leong, S.L. Microbial Populations during Maize Storage in Cameroon. *Afr. J. Biotechnol.* **2012**, *11*, 8692–8697.
38. Kurtzman, C.P.; Robnett, C.J. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Leeuwenhoek* **1998**, *73*, 331–371. [CrossRef]
39. Cenis, J.L. Rapid Extraction of Fungal DNA for PCR Amplification. *Nucl. Acids Res.* **1992**, *20*, 2380. [CrossRef] [PubMed]
40. O'Donnell, K.; Kistler, H.C.; Cigelnik, E.; Ploetz, R.C. Multiple Evolutionary Origins of the Fungus Causing Panama Disease of Banana: Concordant Evidence from Nuclear and Mitochondrial Gene Genealogies. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2044–2049. [CrossRef] [PubMed]
41. Glass, N.L.; Donaldson, G.C. Development of Primer Sets Designed for Use with the PCR to Amplify Conserved Genes from Filamentous Ascomycetes. *Appl Environ Microbiol* **1995**, *61*, 1323–1330. [CrossRef] [PubMed]
42. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Elsevier: Amsterdam, The Netherlands, 1990; pp. 315–322.
43. Hugerth, L.W.; Wefer, H.A.; Lundin, S.; Jakobsson, H.E.; Lindberg, M.; Rodin, S.; Engstrand, L.; Andersson, A.F. DegePrime, a Program for Degenerate Primer Design for Broad-Taxonomic-Range PCR in Microbial Ecology Studies. *Appl. Environ. Microbiol.* **2014**, *80*, 5116–5123. [CrossRef]
44. Cunningham, J.L.; Bramstång, L.; Singh, A.; Jayarathna, S.; Rasmusson, A.J.; Moazzami, A.; Müller, B. Impact of Time and Temperature on Gut Microbiota and SCFA Composition in Stool Samples. *PLoS ONE* **2020**, *15*, e0236944. [CrossRef]
45. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2—Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **2010**, *5*, e9490. [CrossRef]
46. Environment for R. Posit Team RStudio: Integrated Development Environment for R. Posit Software, 2024, PBC, Boston, MA. Available online: http://www.posit.co/ (accessed on 15 December 2024).
47. Ryu, C. *R Package, Version 0.6.3, dlookr: Tools for Data Diagnosis, Exploration, Transformation*; R Foundation for Statistical Computing: Vienna, Austria, 2024.
48. Sjoberg, D.D.; Whiting, K.; Curry, M.; Lavery, J.A.; Larmarange, J. Reproducible summary tables with the gtsummary package. *R J.* **2021**, *13*, 570–580. [CrossRef]
49. Kassambara, A. *CRAN: Contributed Packages, Version 0.7.2*; Rstatix: Pipe-Friendly Framework for Basic Statistical Tests; R Foundation for Statistical Computing: Vienna, Austria, 2023.
50. Dósea, R.R.; Marcellini, P.S.; Santos, A.A.; Ramos, A.L.D.; Lima, Á.S. Qualidade microbiológica na obtenção de farinha e fécula de mandioca em unidades tradicionais e modelo. *Cienc. Rural* **2009**, *40*, 411–416. [CrossRef]
51. Okolo, E.; Makanjuola, A.T. Microbial Evaluation of Garri Sold Within Ahmadu Bello University Main Campus, Samaru—Zaria, Kaduna. *Sci. World J.* **2021**, *16*, 259–265.
52. Kadariya, J.; Smith, T.C.; Thapaliya, D. *Staphylococcus Aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. *Biomed. Res. Int.* **2014**, *2024*, 827965.
53. Bintsis, T. Foodborne Pathogens. *AIMS Microbiol.* **2017**, *3*, 529–563. [CrossRef] [PubMed]
54. Prescott, L.M.; Harley, J.P.; Klein, D.A. *Microbiology: Food and Industrial Microbiology*, 5th ed.; McGraw-Hill: Boston, MA, USA, 2002; pp. 978–981.
55. Adams, M.; Moss, M.O.; McClure, P. *Food Microbiology*; Royal Society of Chemistry: London, UK, 2015.

56. Gnonlonfin, G. Mycoflora and Natural Occurrence of Aflatoxins and Fumonisin B1 in Cassava and Yam Chips from Benin, West Africa. *Int. J. Food Microbiol.* **2008**, *122*, 140–147. [CrossRef] [PubMed]
57. Wareing, P.W.; Westby, A.; Gibbs, J.A.; Allotey, L.T.; Halm, M. Consumer Preferences and Fungal and Mycotoxin Contamination of Dried Cassava Products from Ghana. *Int. J. Food Sci. Technol.* **2001**, *36*, 1–10. [CrossRef]
58. Ono, L.T.; Taniwaki, M.H. Fungi and Mycotoxins in Cassava (Manihot Esculenta Crantz) and Its Products. *Braz. J. Food Technol.* **2021**, *24*, e2020240. [CrossRef]
59. Okoth, S.; De Boevre, M.; Vidal, A.; Diana Di Mavungu, J.; Landschoot, S.; Kyallo, M.; Njuguna, J.; Harvey, J.; De Saeger, S. Genetic and Toxigenic Variability within Aspergillus Flavus Population Isolated from Maize in Two Diverse Environments in Kenya. *Front. Microbiol.* **2018**, *9*, 57. [CrossRef]
60. Frisvad, J.C.; Hubka, V.; Ezekiel, C.N.; Hong, S.-B.; Nováková, A.; Chen, A.J.; Arzanlou, M.; Larsen, T.O.; Sklenář, F.; Mahakarnchanakul, W.; et al. Taxonomy of *Aspergillus* Section *Flavi* and Their Production of Aflatoxins, Ochratoxins and Other Mycotoxins. *Stud. Mycol.* **2019**, *93*, 1–63. [CrossRef]
61. Benkerroum, N. Aflatoxins: Producing-Molds, Structure, Health Issues and Incidence in Southeast Asian and Sub-Saharan African Countries. *Int. J. Food Saf. Nutr. Public Health* **2020**, *17*, 1215. [CrossRef]
62. Mesquita, J.S.; Araújo, S.K.; Pereira, F.C.S. Análise micológica de farinha de mandioca vendida nas feiras do produtor na cidade de Macapá-AP. *Rev. Ciência Soc.* **2017**, *1*, 103–112.
63. Gomes, L.P.; Silva LJ, G.; Fernandes GS, T. Identificação dos principais gêneros fúngicos nas farinhas de mandioca comercializadas nos principais mercados de Manaus. *Rev. Igapó* **2007**, *1*, 60–64.
64. Abass, A.B.; Adegoke, G.O.; Awoyale, W.; Gaspar, A.; Mlingi, N.; Andrianavalona, V.; Randrianarivelo, R.; Sulyok, M.; Mneney, A.; Ranaivoson, L.R. Enumeration of the Microbiota and Microbial Metabolites in Processed Cassava Products from Madagascar and Tanzania. *Food Control* **2019**, *99*, 164–170. [CrossRef]
65. Pitt, J.I.; Hocking, A.D. *Fungi and Food Spoilage*, 4th ed.; Springer: Cham, Switzerland, 2022.
66. Rodrigues, E.B.; Araújo, A.M.; Sobral FO, S.; Romão, N.F. Avaliação da Presença de Bolores e Leveduras em Farinha de Mandioca (*Manihot esculenta* Crantz) Comercializadas a Granel em Feiras Livres do Município de Ji-Paraná-Ro. *S. Am. J. Basic Educ. Tech. Technol.* **2015**, *2*, 1–8.
67. Pontes, C.G.C. Identificação de Fungos Contaminantes em Farinha de Mandioca (Manihot esculenta Crantz). Bachelor's Thesis, Universidade Estadual da Paraíba, Campina Grande, Brazil, 2012.
68. Aguoru, C.U.; Onda, O.; Omoni, V.T.; Ogbonna, I.O. Characterization of Molds Associated with Processed Garri Stored for 40 Days at Ambient Temperature in Makurdi, Nigeria. *Afr. J. Biotechnol.* **2014**, *13*, 673–677. [CrossRef]
69. Ghelardi, E.; Celandroni, F.; Salvetti, S.; Barsotti, C.; Baggiani, A.; Senesi, S. Identification and Characterization of Toxigenic *Bacillus Cereus* Isolates Responsible for Two Food-Poisoning Outbreaks. *FEMS Microbiol. Lett.* **2002**, *208*, 129–134. [CrossRef] [PubMed]
70. Marc, A.R. Implications of Mycotoxins in Food Safety. In *Mycotoxins and Food Safety—Recent Advances*; IntechOpen: London, UK, 2022; pp. 903–907.
71. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance). *Off. J.* **2023**, *L 119*, 103–157. Available online: http://data.europa.eu/eli/reg/2023/915/oj (accessed on 18 June 2024).
72. Gacheru, P.; Abong, G.; Okoth, M.; Lamuka, P.; Shibairo, S.; Katama, C. Microbiological Safety and Quality of Dried Cassava Chips and Flour Sold in the Nairobi and Coastal Regions of Kenya. *Afr. Crop Sci. J.* **2016**, *24*, 137. [CrossRef]
73. Chiwona-Karltun, L.; Brimer, L.; Jackson, J. Improving Safety of Cassava Products. In *Root Tuber and Banana Food System Innovations*; Thiele, G., Friedmann, M., Campos, H., Polar, V., Bentley, J.W., Eds.; Springer: Cham, Switzerland, 2022; pp. 241–258.
74. Food and Agriculture Organization of The United Nations (FAO) and World Health Organization (WHO). *Codex Standards 176-1989*; FAO/WHO Codex Standards for Edible Cassava Flour. Codex Alimentarius Commission; FAO: Rome, Italy; WHO: Geneva, Switzerland, 1995.
75. Ghougal, K.; Moreno Roldán, E.; Espigares Rodríguez, E. Risk factors related to bacterial contamination by Enterobacteriaceae and fecal coliforms and the prevalence of *Salmonella* spp. in Algerian farms, slaughterhouses and butcheries: A two-year follow-up study. *AIMS* **2021**, *6*, 768–785. [CrossRef]
76. Raposo, A.; Pérez, E.; de Faria, C.T.; Ferrús, M.A.; Carrascosa, C. Food spoilage by *Pseudomonas* spp.—An overview. In *Foodborne Pathogens and Antibiotic Resistance*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2017; pp. 41–71.

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Cassava is vital for food security in Mozambique, yet its processing generates large volumes of underutilised residues. This thesis examines how cassava systems can simultaneously support safe food production and sustainable bioprocessing. By applying microbial fermentation, cassava residues were converted into ethanol and microbial lipids, highlighting opportunities for a circular bioeconomy that links food safety with resource valorisation.

Andreia Massamby received her graduate education at the Department of Molecular Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. She holds an M.Sc. in Crop Protection and a B.Sc. in Agronomic Engineering from Eduardo Mondlane University (UEM), Maputo, Mozambique.

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