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Nutritional Characterization of the fruit *Strychnos madagascariensis* and their products: flour and oil

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Dissertation Thesis

Nutritional Characterization of the fruit *Strychnos madagascariensis* and their products: flour and oil

Dissertation presented to Faculty of Nutrition and Food Science to obtain the degree of Doctor of Philosophy (PhD) in Food Consumption and Nutrition Sciences

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Personal Contribution to the Research Papers Presented in this Dissertation

The candidate declares that has made a decisive contribution to the realization of the experimental work, from the programming to the execution of the experimental work. The candidate contributed in the interpretation and discussion of the results presented in all the articles of this dissertation, as well as in their writing.

However, obtained collaboration of several researchers from different institutions.

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RESUMO

As frutas nativas de África têm recebido atenção por parte da comunidade científica, por poderem ter um contributo significativo na alimentação da população dos países em desenvolvimento, diversificando as dietas e suprindo deficiências de micronutrientes. Além disso, podem ser valorizadas pela indústria agroalimentar, tornando-se posteriormente uma potencial fonte de rendimento para as comunidades rurais. Em Moçambique, tal como noutros países da África subsariana, vive-se a insegurança alimentar, havendo vários indicadores de desnutrição. Nesta região, existem várias frutíferas subvalorizadas com potencial para aliviar essas deficiências. Embora exista um crescente interesse no género *Strychnos*, conhecido pelas frutas comestíveis e pela tolerância à seca, a fruta da *S. madagascariensis*, consumida em tempos de escassez de alimentos básicos e utilizada como medicina popular, tem recebido pouca atenção.

Para promover e expandir a utilização desta fruta ("macuácua"), bem como de seus produtos (a farinha "*nfuma*" e o seu óleo), o conhecimento da sua composição nutricional é essencial para determinar seu verdadeiro valor e assim suportar decisões mais racionais sobre sua utilização sustentável e valorização. Assim, os objetivos específicos desta tese foram: (i) Caracterizar a polpa da *S. madagascariensis in natura* quanto às propriedades físico-químicas e nutricionais, explorando potenciais diferenças devido à origem geográfica; (ii) Conhecer em detalhe, a composição nutricional da *nfuma* produzida de forma tradicional, tradicional, avaliando a sua adequação nutricional; (iii) Determinar a composição do óleo extraído da *nfuma* através de prensagem, avaliando seu potencial nutricional e tecnológico; (iv) Elaborar recomendações com as melhores práticas na elaboração dos produtos derivados da macuácua, do ponto de vista nutricional, de segurança e tecnológico. Para concretizar estes objetivos, os frutos foram colhidos em 4 distritos do sul de Moçambique (Chicualacuala, Chókwè, Manhiça, Marracuene); as farinhas foram produzidas localmente; os óleos extraídos em laboratório e as análises realizadas através de métodos AOAC ou métodos previamente validados.

As frutas da *S. madagascariensis*, apresentam cor alaranjada e pH ligeiramente ácido. A fruta apresentou de 61,2 a 77,6% de humidade, 15,6 a 23,0% de hidratos de carbono, > 1,5% de proteína e 4,0 a 12,5% de lipídios. De destacar o elevado teor de carotenos (5,8 a 11,4 mg/100g), a partir do quais se prevê, através do consumo de 50 g de polpa, 100%

do valor diário de referência (DRV) de vitamina A para crianças (1 aos 6 anos). Esta fruta também contém quantidades consideráveis de vitamina E (3,3 a 8,4 mg/100g) e uma atividade antioxidante moderada (41,9 - 47,5 mg TE/100g de polpa, através do método DPPH). A composição mineral revela o K (> 500 mg/100 g) como o principal macromineral seguido do Mg > Ca > Na. O elemento traço principal foi o (Mn > 500 µg/100 g). Foi possível verificar que a origem da fruta influenciou a composição nutricional e antioxidante. A polpa das frutas de Chókwè apresentou maior capacidade antioxidante, gordura, proteína e carotenos, enquanto a polpa das frutas de Chicualacuala apresentou maior teor de minerais e vitamina E.

A farinha destacou-se pelo alto teor de gordura (26,3–27,8%), fibra (> 6%), vitamina E (6,7 a 8,0 mg/100 g) e carotenos (2,2 a 2,6 mg/100 g). Os principais aminoácidos da foram a Arg, Asp e Glu, sendo a Lys o limitante. Assumindo o consumo diário de 50 g de *nfuma* pelas crianças, 82% das recomendações de vitamina A são atingidas, enquanto o consumo de 100 g pelos adultos, contribui para 132% e 60% das recomendações de Mn e vitamina A, respetivamente. Assim, o consumo de *nfuma* pelas crianças de Moçambique pode contribuir para aliviar a deficiência de vitamina. Considerando as referências de ingestão diária para os elementos essenciais, a *nfuma* (100 g) contribui significativamente para a ingestão de Mg, K e Mn (22 - 26%, 40% e > 100% das DRV, respetivamente). Apesar das vantagens nutricionais desta farinha, esta pode ser fonte de Ni, destacando a importância do estudo de boas práticas para a sua elaboração.

O óleo da *S. madagascariensis* revelou ser rico em ácido oleico (62-63%), seguido de ácido palmítico (20%) e ácido linoleico (7%). Quantidades consideráveis de tocoferóis (25 - 34 mg/100g), carotenos (8 - 10 mg/100g), bem como esteróis (~430 mg/100g) e álcoois triterpénicos (~190 mg/ 100g) foram observadas. No entanto, seu alto teor de ácidos gordos livres (22 - 25%) revela uma intensa hidrólise enzimática durante a secagem, cuja extensão poderá ser reduzida com a otimização de processo tecnológico.

Esta tese fornece o reconhecimento científico essencial para fundamentar algumas crenças populares atribuídas à *S. madagascariensis*, revelando os perfis químicos e nutricionais da fruta e dos seus produtos; e indicando o seu potencial para a saúde e tecnológico, sugerindo medidas para o melhoramento e maior valorização.

Palavras-chave: Frutas nativas de África, *S. madagascariensis*, *nfuma*, óleo comestível, óleo monoinsaturado, compostos bioativos.

ABSTRACT

African native fruits have been receiving considerable attention from the scientific community as they can be important contributors to the diet of people in developing countries, diversifying diets, and addressing micronutrient deficiencies. Moreover, they can be exploited by the agro-industry and become a source of income in poor rural communities in the future. Mozambique, such as other Sub-Saharan countries, experiences food insecurity and the multiple burden of malnutrition. In this region, there are several underutilized fruit species with the potential to alleviate these deficiencies. Although a rising interest has developed around the genus *Strychnos* (monkey orange), recognized by its edible fruits and drought tolerance, the fruit of *Strychnos madagascariensis*, consumed as a food alternative in times of scarcity of basic foods, and as a multipurpose folk medicine, has received little attention.

In order to promote and expand the utilization of this fruit ("macuácua"), as well as their food products (*nfuma* flour and its oil), knowledge about their nutritional composition is essential to determine their true value and support more rational decisions about its sustainable utilization and valorization. Therefore, the specific objectives of this thesis were: (i) To characterize the pulp of *S. madagascariensis* regarding its physicochemical and nutritional properties, exploiting potential differences of the fruits from different geographical locations of the south of Mozambique; (ii) To know in depth the nutritional composition of *nfuma* produced according to traditional procedures, estimating its nutrients adequacy. (iii) To determine the compositional basis of the oils physically extracted from *nfuma* while evaluating its nutritional and technological potential. (iv) To elaborate recommendations regarding the best practices in the production of macuácua products from a nutritional, safety, and technological approach.

To achieve these objectives, the fruits were collected in 4 districts of southern Mozambique (Chicualacuala, Chókwè, Manhiça, Marracuene); *nfuma* flours were locally produced; oils were physically extracted in laboratory, and analysis were carried out applying AOAC methods or previously validated methodologies.

The fruits of *S. madagascariensis* are yellow-orange and acid-neutral pH, with 61.2 - 77.6% of moisture, 15.6-23.0% of carbohydrates, less than 1.5% protein and 4.0 - 12.5% lipids. The macuácua fruit reveals outstanding amounts of carotenes (5.8 to 11.4 mg/100 g), and the consumption of 50 g of the fruits provides at least 100% of toddler's vitamin A dietary reference value (DRV). This fruit carries considerable amounts of vitamin E (3.3 to 8.4 mg/100 g) and an overall moderate antioxidant activity (41.9 - 47.5 mg TE/100g of pulp by DPPH method). The mineral composition reveals K (> 500 mg/100 g) as the main macromineral followed by Mg > Ca > Na. The main trace element was Mn (> 500 µg/100 g). It was observed that the origin influences the nutritional and antioxidant aspects of the fruits. The pulp from Chókwè displayed the higher antioxidant content, as well as fat, protein, and carotenes, while the sample from Chicualacuala had the highest mineral and vitamin E content.

Nfuma stands out for its high content of fat (26.3–27.8%), fiber (> 6%), vitamin E (6.7 to 8.0 mg/100 g) and carotenes (2.2 to 2.6 mg/100 g). The main amino acids of *nfuma* protein were Arg, Asp and Glu, and Lys was the limiting one. Assuming the daily consumption of 50 g, *nfuma* provides 82% of Vitamin A DRV for toddlers, while the consumption of 100 g contributes to 132% and 60% of Mn and vitamin A DRV for adults, respectively. Thus, the consumption of *nfuma* by children may contribute to alleviate vitamin A deficiency due to its high β-carotene. Considering the EDI of essential elements, *nfuma* (100 g) contributes significantly to the daily intake of Mg, K, and Mn (22 - 26%, 40% and > 100% of DRVs, respectively). Despite the nutritional advantages, this flour can be a source of Ni, highlighting the importance of studying good practices in its preparation.

The oil of *S. madagascariensis* is characterized by a high content of oleic acid (62-63%), followed by palmitic acid (20%), linoleic acid (7%). Analysis of liposoluble vitamins reveals the presence of considerable amounts of tocopherols (25-34 mg/100g), carotenoids (8 - 10 mg/100g), as well as sterols (~430 mg/100g) and triterpenic alcohols (~190 mg/100g). However, its high content of free fatty acids (22-25%) reveals an intense enzymatic hydrolysis during the drying step, whose extent can potentially be reduced with the optimization of the technological process.

At the end, this thesis provides scientific recognition to support some of *S. madagascariensis* folklore uses, revealing the chemical and nutritional profiles of the fruit and its products, pointing health and technological potential that deserves to be technologically approached for wider valorization.

Keyword: African native fruits, *Strychnos madagascariensis*, *nfuma*, edible oil, monounsaturated oil, bioactive compounds

GENERAL SCOPE AND THESIS OUTLINE

General Scope

Food security is a known problem of Mozambique, especially in the rural areas, where native fruits grow wildly. Thus, it is considered important to explore the potentialities of the different parts of the fruits, in order to further encourage rural development with domestication of its plants, therefore creating conditions for the sustainable production of these fruits. Agricultural systems are considered sustainable as long as the production is maintained at current levels. A dynamic concept is more appropriate and responds to both evolution and development of the society. In this way, the goal of sustainable agriculture must assure efficient management of available resources, where production is kept at a level that is necessary to meet the growing aspirations of an equally growing population, without degrading the environment. Considering the nutritional characteristics of a native fruit, as well as the development of sustainable agriculture in Mozambique, a door will be opened for the development of minimal processing of S*trychnos madagascariensis* fruit, its dried pulp flour *nfuma* and its oil, not yet popular and undervalued.

Agriculture is significant for the Mozambican economy, representing 25% of the Mozambican Gross domestic product. and plays a crucial role as a source of food to most of the population, however, food insecurity is at a high level (Fenita and Abbas, 2017). Nearly 70% of Mozambicans reside in rural areas and are agriculture-dependent for subsistence, with nearly half of the country's population suffering from absolute poverty. Several fruits of southern Africa have been studied and in a general way they present an exceptional potential due to their abundance, nutritional value, and palatability. Accordingly, the knowledge of the nutritional value of the fruit of *Strychnos madagascariensis* could also contribute to the reduction of food insecurity. The government, through the Ministry of Agriculture and Food Security, can develop policies to domesticate their plants in a sustainable and organized way, reflecting on health and economic benefits for communities.

Strychnos madagascariensis fruit can be immediately consumed in natura but is usually processed as a flour by the local communities to increase the stability and shelf life of the fruit and to be mainly consumed when there is basic food shortage. Several health claims are assigned to this fruit, used in folklore medicine, however, there is still a paucity of scientific evidence on nutritional and phytochemical properties.

Considering the above-mentioned facts, increase the interest and the need in the study of the nutritional value and potential applications of the fruit of *Strychnos madagascariensis* and its products. The results provided by this thesis will contribute to the scientific knowledge on this area, improvement of food consumption habits and nutrition of the population of Mozambique through the nutritional adequacy of these foods, as well as the manufacture good practices recommendations. At the end, the dissemination of these findings will contribute to improve the nutrition and economic status of communities.

Thesis outline

This dissertation is organized in chapters that include the Introduction, Material and Methods, the chapters with main results and an overall discussion and conclusion at the end, (Figure 1) as follows:

Chapter 1 - Introduction

In this chapter, the contextualization, the literature review and the objectives of the thesis are presented.

Chapter 2 - Materials and methods

The main procedures for sampling from fruits to flours and oils and a brief presentation of the methods of analyses are described in this chapter.

Chapter 3 - Nutritional and antioxidant activity of *Strychnos magacascariensis* from the southern districts of Mozambique

In this chapter, the fruit was characterized in terms of physical-chemical parameters, minerals and antioxidant activities.

Chapter 4 - Characterization of *Strychnos madagascariensis* fruit flour produced by local Mozambican communities and its nutritional adequacy

In this section the macro and micronutrients of *nfuma* are presented, as well as the evaluation of nutritional adequacy.

Chapter 5 - Chemical characterization of the oil separated by mechanical pressing from *Strychnos madagascariensis* dried fruit pulp flour

Characterization of oil extracted from the flour of *S. madagascariensis* and evaluation of its nutritional and technological potential are presented in this chapter.

Chapter 6 – Discussion and Conclusions

Overall discussion of the knowledge acquired during this work is depicted. Conclusions and further research directions are also presented in this chapter.

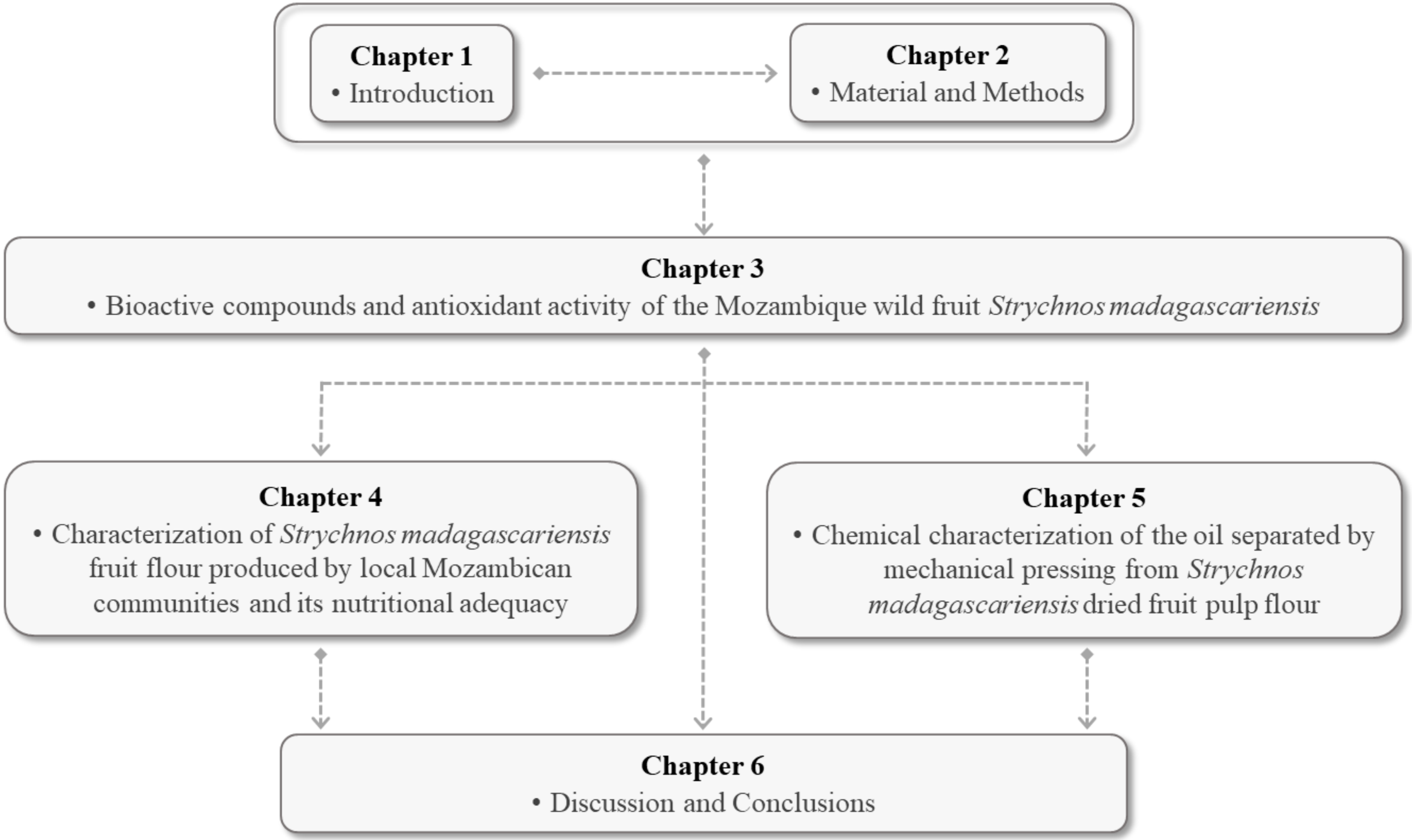

Figure 1: Thesis Outline

LIST OF PUBLICATIONS AND COMMUNICATIONS

Peer-reviewed articles

1. **Sandra S. I. Chemane**, Catarina Vila-Real, Edgar Pinto, Eulalia Mendes, Susana Casal, Maida Khan, Ana M. P. Gomes, Olivia Pinho and Olga Viegas. **Nutritional and antioxidant activity of *Strychnos magacascariensis* from the southern districts of Mozambique** (submitted);

2. **Sandra S. I. Chemane**, Susana Casal, Eulalia Mendes, Edgar Pinto, Maida Khan, Olívia Pinho, and Olga Viegas. **Characterization of *Strychnos madagascariensis* fruit flour produced by local Mozambican communities and its nutritional adequacy** (submitted);

3. **Sandra S. I. Chemane**, Susana Casal, Rebeca Cruz, Isabel M.P.L.V.O. Ferreira, Maida Khan, Olívia Pinho, and Olga Viegas. **Chemical characterization of the oil separated by mechanical pressing from *Strychnos madagascariensis* dried fruit pulp flour** (submitted).

Communications in scientific meetings

Oral presentation

1. **Chemane, S**., Casal, S., Pinho, T., Khan, M., Pinho, O., Viegas, O. **Characterization of the oil extracted from the *Strychnos madagascariensis***. XVIII Congresso de Nutrição e Alimentação da Associação Portuguesa de Nutrição (APN) 2019. Porto, 16 -17 May 2019.

Poster presentation

1. **Chemane, S**., Vila-Real, C., Mendes, E., Casal, S., Khan, M., Gomes, A., Pinho, O., Viegas, O. **Nutritional value, bioactive compounds, and antioxidant activity characterization of *Strychnos madagascariensis* fruits from Mozam-**

bique. XIV Congresso de Nutrição e Alimentação da Associação Portuguesa de Nutrição, Portugal, 9 - 10 September, 2020.

2. **Chemane, S**., Mendes, E., Casal, S., Khan, M., Pinho, O., Viegas, O. **Nutritional Value of *Strychnos madagascariensis* Fruit and Flour.** 3rd Global Summit on Nutritional Science & Food Chemistry Scientific Federation Abode for Researches/ 150th Conference Scientific Federation, Ozaka, Japan, 10-12 June, 2019.

3. **Chemane, S.,** Casal, S., Khan, M., Pinho, O., Viegas, O. **Caracterização nutricional da farinha do fruto *Strychnos madagascariensis* - comparação das farinhas produzidas na comunidade *vs* laboratório.** 3º Congresso Nacional de Nutrição e Encontro Nacional de Nutricionistas, Universidade de Lúrio, Mozambique, 21 - 23 November, 2018.

LIST OF ABBREVIATIONS

FAO–Food and Agriculture Organization

SETSAN– Technical Secretariat for Food Security and Nutrition

AFS–Agregados Familiares em Insegurança Alimentar

BMI–Body mass index.

IPC-InSAA–Ranking of Acute Food Insecurity in Phases

HPLC–high performance liquid chromatography

ICP–inductively coupled plasma

MS–mass spectrometry

GC–gas chromatography

FLD–fluorescence detection

ABTS–2,2-Azinobis, 3-ethylbenzthiazoline-6-sulphonic acid radical

DPPH– Diphenyl-1-picrylhydrazyl radical

FDA–Food and drug administration

FRAP–Fluorescence recovery after photo bleaching

LDH–Lactic acid dehydrogenase

LSD–Lysergic acid diethylamide

ORAC–Oxygen radical absorbance capacity

TDF–Total fibers

IDF–Insoluble dietary fibers

SDF–Soluble dietary fibers

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CHAPTER 1- INTRODUCTION

In this chapter, the state-of-the-art and identification of the novelty and relevance of the research of this thesis are described, as well as the general and specific objectives.

1.1. Contextualization

Food security is a very complex subject, which is becoming more relevant in national debates, as well as at international level. This topic has been a priority of many international organizations’ agendas, playing a significant role in many African countries. In Mozambique, 54% of households cannot afford a nutritious diet that meets minimum nutrient needs. Chronic food insecurity affects Mozambican households and stems from the insufficient food access and production, due to the weather-related shocks and also due to a prolonged civil war, political strife, and economic recession (Technical Secretariat for Food Security and Nutrition - SETSAN, 2014).

Mozambican diet is largely characterized by low amounts of animal source proteins, fresh fruits and vegetables, and dairy products, on the other hand cassava, maize, sorghum, millet, and rice are staple foods (FAO, 2011). Thus, micronutrient deficiency rates are feared to be high, and chronic malnutrition manifests itself in the first years of life (43% of prevalence in children under 5 years old), being responsible for the death of one third of children under 5 years old. It also leads to some of the most irreversible damages during all life, cycle such as: short structure, which decreases both productive and physical capacities and cognitive function, resulting in poor school performance, and increases the risk of degenerative diseases (SETSAN, 2014).

Chronic malnutrition is defined as a form of growth failure that causes both physical and cognitive delays in growth and development and differs from acute malnutrition, defined as low body mass index (BMI). Acute malnutrition may come at any time of life, although it can be recovered. Chronic malnutrition is caused by acute malnutrition between conception and the first 2 years of age and is irreversible. In general, in Mozambique food insecurity is higher in rural areas compared to urban areas, where higher income rate occurs, together with subsidized prices of basic products, increased food availability and diversity.

Several studies are being performed in Mozambique to evaluate food security and nutrition under the coordination of the SETSAN. SETSAN was created in 2010, with main objective the evaluation of the state of food security in Mozambique and reversion of the situation of food and nutritional insecurity, through analysis and suggestion of corrective measures to revert undesirable situations of public health.

About two thirds of children 6–59 months of age are affected by vitamin A deficiency and anaemia. Despite the influence of the infection in these two indicators, the low intake of micronutrients is the main cause. In 2010, recognizing the high child stunting rates and its effect on the country's economic development, the Government of Mozambique developed the Multisectoral Action Plan for the Reduction of Chronic Malnutrition 2011–2014, to reduce the prevalence of chronic malnutrition (Government of the Republic of Mozambique, 2010), introducing micronutrient delivery interventions in the country through point-of-use food fortification (micronutrient powders) or industrial food fortification (including wheat and maize flour with iron and vegetable oil and sugar with vitamin A). However, there is a low coverage of vitamin A supplementation in routine child health services, thus food-based strategies to promote dietary diversity, such as through complementary feeding recipes and cooking demonstrations are key feature of health programming at the health facility and community level. Recognizing that porridge, a typical first food for young children made from local cereal or tuber flours prepared with water, is not sufficient to meet the nutritional needs of infants, the Ministry of Health promotes the “enrichment” of porridge by adding locally available nutritious foods, such as legumes, ground nuts, seeds, and green leafy vegetables as well as the addition of a teaspoon of oil to increase the energy density of the porridge (Picolo et al., 2019).

According to SETSAN (2014), agriculture plays a very important role to food and nutritional security, not simply as a source of food, but also as a source of employment, which provides income to rural populations. Furthermore, Mozambique ranks third among African countries most exposed to multiple weather-related hazards and suffers from periodic cyclones (Idai, Kenneth and Desmond), particularly in the centre and north of the country. Due to the Cyclone Idai (March 2019), hundreds of rural communities experienced food shortages and fell into a nutrition crisis. Six weeks later, Cyclone Kenneth made landfall in northern Mozambique. In these areas, over 80% of the population is dependent on farming/agriculture as primary source of income, and 76% of households, headed by women, depend on subsistence farming. These smallholders have sustained significant crop loss, damaged land, and lost access to seed supplies to prepare for the planting season. Farming families, which had already been affected by drought, lost all or large portions of their seed stores and the cyclones wiped out the lower-than-usual harvests (SETSAN, 2019). Figures 2 and 3 depict the classification of food insecurity in 2019/2020.

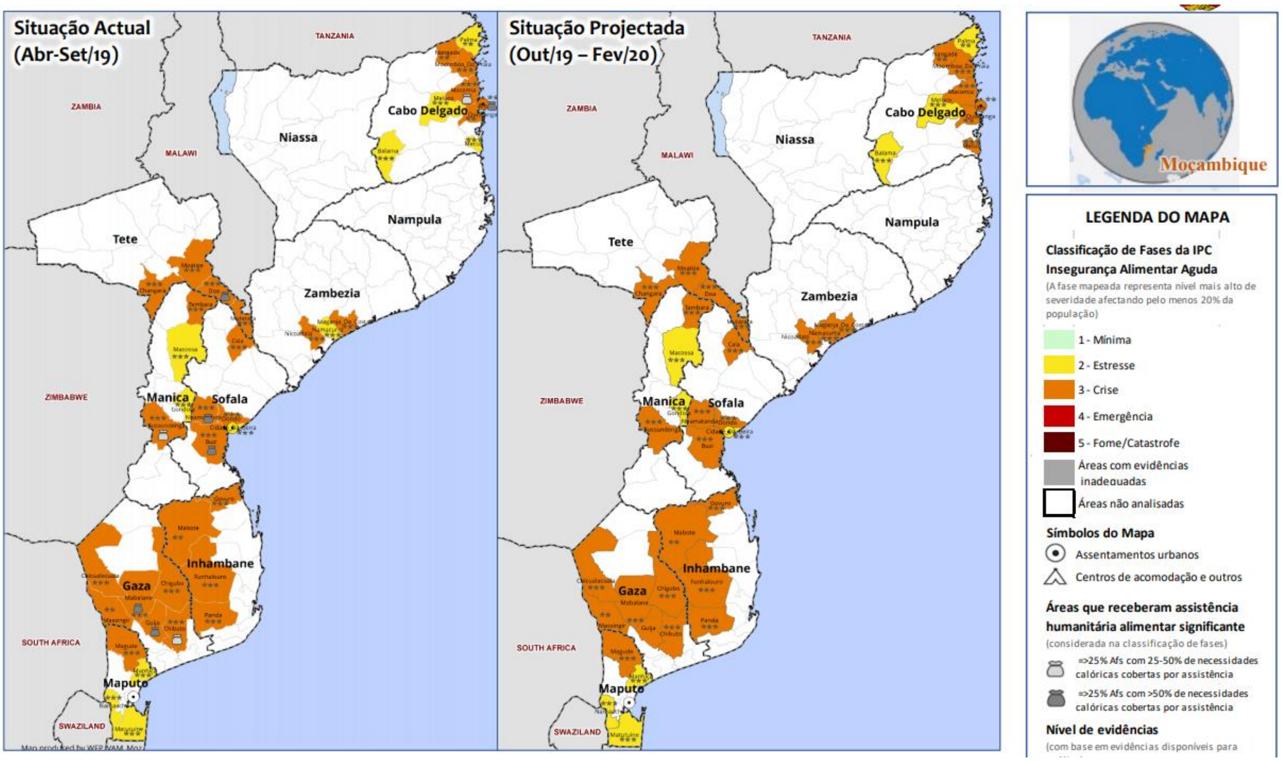

Figure 2: Classification of acute food insecurity, 2019/2020 (Source, SETSAN 2019)

Food insecurity vulnerabilities are higher in households that depend on informal occasional work and food assistance, wage earners and pensioners. The second group, which is more vulnerable, are families involved in the production and commercialization of agricultural products and livestock, as they are not involved in any other extra agricultural activity. They end up getting less products due to low productivity in the sector, meaning that they get lower earnings and less access to other products available on markets. Furthermore, low diversification of products negatively affects the overall quantity and quality of family's diet (SETSAN, 2014).

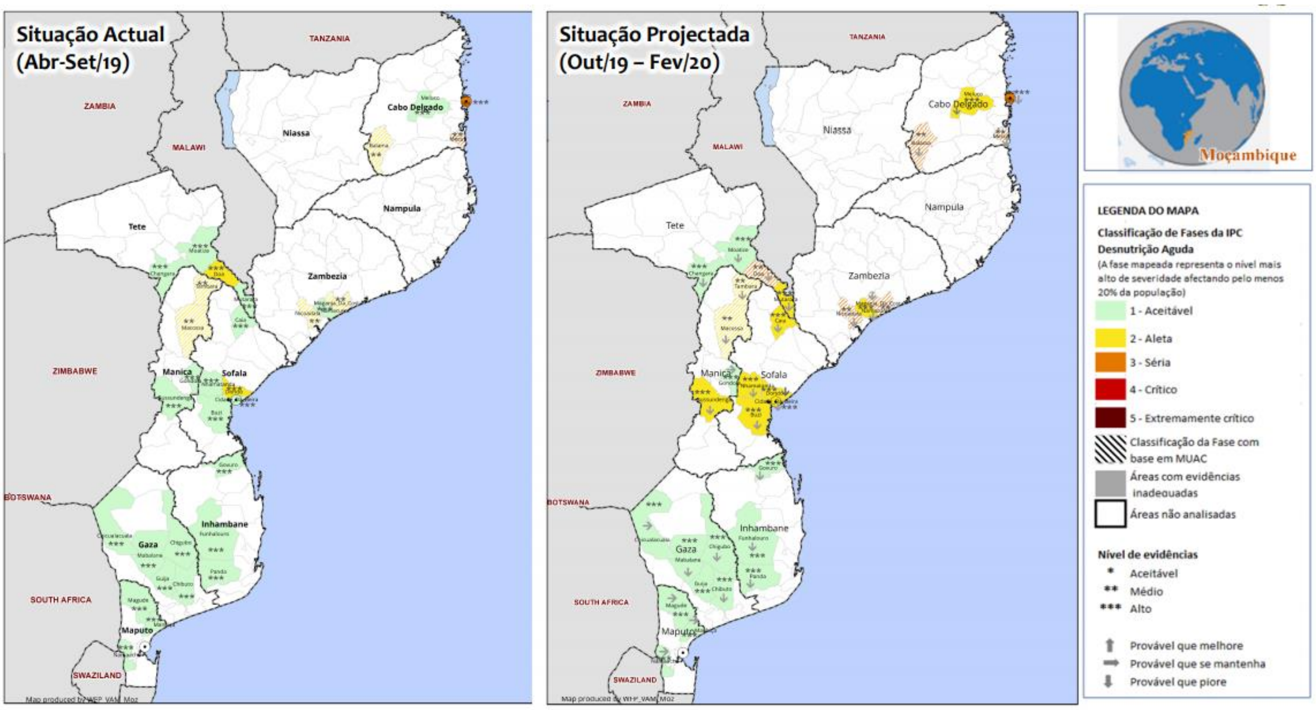

Figure 3: Acute Malnutrition Classification-2019/20 (Source, SETSAN 2019)

Table 1 shows acute food insecurity and acute malnutrition. It is expected that 1.6 million of people will need intervention to improve their diet. On the other hand, 6.500 children are under acute malnutrition and 61.000 under moderate malnutrition (SETSAN, 2019).

According to FAO (2011) Sub-Saharan Africa experiences food insecurity and the multiple burden of malnutrition. In that region, several fruit species have the potential to alleviate these deficiencies. Though abundant, these fruits remain underutilized. Moreover, several crops are drought tolerant, they represent an important component of agro-biodiversity with potential to contribute to climate change adaptation, food security and sovereignty in poor rural and could be exploited by agro-industry and become a source of income for poor rural areas in the future (Mabhaudhi et al., 2017).

A rising interest has developed around the genus *Strychnos* (Monkey orange) as a new food crop due to nutritive potential of specific species within this genus (Ngadze et al., 2017a). These species are known for their production of edible fruits, fast grow when cultivated and high drought tolerance, making them suitable for cultivation in arid regions. (Omotayo et al., 2021; Govender 2008).

Table 1: Acute food insecurity and acute malnutrition situation in Mozambique-2019/2020 (Source: SETSAN 2019)

Acute Food Security				Acute Malnutrition		
1.6 million people need intervention **to improve their diet,** rebuild and restore assets and livelihoods	Methodology	April-Set 19	Oct 19-Feb 20	2 Currently (April to Set/19	**Severe** acute malnutrition	6.55 Children
	IPC (39 districts)	1.358,046	1.689,408	6 Projected (Oct/19 to Feb/20	**Moderate** acute malnutrition	61.000 Children
	Secondary data (24 districts)	290.6	305.13	Number of districts in phase 3 that need an **urgent increase in prevention treatment**	67.5 is the number of children who need treatment for malnutrition	
	Total 63 districts	1.648,646	1.994,538			
	Results of IPC Classification					
	Phase 5	The current Catastrophe	The projected			
	Phase 4	188.589 current	Emergency 254.793 projected			
	Phase 3	1.168,377 current	Crisis 1.424,615 projected			
	Phase 2	1.747,834 current	Stress 1.600,589 projected			
	Phase 1	1.878,082 current	Minimum 1.693,964 projected			

Monkey orange fruits (member of the Loganiaceae family, belonging to the *Strychnos* genus) contain high micronutrients content and phenolic compounds and are widely distributed throughout sub-Saharan Africa. Up to 75 *Strychnos* species have been recognized in Africa. Although 20 species produce edible fruits, the most consumed species are *S. innocua, S. cocculoides, S. pungens, S. spinosa, S. icaja and S. madagascariensis.* Some fruits from S*trychnos* spp, has been identified to contribute with more than 100% of the recommended daily intake of vitamin C, Fe and Zn, especially for children between 4 and 8 years old, and pregnant women (Gadaga et al., 2009; Ngadze et al., 2017a). Furthermore, over 65% of Zimbabweans that live in rural areas are under food insecurity, especially during prolonged dry periods. Malnutrition problems, due to vitamin and mineral deficiencies, have public health significance in the country. Monkey oranges are fruits with a potential for contributing to alleviate vitamin and micronutrient deficiencies of the vulnerable rural population, particularly children and women, by complementing the staple food diet (Ngadze et al., 2017a).

Currently, the knowledge of fruits processing of the *Strychnos* spp is vague because it is usually undervalued and considered a "food for poor". The disappearance of cultural habits with urbanization and the increase of cultivation of exotic fruits and their commercialization have led to further reduction in the use of native fruits, besides the lack of awareness of their potential health benefits. In addition, the lack of standardized processing techniques of these fruits keeps them away from most diets and undermines nutritional security (Ngadze et al., 2019).

In Mozambique, as in Zimbabue, monkey oranges fruits ripen and are harvested from September to December, the so-called “lean season”– a time of cultivated food shortages in Sub-Sahara Africa, namely low maize stocks and the unavailability of freshly-gathered vegetables. The season of prolonged food scarcity often increases reliance on consumption of indigenous fruits by the rural communities (Khan et al., 2016; Ngadze et al., 2017a,b).

In order to improve the availability of *Strychnos* fruits outside its production season, the processing and preservation techniques used at a household level need to be improved and their effects on nutritional quality exploited, improved and disseminated. Ngadze et al. (2017a) in their review, referred that a sustainable solution to malnutrition in rural areas in transition countries could be improved through the production of *Strychnos* spp fruits.

During the non-productive periods, the consumption of native fruits has been an alternative for the food subsistence of rural communities in Mozambique (Khan et al., 2016). Native food plants, as fruits, vegetables and tubers have been a food option for communities on their diet, as well as for medicinal purposes. In Mozambique, there is a nature, and a unique flora, which is a resource to meet the primary needs of Mozambican population. Moreover, it is estimated that 70% of the Mozambican population, mostly in rural areas uses traditional medicinal plants for their health care (Carvalho, 1968).

Strychnos madagascariensis (macuácua), according to the communities of the south of Mozambique, is consumed both as food and as medicine. The fresh fruit has been used in the preparation of tea, which is considered to lower blood pressure. During food shortages in rural communities, people produce and consume a flour-like product, called *nfuma*, obtained from the dried pulp of this fruit for their survival. According to those communities, the consumption of an amount equivalent to five tablespoons of *nfuma* and then drink water, can provide enough energy for all day, suggesting that this product is probably energetic and nutritious, and well accepted for consumption by the communities. In this way it seems to be important to characterize the fruit of S. *madagascariensis* and the respective products both chemically and nutritionally and provide information to recommend that as a nutritional and safe food source.

1.2. Literature Review

In ancient times, native fruits were used worldwide as unique sources of drugs and became the most common human use of biodiversity (Hamilton, 2004; Hiremath and Taranath, 2010). According to World Health Organization (2002), 80% of people in developing countries still rely on medicinal plants to meet their primary health needs. There is a global consensus on the benefits of Phyto pharmacy, and currently medicinal plants occupy a key position in research and plant medicine. In this context, efforts have been carried out, by performing several studies aiming to preserve these relevant plants in all aspects, denying the progressive loss of knowledge associated with the rural exodus, and the exposition to the threats to the Plant Genetic Resources (Vicente et al., 2006). In Mozambique, as in other African countries, there is an important repository of biological diversity. This diversity is used by about 90% of the population for the needs of housing, food, energy and health. According to Krog et al. (2006), in Mozambique around 15% of all native plants (approximately 5,500 plant species) are used by rural communities for medical purposes playing a key role in basic health care. Despite a long history of Plants in Mozambique, research on this subject is still in initial stage (Carvalho, 1968; Oliveira and De Carvalho, 1975) and is still poorly disseminated, focusing mainly on markets for medicinal plants and commercial issues in the province of Maputo (Krog et al., 2006).

On this topic, various ethnobotanical articles are available in Southern Africa covering a wide range of uses for food, beverages, medicine, cosmetics and various arts and crafts (Van Wyk et al., 2008). De Beer and Van Wyk (2011) believe that an effort is required to develop innovative products with exceptional quality from plants and fruits at regional and global markets. They want to encourage a new generation of entrepreneurs to harness the energies between indigenous knowledge, scientific research, and modern technologies driving the sustainable development in the Southern African Region for the benefit of people. The diversity of food plants and potential new crops available, as well as the progress that has been made in the economic botany in South Africa, underline the emphasis given on the development of indigenous food products including fruits, vegetables, herbal teas and alcoholic beverages, in which flavors and spices have been also highlighted (De Beer and Van Wyk, 2011).

1.2.1. Native Fruits

Native fruits in Africa are source of untapped potential for food and nutrition security. They are not only an important source of vitamins and other nutrients for children in rural areas, but also a survival alternative of local communities at times, such as tsama (*Citrillus lanatus*) in the Kalahari, nara (*Acanthosicyous horridus*) in Namibia and mongongo (*Schinziophyton rautanenii*) in northern Botswana (Van Wyk and Gericke, 2000). Other fruits are important trade products and are sold along roadsides in many parts of southern Africa. Different studies showed evidence about the potential of production and valorisation of native fruits. Ackhurst (1996), evaluated fruits of Southern Africa in terms of their abundance, nutritional value, palatability, size and yield. In this study he proposed that the following fruits have an exceptional potential: marula (*Sclerocarya birrea*), sourplum (*Ximenia caffra*), blue sourplum (*X. americana*), pappea (*Capensis*), baobab (*Adansonia digitata*), mobola (*Parinari curatellifolia*), African mangosteen (*Garcinia livingstonei*), the common mushroom (*Ficus* *sycamorus*), the green monkey apple and the black monkey apple (*Strychnos madagascariensis*), Wild basil (*Annona senegalensis*) and wild palm (*Phoenix reclinata*).

Omotayo and Aremu, (2020), review information on 10 indigenous fruit trees that are considered to be underutilized and explored their occurrence, distribution, nutritional components, phytochemicals, and medicinal potentials, as well as their associated challenges and prospects. Several other fruits have the potential as new commercial crops and progress has already been made with marula (*Sclerocarya birrea*) and a few others. In communities, in Zimbabwe, some studies with processed native fruit products such as baobab (*Adansonia digitate*) have been conducted (Nyanga et al., 2013; Mpofu et al., 2014). Ham et al. (2008) discussed the commercial potential and opportunities to develop new companies based on native fruits. Based upon these fruits, alcoholic beverages can be made, with a commercial value, such as-marula, which is consumed at an international level.

Nowadays, the most interesting potential source of new products is the rich diversity of traditional alcoholic beverages, called wine, beer and brandy. It is hoped that growing awareness of the value of diversity and its potential in tourism will lead to the revival of the traditional art of brewing (using old recipes with special methods and ingredients) so that more people in the future can appreciate the regional diversity of Tastes (Van Wyk and Gericke, 2000). The importance of native food products has been increasingly highlighted.

In this way, a wide range of new small-scale products and development activities are currently underway in several parts of south Africa. This should be evident when visiting any traditional market or farm and observing the intention to several innovative ways in which traditional products are offered for sale. It is noteworthy that it will be very relevant in terms of public health to harness the potential in sugars to produce foods with nutritional value instead of alcohol, which is widely consumed in our populations. In Africa, native fruits also supplement the diet in rural populations by providing essential nutrients (Nhukarume et al., 2010; Bille et al., 2013) and in times of shortages of staple foods serve as a source of livelihood (Mithöfer and Waibel 2003; Legwaila et al., 2011). Hundreds of native fruit trees are known locally and are unknown in global markets (Jamnadass et al., 2011). Since these products are not present in global markets, only poor communities use them. In Mozambique, a large number of wild food plants are widely distributed throughout the country. Fruits and nuts are sold at informal markets during the harvest season and are consumed in different ways, being mostly appreciated by children. The importance of wild fruits in the diet depends largely on the availability of the fruits, since cultivated fruit trees are not particularly common in the dry regions of the country. Depending on the season, the fruits are either eaten raw, pressed for juice making, cooked with sugar, or used as flour to make porridge; the seeds or nuts are roasted to be eaten as snacks. The choice of fruit species varies according to region and cultural traditions (Magaia et al., 2013).

1.2.2. Commercial potential of native plant foods

Africa contributes to the world's major food crops, not only in the total number of species used in international markets, but also in the relevance of their products. In 1992, 2155 African species were used as food, representing 4.3% of African flora. According to Fox and Norwood (1982), 1002 plant foods from South Africa represented 4.4% of native flora. Van Wyk (2005) published a review of food plants from worldwide and demonstrated that Africa contributed substantially with 119 species, compared with 126 from Europe, 68 from Central America and 97 from South America.

Native plants have great nutritional and commercial value and are divided in different groups such as: cereals, seeds, nuts and pulses, leaves and fruits.

Cereals

Indigenous cereals have been largely replaced by corn, wheat, barley and oats. However, sorghum (*Sorghum bicolor*), finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) may have the potential to be developed and commercialized as traditional foods because they are based on grain or malt. All these three species are still popular for the production of traditional malted beer, especially due to the sweetness of their malts (Van Wyk, 2011). Malted beer is a daily food for traditional communities (Quin, 1959) and the sorghum beer “umqombothi”, also known as traditional African beer, remains important also in urban areas. Sorghum beer accounted for 23.6% of all commercial alcoholic beverages consumed in South Africa, compared with 42.6% and 17.4% for malt beer and wine respectively.

Indigenous grains also provide security for small farmers because they are tolerant to poor soils and drought periods. It may be possible to encourage the production of

Maltese beers and traditional foods for specialty restaurants, generating unique experiences for tourists in various regions of South Africa (Fox and Norwood, 1982).

Seeds, nuts and pulses

Seeds, nuts and pulses present historical interest in Southern Africa and also of considerable interest as new potential crops for dry regions (Bostid, 1979) especially regarding the development of new products with lucrative health food and snack food markets. Several seeds are also a source of valuable oils that have both culinary and cosmetic uses. Considering pulses, there are many traditional recipes using cooked, pounded, or crushed African indigenous beans (Van Wyk, 2011).

Leaves

Green leaves from several native species are consumed fresh or cooked in different dishes and can also be quickly cooked. They also can be sun dried and stored for later use or for sale in local markets, namely *Amaranthus*, *Cleome*, *Corchorus* and *Vigna*. These products are ideal for processing as canned foods; canned *Amaranthus*, for example, is becoming more regularly available (Van Wyk, 2011).

Myrothamnus flabellifolius is a traditional spice in South Africa and is used as flavour in tea. It presents a remarkable antimicrobial activity and may therefore be of interest in the development of healthy beverages, throat lozenges, mouthwashes and dental care products. The rhizomes and roots of *Siphonochilus aethiopicus* have a delicious spicy taste and are used to season biscuits and other confectionery. It also has a considerable potential in the development of novel functional foods (Van Vuuren and Viljoen, 2006).

It is interesting to note that the main phenolic compound in honeybush tea is mangiferin (De Nysschen et al., 1996, Joubert et al., 2011), a xanthone with considerable medicinal interest that is currently being commercially developed as a new drug in Cuba (the product is known as Vimang) (Archer, 1982; Rood, 2008). Several other indigenous species are widely used in the same way as black tea (but often with implicit or explicit claims of health benefits). The popularity of healthy foods and functional foods suggests that there are some opportunities for developing new herbal beverages, with carefully selected plant extracts and other ingredients chosen for their health benefits. Moreover, they can contribute greatly as a flavour (Moolla and Viljoen, 2008).

Fruits

The native fruits are rich sources of vitamins, minerals, proteins, and valuable phytochemicals. They also have recognized medicinal value and used as diverse therapeutic remedies by many ethnic groups in Africa (Omotayo and Aremu, 2020). Several native fruits such as *Strychnos* spp fruits are widely distributed in Southern Africa but are still underutilized and little attention has been given to their commercialization due to limited knowledge and information. Products are wasted due to limited harvesting time, process control and storage conditions, affecting nutritional quality, shelf life and sensory quality. Traditional processing techniques make insufficient use of these food resources in communities (Ngadze et al., 2019). The *Strychnos* spp has the potential to generate income for actors in the value chain within local and regional markets, as in the case of baobab (*Adansonia digitata*) fruit and other African indigenous fruits that reportedly reduce poverty by 33% during the critical period of the year (Mithöfer and Waibel, 2003; Chadare et al. 2008). *Adansonia digitata* is becoming popular in Europe and has been placed in European market as a novel food ingredient (European Commission, 2008).

The most interesting potential source of new products is the rich diversity of traditional alcoholic beverages, inaccurately called “wine”, beer and brandy. It is expected that a growing awareness of the value of this diversity and its potential for tourism will lead to a revival of the traditional art of brewing (using old recipes with special methods and ingredients) as mentioned before, and in future more people can appreciate the regional diversity of tastes (Van Wyk and Gericke, 2000). These alcoholic beverages are traditionally distilled from fermented fruits (*mampoer*), fermented juice from sugarcane (*shiwayawaya*) and from golden syrup or palm wine laced with sugar (*skokiaan*). Brandy flavoured with buchu (*Agathosma betulina*) is an old favourite beverage of the Cape to prepare an excellent cocktail when mixed with lemonade and/or tonic water.

Strychnos spp is a genus of plants used since the ancient times. Populations have used it not only as food source but also to treat various diseases, and therefore scientists currently use it to obtain compounds to treat several diseases (Van Vuuren and Viljoen, 2006).

1.2.3. *Strychnos* Species

Strychnos is the largest genus of Loganiaceae with about 200 species and can be subdivided into three groups of geographically separated species: Central and South America (at least 73 species), Africa including Madagascar (75 species), and Asia including Australia and Polynesia (about 44 species). From these species, the *S. xantha* is sister to the expanded representation of Southern African taxa, while *S. aculeata* is sister to all African taxa. The uncertain relationships between *S. innocua, S. madagascariensis* and *S. gerrardii* were partially resolved in the phylogenetic analysis of combined datasets. *S. innocua* is sister to the other two species in a well-supported clade. *S. gerrardii* and *S. madagascariensis* are also similar in taxa that are not yet reciprocally monophyletic, but have other characteristics that distinguish them (Neuwinger, 1996).

In the past it was thought that the *Strychnos* spp contained strychnine and convulsive analogues, particularly the Asian species, while the American species had curative power. This theory is now completely outdated. In fact, strychnine was isolated from *S. nux vomica L.* and many other different species from Asia, but also from *Strychnos* from other parts of the world. In addition, curing alkaloids are not isolated only from American species. The presence of strychnine seems to be quite rare in species of *Strychnos* and some species are totally harmless.

Marini-Bettolo et al. (1972) described the "*Strychnos* discovered in African continent is a judiciously called *Strychnos innocua*". African *Strychnos* and its alkaloids have been the subject of an excellent review for about 20 years describing their phytochemistry and pharmacology (Ohiri et al., 1983). There are good results in terms of pharmacology as well as about nutritional aspects, which have been stimulating the research. In addition, it is important to verify that some *Strychnos*, such as *Strychnos icaja*, contain different types of alkaloids, some with a tetanizing effect associated with others that induce muscle relaxant effects (Kambu et al., 1980). These species do not present an exclusively strychnine effect in all their extracts.

The species *Strychnos* spp (monkey orange) was identified among the priority fruit trees in ethnobotanical research in southern Africa (Saka et al., 2004), particularly in the dry zones of Zimbabwe (Mpofu et al., 2014). This fruit tree is found in areas of prolonged drought because it can survive without water during a long period of time and producing many fruits (Ngadze et al., 2019). The fact that this fruit is produced in the forests and in large quantities, means that it is not used and as such it stays in the trees without being picked up (Mapaura and Timberlake, 2004).

The fruits of S*trychnos* spp become mature and are harvested from September to December when maize stock is low and there is also little fresh vegetable availability. In this period of shortage, communities depend on products such as these fruits to suppress their basic needs (Ngadze et al., 2017a,b). Fruits are consumed fresh, but in dry climates they are processed to supplement food needs (Ngadze et al., 2017b; Saka et al., 2004). In rural communities, 46% of families process native fruits into juices or porridge (Kalaba et al., 2013), with other food products that can be used to supplement the cereal-based staple substitute. *Strychnos* species, (monkey orange fruits) contain high amounts of micronutrients and phenolic compounds and are widely distributed throughout sub-Saharan Africa. Nutrients from monkey orange fruits can easily be extracted by traditional and pectinase maceration techniques (Ngadze et al., 2017b, 2018).

Among the S*trychnos* spp, *Strychnos cocculoides* stands out, a fruit that is highly appreciated because of its perceived health benefits, taste and resistance to drought. The pulp of this fruit has a typical sweet-sour flavor (Saka et al., 2007), which contributes to consumer acceptance. Moreover, the fruit has plenty of fiber (4 g/100g) and a high micronutrient content: iron (70–140 mg/100g), and vitamin C (34 mg/100g) content (Ngadze et al., 2017a).

Bello et al., (2008) studied the chemical composition and anti-nutrient composition of some *Strychnos* fruits unknown in Nigeria and found that these fruits present low level of anti-nutritional, high elemental composition, proteins, lipids, carbohydrates, and ascorbic acid and can serve as a supplemental source of essential nutrients for humans and livestock. The pulp and seeds of *Strychnos innocua* are rich in potassium, magnesium, iron, zinc, manganese and ascorbic acid. However, they also have a high content of oxalate and phytic acid.

In Mozambique there are 19 varieties of *Strychnos* distributed all over the country namely: *S. angolensis, S. matopensis, S. lucens, S. panganensis, S. xantha, S. usambarensis, S. mitis, S. decussata, S. henningsii, S. myrtoides, S. xylophylla, S. mellodora, S. potatorum, S. pungens, S. gerrardii, S. cocculoides, S. spinosa and S.*

madagascariensis, are the species which can be found in the north, centre and south while the others occur only in certain areas of the country (Burrows et al., 2018).

1.2.4. Prejudicial Compounds

The toxicity of the *Strychnos* species differs according to the part of the plant and is mainly related to the presence of strychnine. Fruit pulp, of different African and Asian species, for instance *S. nux vomica*, are consumed by both animals and humans. However, the seeds are poisonous. When animals feed on different species of *Strychnos*, this is presumably an indication of the presence of little or no alkaloids. In India, cows are known to be grazed on leaves of *S. nux vomica* (Jordan et al., 2004). The roots are known as strychnine biosynthesis and have the richest alkaloid content and have a higher pharmacological activity than other parts of plants among the *Strychnos* spp.

1.2.5. Beneficial Effects

Alkaloids are one of the most important classes of natural products that provide medication since ancient times. Alkaloids are the physiologically active nitrogenous bases derived from biogenetic precursors. Some alkaloids are well known because of their toxicity or as they are used as psychedelics such as: cocaine, morphine or semisynthetic. At the same time, alkaloids have been successfully used to treat parasite infections. The most outstanding example is quinine, from *Cinchona succirubra* (Rubiaceae) used for the treatment of Malaria for more than three centuries (Nuzillard et al., 1996; Jordan et al., 2004). In the review of Brandão et al. (2010) the importance of plants was demonstrated as a source of compounds for chemotherapeutical medicines, describing the development of anticancer agents from plants by the pharmaceutical industry and the difficulties to release them in trademark (Table 2). These include the well-known paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, vindesine, etoposide, teniposide, and other molecules (Jordan et al., 2004; Brandão et al., 2010).

Subsequently, phytochemical investigation of several *Strychnos* species has shown great structural diversity of the alkaloid constituent which also causes various biological effects such as convulsive and relaxant effects on muscles, antimicrobial, antitumor and has antihypertensive properties. Ethnobotanical field work conducted in different regions of Madagascar revealed that infusion of three *Strychnos* species (*S.*

mostueoides, S. myrtoides and *S. diplotricha*), were used in association with sub curative doses of chloroquine to treat chronic malaria (Rasoanaivo et al., 1996).

Table 2: Some examples of drug alkaloids and their clinical use (adapted from Brandão et al., 2010)

Class	Drug	Status	Indications
Vinca alkaloids *Catharanthus roseus*	Vimblastine (Velban®)	Approved by FDA (Eli Lilly)	Hodgkins disease and testicular cancer
	Vincristine (Oncovin®)	Approved by FDA (Eli Lilly)	Leukemia, lymphomas
	Vinorelbine (Navelbine®)	Approved by FDA (Asta Médica)	Solid tumors, lung cancer and acute lymphoblastic leukemia
	Vindesine (Eldisine®)	Approved by FDA (Eli Lilly)	Acute lymphoblastic leukemia and lung cancer
Taxanes yem, *Taxus brevifolia* Nutt. (Taxaceae)	Paclitaxel (Taxol®)	Approved by FDA (Bristol-Myers)	Lung, ovary and breast cancer
	Docetaxel (Taxotere®)	Approved by FDA (Sanofi-Aventis)	Lung and prostate cancer

African people have been using traditional remedies for the treatment of various diseases such as hypertension, skin diseases, diarrhea, and dysentery among others. A survey taken in consideration the edible and medicinal plants native to South Africa as well as to Mozambique was carried out by Mithöfer and Waibel (2003) and Chadare et al. (2008). From this study it was possible to select several plants that communities use for consumption and treatment of some diseases.

According to Ngadze et al., (2019) the fruits of *Strychnos* spp (monkey orange) have health benefits for children and people with low immunity. It means that the regular consumption of these fruits may increase not only weight but also disease resistance. The positive perception of these fruits offers a good opportunity to improve nutritional security, although these resources are still not fully used. Micronutrients have physiological importance to human health because of their role in growth, bone formation, enzyme activity and energy metabolism, among others (Martínez-Ballesta et al., 2010; Pereira et al., 2018). Previous studies have also identified phenolic compounds in *Strychnos* spp species such monkey orange fruit pulp, which are effectively extracted by maceration with pectinase enzymes (Ngadze et al., 2018). The identified phenolic compounds belong to the phenolic acid, flavonoid, iridoid and phenolic apio glucoside fractions.

1.3. *Strychnos madagascariensis*

Strychnos madagascariensis, called in Mozambique as macuácua, is a tree from 5 to 8 meters with high branches, a light grey bark with white spots that darken with age. The leaves are simple, green grouped at the ends of thick branches. The flowers are greenish-yellow and grouped at the base of the leaves, appearing usually after heavy rains. The fruits of macuácua are round shaped, with a thick woody shell, green coloured for most of the year, becoming yellow to orange colour when ripen. Whole fruits weight around 450 g and contain hard seeds imbedded in a fleshy and juicy pulp with a sweet and slightly bitter taste (Khan et al., 2016).

Figure 4: ***Strychnos madagascariensis*** **tree**

S. madagascariensis is found mainly in Botswana, South Africa (Limpopo, North-West, Mpumalanga, KwaZulu-Natal), Swaziland, Mozambique and other African countries (Schmidt et al., 2002; Burrows et al., 2018) as can be seen in Figure 5.

Figure 5: Distribution of *Strychnos madagascariensis* in Africa (Source: www.waspweb.org)

In Mozambique *S. madagascariensis* trees grow in all provinces (Figure 6) and are characterized by their seasonality, being harvested between August and December. In the south of Mozambique, they are locally called macuácua by native people.

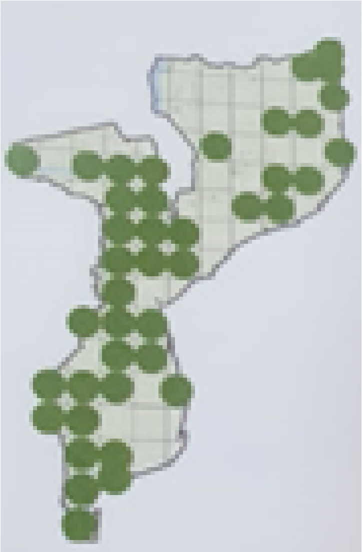

Figure 6: Distribution of *S. madagascariensis* in Mozambique (Source: Burrows et al., 2018)

S. madagascariensis fruit is consumed in several ways, such as fresh or in the form of tea and flour. In some African countries, the fruit can also be used to make alcoholic beverages after fermentation under sun exposure, and the pulp extract produces an appreciated sweet beverage when mixed with honey (Van Wyk and Gericke, 2000). Population of the south of Mozambique consume this fruit in natura, as tea, and also process it into a flour-like product from pulp, known as *nfuma* (Figure 7b). This flour is consumed during the basic food shortages, becoming an important source of nutrients, and described by community people as "the consumption of few spoons of flour gives enough energy to work on the farm". During the preparation of the flour, in the crushing step, some women from the communities collect oil that comes from the flour, keeping it for culinary uses. Furthermore, the *nfuma* consumers in the communities reported that "by consuming this flour, they control cardiovascular diseases, such as arterial hypertension".


(a)
(b)

Figure 7: Macuácua tea (a) and macuácua flour *nfuma* (b)

Khumalo et al. (2002) carried out a study with *S. madagascariensis* from Zimbabwe and other two native fruits from the same area and found that they are rich in fatty acids. For that, authors obtained the oil from the inner skin of the *S. madagascariensis* fruit shell and from the seeds of the other fruits (Table 3) and evaluated their potential as starting materials in lipopolysaccharides production of cocoa butter equivalents using transesterification reactions. The oils evaluated in this study are edible and already being used by rural communities in food. Although their nutritional properties are poorly studied, they seem to have potential for the development of various marketable foods. Moreover, these oils have the advantage of being available and in most cases, the material from which the oils are derived is treated as by-products. The oils of *S.*

madagascariensis and *Ximenia caffra* are ideal starting materials for the synthesis of cocoa butter equivalent.

Table 3: Triglyceride composition of three oils derived from indigenous fruits (Adapted from Khumalo et al., 2002)

Oil	% Oleic acid in non-hydrolyzed acid	% Oleic acid in 2-monoglyceride hydrolysate Oil	Enrichment of oleic acid in 2-monoglycerides
*S. madagascariensis**	67.7	75.0	1.1
Trichelia emetic#	27.8	50.2	1.8
Ximenia caffra#	58.7	86.1	1.5

***Obtained from the inner skin of the fruit shell; # Obtained from seeds**

Despite the use of these underutilized oils as cooking oil may not be economically attractive, the modification of these oils into value-added products could be economically advantageous, especially for the medium-sized confectionery industries of the countries, where these plants are found. Thus, macuácua oil from the pulp of the fruit probably has potential, however, still needs to be properly studied. Various authors have reported the use of *S. madagascariensis* as medicine as described on Table 4. However, no scientific studies were carried out considering the beliefs of the traditional communities and folk applications, in order to provide scientific support for pharmacological effects of this plant and its fruit for certain diseases.

Table 4: *Strychnos madagascariensis* applications as medicinal plant

Part of the plant used	Medical use	Method of preparation	Administration	References
Bark, roots and leaves	Treatment of diarrhea and dysentery	Root, bark and leaves are crushed and mixed with cold water	Drink the infusion (60 mL) twice a day, until the diarrhea decreases	Van Wyk et al., 2008 De Wet et al., 2010
Roots	Fever	Peel the roots and put them in the infusion	To be drink as a juice	Jansen et al., 1990 De Wet et al., 2013
Leaves and fruit pulp	Sores	Crush the leaves and use the pulp	Apply directly on sores	De Wet et al., 2012
Seeds	Treatment of hypertension	Ten seeds dry in the sun, then grind until powdered	Take two table spoons three times a day	Eddouks et al., 2002 Gbolade et al., 2012

According to Shaffer (2008) only the fruit pulp of *S. madagascariensis* is edible, as consumed in Mozambique (fruit pulp and its products) because it is believed that seeds may contain the toxic alkaloid, strychnine. However, there are contradictory claims in relation to the toxicity of the seeds. For example, Govender (2008) reported the consumption of seeds and Van Rayne et al. (2020) characterized their nutritional and anti-nutritional characteristics and its potential for food. The toxicity of the seeds has not been proven and it is not supported by any ethnobotanical data. The fruit is considered an underutilized commodity as its full potential as a food source has not yet been investigated (Van Rayne et al., 2020). Underutilized food plants typically provide higher nutritional value in comparison to those domesticated and commercialized (Bjarklev et al., 2019).

Van Rayne et al., (2020), studied the seeds of *S. madagascariensis*, and determined the nutritional composition and the functional properties of the seed flour with the objective of evaluating its safety as a potential food source. The results of this study are presented in Table 5.

Table 5: Nutritional composition and strychnine content of *S. madagascariensis* seed flour (per 100 g) and its contribution to Nutrient Reference Values (NRV) (Source: Van Rayne et al., 2020)

Nutricional Category	Composition	NRV	Percentage Contribution to NRVs
Calories	189.03 kcal ± 0.00	2000 kcal	9.45%
Total Fat	0.95 g ± 0.00	44.44-77.78 g	2.14%
Total Carbohydrates	89.85 g	225-300 g	34.94%
Total fiber	53 g ± 3.65	25 g	200%
Sugar	41g ± 4.69	<50 g	82%
Starch	0 g ± 0.00	150-225 g	0%
Protein	8.27 g ± 0.13	49.8 g	16.61%
Strychnine	0.08% ± 0.04	N/A	N/A
Ca	148.03 mg ± 6.00	700 mg/day	21.15%
Mg	79.25 mg ± 1.00	285 mg/day	27.80%
K	594.36 mg ± 4.00	3500 mg/day	16.98%
P	94.20 mg ± 2.00	2400 mg/day	3.93%
Na	16.45 mg ± 1.00	1600 mg/day	1.03%
Zn	1.36 mg ± 0.02	42 mg/day	3.24%
Mn	9.86 mg ± 0.1	12.2 mg/day	80.82%
Fe	15.78 mg ± 0.4	6.7 mg/day (males) 11.4 mg/day (females)	235.52% 138.42%
Cu	0.72 mg ± 0.01	10 mg/day	7.2%

Nutrient reference values (NRVs) are a set of values that are used as a reference point for the daily intake of micro and macronutrients, which are required to maintain a healthy life. It is indicated in this table the NRVs for both micro and macronutrients as well as the percentage contributions of *S. madagascariensis* seeds to these values. The total fiber, sugar, Mn and Fe contribute greatly towards these daily recommended values (200, 82, 81 and 236/ 138%, respectively). Authors highlight the potential of *S. madagascariensis* seeds as a food alternative or supplement, aiming to alleviate food insecurity and mineral deficiencies.

There are some studies reporting the *S. madagascariensis* application in traditional medicine, using roots, stems, leaves and seeds. However, scarce information was found in relation of the pulp of the *S. madagascarienis* fruit, its nutritional value, as well as about *nfuma* prepared by the communities, a flour which provides energy to support the lack of basic staple foods during shortages. The potential application and nutritional value of the oil from *nfuma* was also unknown. Thus, the study of the nutritional properties of the fruit of *S. madagascariensis* and their products is needed, providing to the communities nutritional and economic advantages.

1.4. General and specific objectives of the thesis

1.4.1. General objective of the thesis

To study the nutritional properties of macuácua, the fruit of *S. madagascariensis*, as well as exploit the nutritional and technological potential of its flour (*nfuma*) and oil produced by local communities, in order to determine the true value of their practices and support more rational decisions about its sustainable utilization and valorization.

1.4.2. Specific objectives of the thesis

The specific objectives of this thesis are:

1. To characterize the macuácua pulp in terms of its physicochemical properties, proximate composition, as well as to know the mineral content and bioactive compounds.
2. To exploit potential differences between the nutritional and antioxidant values of these fruits from different geographical locations within the south of Mozambique.
3. To detail the nutritional composition of *nfuma*, in terms of sugars, fatty acids and amino acids profiles as well as vitamin and mineral content.
4. To evaluate *nfuma* adequacy in terms of nutrients considering current nutritional recommendations.
5. To determine the compositional basis of the oils physically extracted from *nfuma* produced following traditional procedures, while evaluating its nutritional and technological potential.
6. To compare the overall quality of the oils produced by the communities with oil produced at laboratory, under controlled conditions.
7. To elaborate recommendations regarding the best practices in the production of macuácua products from a nutritional, safety, and technological approach.

CHAPTER 2-MATERIAL AND METHODS

In this chapter are described the main procedures of sampling from fruits harvesting to production of flours and oils, both in community and in laboratory.

2.1. Sample Preparation

Macuácua fruits and its products were collected from four districts selected from the provinces of Maputo and Gaza located in the south of Mozambique. Although macuácua is distributed all over the country, the consumption as food occurs mainly in the provinces of the south. The districts selected were Marracuene, and Manhiça in Maputo province and, Chókwè and Chicualacuala in Gaza province.

The ripe fruits were collected in the summer period from October to December (average temperature of 30 ^{o}C). In each district, 40 fruits were randomly collected from 8 trees, in 3 independent times, resulting in 960 fruits collected in each district. A total of 3840 fruits were collected for this study.

Around 2,880 fruits were used for traditional flour preparation by communities, whose chemical and nutritional characterization is presented in Chapter 4. From the flour produced in the communities, it was performed the mechanical extraction of the oil which results are presented on Chapter 5. The remaining fruits from Marracuene were used in the production of flour and extraction of its oil under controlled conditions at laboratory, being this oil used as control in Chapter 5. Flour and oils from laboratory were prepared in *Departamento de Engenharia Química, Faculdade de Engenharias* and in *Departamento de Química da Faculdade de Ciências, Universidade Eduardo Mondlane,* Maputo, Mozambique.

The experimental work of this PhD thesis was performed in REQUIMTE, Laboratory of Bromatology and Hydrology and Laboratory of Applied Chemistry from Department of Chemical Sciences (Faculty of Pharmacy, University of Porto) and Universidade *Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia*.

2.1.1. Fruit characterization

From the harvested fruits, 18 fruits from each community were used to determine physicochemical composition and bioactive compounds. The results are presented in Chapter 3.

2.1.2. Preparation of macuácua flour (*nfuma*) and its oil

Macuácua flour and oil were processed traditionally at the community and at the laboratory. The schematic representation of the macuácua flour and oil extraction processes are summarized in the figure below (Figure 8).

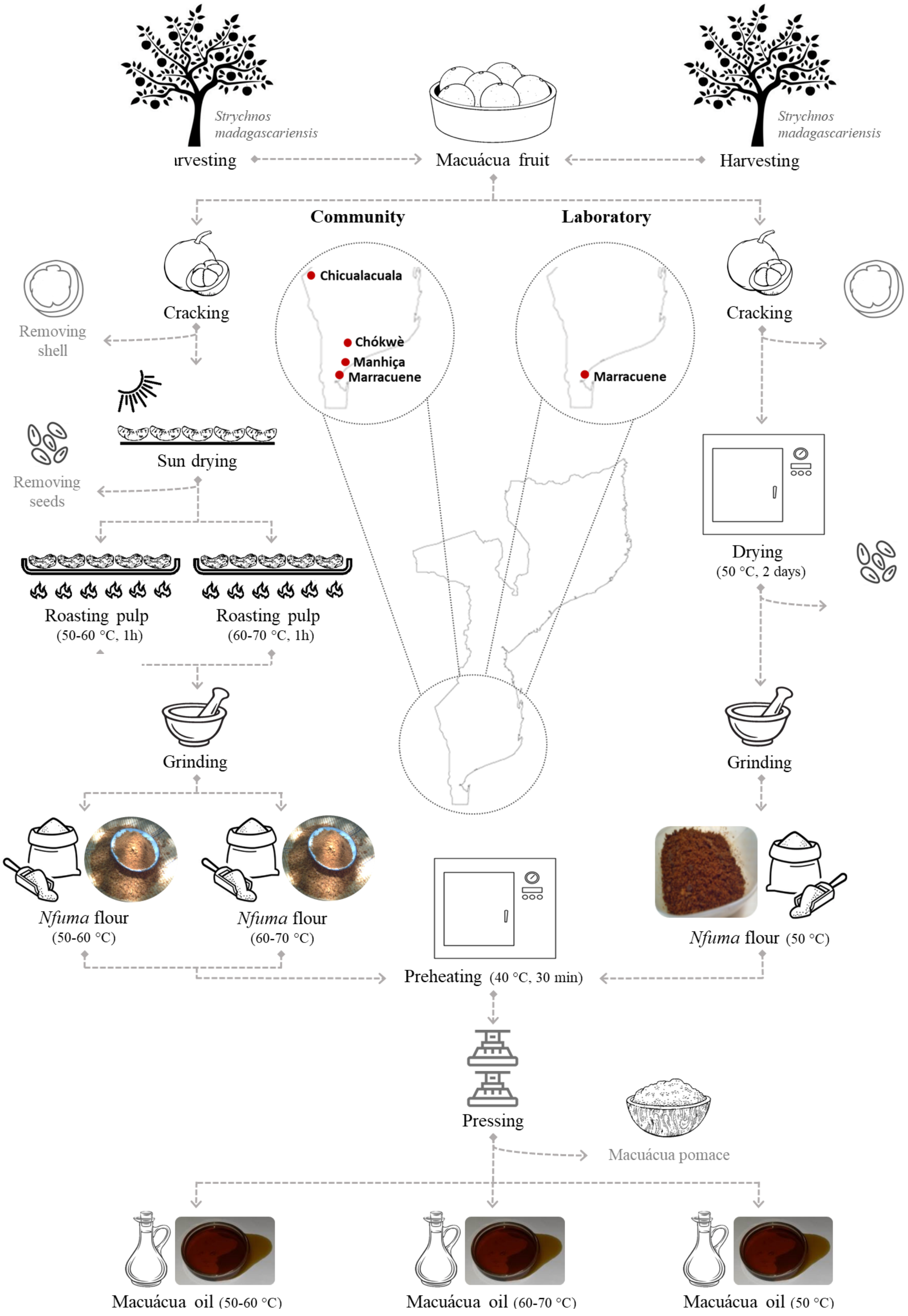

Figure 8: Processing of macuácua flour and oil at comunity level and laboratory level

2.1.2.1. Processing of flour at community level

Macuácua flour is usually produced by the communities in Mozambique for domestic consumption but also for commercialization without any quality control. In this work, macuácua flour was produced with the communities from the four districts selected. The processing of the flour with the community was done three times in different moments in each district, resulting in 6 samples of 5 kg for each location resulting in a total number of 24 samples of flour produced under two different temperatures. The flour was preserved in closed containers under normal environmental conditions for subsequent oil extraction. For flour processing, the pulp was firstly sun dried and then roasted on a metallic plate heated over a low heat at temperatures of 55 ± 5 ^{o}C and 65 ± 5 ^{o}C, for around one hour. The product was then cooled naturally until room temperature. The dried pulp was ground until the flour was obtained. The process of obtaining the flour took place in each of the locations under two different temperature conditions**.**

2.1.2.2. Processing of flour and its oil at laboratory level

Ripe fruits from communities of Marracuene were collected for analyses in the laboratory and for flour and oil production. At laboratory level, the flour was prepared under controlled conditions. Briefly, the fruits were selected, cleaned, and then cracked to separate the pulp. The seeds containing pulp were then removed and let to dry in the oven (memmert UN 110) for two days at a temperature of 50 ± 2 ^{o}C. The dried pulp was removed from the seed, and ground into flour using a crusher and then the oil was extracted and used as control in Chapter 5.

2.1.3. Oil extraction from *nfuma*

The oil was extracted from the flour using a hydraulic hand press machine (carver MENOMONEE FALLS, WIS 53051 USA Serie 24000-103). The flour was previously warmed in a Becker of 0.5 kg capacity and placed in oven (memmert UN 110) at 40 ± 2 ^{o}C for 30 minutes. Flour samples were pressed for oil extraction. Oil samples were placed in 50 mL containers, then closed and protected with aluminium foil and stored at 5 ± 1 ^{o}C in a refrigerator.

2.2. Methods of Analysis

The procedures used to study the fruit, the flour and oil are described in detail in the Chapters 3, 4 and 5. The determination of macronutrients procedures are described in the Association of Official Analytical Chemists (AOAC, 2005). Fibers were determined based on AOAC 32-05.01 method and AOAC 985.29 method and carbohydrates were calculated using the differential method. pH was determined using a pH meter (pH meter, Crison micro pH 2002), the total sugars (^{o}Brix) using a refractometer (PR 32α, Atago Co., Ltd., Japan), colour (colorimeter Chroma Meter CR-400, Konica Monilta, Sensing, Inc., Japan)

The total phenolic compounds were estimated as the concentration of gallic acid equivalents (GAE, mg/L), according to the Folin-Ciocalteau method (Re et al., 1999) and antioxidant activity was determined using using ABTS (2,2-Azinobis, 3-ethylbenzthiazoline-6-sulphonic acid radical) and DPPH (diphenyl-1-picrylhydrazyl radical) methods.

Extraction of vitamins A and E were carried out according to the HPLC method (detection of fluorescence by HPLC) and Total Carotenoid Content (TCC) estimation was based on the method described by Cruz and Casal (2018). Mineral analysis was performed after wet digestion according to Pinto et al. (2020). The low molecular sugars (mono and disaccharides) were determined by high performance liquid chromatography with refractive index detection (HPLC-IR), as described by Santos et al. (2016) with some modifications.

Fatty acid composition of extractable lipids were evaluated as methyl esters by gas chromatography with flame ionization detection, using alkaline trans-esterification with methanolic potassium hydroxide, as detailed in Regulation EEC 2568/91 (European Commission, 1991). Total triglycerides (TG), diglycerides (DG), and free fatty acids (FFA) contents were assayed following ISO 18395:2005 by size-exclusion high-performance liquid chromatography (HPSEC). The quantification of phytosterols was made by gas chromatography with FID detection following the Regulation (EEC) No. 2568/91 (European Commission, 1991). Amino acids were analyzed by high performance liquid chromatography with fluorescence detection (HPLC-FLD), after hydrolysis and derivatization with 9-fluorenylmethyl chloroformate and O-phthaldialdehyde, according with Benhammouche et al. (2021).

CHAPTER 3- NUTRITIONAL AND ANTIOXIDANT ACTIVITY OF *STRYCHNOS MAGACASCARIENSIS* FROM THE SOUTHERN DISTRICTS OF MOZAMBIQUE

To achieve Chapter 3, the fruit of *S. madagascariensis* was characterized in terms of physical-chemical parameters, minerals and antioxidant activities. Fruits from different geographical locations within the south of Mozambique were compared.

ABSTRACT

Macuácua (*Strychnos madagascariensis*), an African fruit collected from different districts of Mozambique, was investigated. The pulp of fruits from 4 districts from the south of Mozambique (Marracuene, Manhiça, Chókwè, and Chicualacuala) were evaluated in terms of physicochemical parameters (pH, total soluble solids, color, moisture, proteins, fat, ashes, fiber, carbohydrates), minerals (Ca, Mg, Na, K, Fe, Zn, Mn, Cu, Cr, Co, Al, Rb, Ni, Sr, Ba, Cd), antioxidants (Carotenes, vitamin E, total phenolics) and its activity against ABTS and DPPH radicals. The results indicated differences in the origin of the pulp. Samples from Chókwè and Manhiça presented the higher % of protein, fat, antioxidants, carotenes, and Chicualacuala presented the most outstanding mineral composition and vitamin E. The results provide new information about poorly studied nutritional and biological aspects of highly consumed Macuácua fruit.

Keywords: orange monkey, nutritional value, bioactive compounds, antioxidants.

1. Introduction

The African continent has several wild fruits and plants with a high agronomic and commercial potential. However, many of these species are not valued due to the lack of knowledge of their physical, chemical, and nutritional properties (Kucich and Wicht, 2016). In fact, fruits, a source of essential micronutrients, are essential in the diet of the rural African population (Khan et al., 2016; Kucich and Wicht, 2016). Scientific studies have proven that African fruits, in general, due to unfavourable edaphoclimatic conditions, develop a relevant content of antioxidant compounds. Then, its consumption must be promoted to solve many problems arising from the nutritional imbalance of rural communities in Africa (Khan et al., 2016; Kucich and Wicht, 2016; Magaia et al., 2013).

It was demonstrated that individuals who eat five or more servings a day of fruits and vegetables have approximately half of the risk of suffering non-transmissible diseases, particularly those in the gastrointestinal tract (Kucich and Wicht, 2016). Fruit consumption is globally insufficient and should be encouraged to reach adequate intake of minerals, vitamins, and all the secondary metabolites, namely phenolic compounds (PCs) with antioxidant activity. Some vitamins such as A, E, carotenoids, and PCs, contribute to fruit characteristics such as colour, flavour, bitterness, astringency and, together with the other food components (moisture, protein, fat, carbohydrates, and fiber), add to chemical stability and sensory perception (Ngadze et al., 2017a; Oboh et al., 2020). Besides, have been related to maintaining the antioxidant balance in the body, reducing the risk of accelerated aging, cancer, cardiovascular diseases, neurodegenerative disease, and inflammation (Márquez et al., 2002; Trujillo-Mayol et al., 2021). Thus, consuming food rich in antioxidants to promote the immune system by keeping the free radicals balanced is recommended (Kucich and Wicht, 2016; Trujillo-Mayol et al., 2021).

The native southern Mozambican flora is rich in different species of wild fruit trees, with fruits of excellent appearance and flavor and rich in nutrients and antioxidants, with broad perspectives of domestication and economic exploitation (Khan et al., 2016; Ngadze et al., 2017a). In this sense, the Mozambican native fruit *Strychnos madagascariensis*, known as macuácua, is consumed as food and medicine, being sometimes the only food option in the poorest communities. This fruit grows mainly in the different districts of the south of Mozambique, namely Marracuene and Manhiça at Maputo province, and districts of Chókwè and Chicualacuala at Gaza province. Particularity in these provinces, the macuácua pulp is transformed into flour named *nfuma* to combat the hunger in the south of Mozambique in famine periods. Thus, this fruit plays a substantial role in food security in this African country (Khan et al., 2016; Magaia et al., 2013).

Nevertheless, it is not well understood if there are differences between the nutritional and antioxidant value of these fruits from these different geographical producers. Therefore, the objective of this study was to characterize the tropical macuácua pulp (*S. madagascariensis)* in terms of physicochemical properties, proximate composition, minerals, and antioxidant activity given by vitamins (A and E), carotenoids, and phenolic contents from these four locations where it principally grows.

2. Materials and Methods

2.1. Chemicals and Materials

Folin−Ciocalteu reagent, sodium carbonate, gallic acid, aluminium chloride, quercetin, potassium chloride, sodium acetate, cyanidin-3-glucoside, formic acid, bradykinin, glucagon, 4-(dimethylamine) cinnamaldehyde, and 2,5-dihydrox-ybenzoic acid (DHB) were purchased from Sigma-Aldrich (Milwaukee, WI, U.S.A.). Methanol and acetone were purchased from Fischer Scientific (Fair Lawn, NJ, U.S.A.). Methanol-d 4 with tetramethyl silane (TMS, 0.03%) was obtained from Across Organics (Fair Lawn, NJ, U.S.A.). Ethanol (100%) was obtained from Decon Labs (King of Prussia, PA, U.S.A.). Sephadex LH-20 was purchased from GE Healthcare (Uppsala, Sweden). All other reagent used were in analytic and pure grads.

2.2. Samples

Macuácua samples were collected in four districts in the south of Mozambique, (Marracuene and Manhiça at Maputo province, and districts of Chókwè and Chicualacuala at Gaza province) and were stored at room temperature two days before being taken to the laboratory in Porto (Portugal). According to Khan et al. (2016) macuácua can be stored at room temperature for 15 days without degrading its properties. The fruit samples were cleaned, washed with tap water, and opened by breaking the shell using a wooden stick. The seeds are wrapped with a fleshy layer (the pulp), which was removed using a knife. The obtained pulp was then homogenized for each sample separately and stored until analysis. Macuácua fruits (n=12, 3 fruits per each community) presented an average weight of 511 ± 76 g, being 115 ± 26, 154 ± 67 and 269 ± 62 g of pulp, seeds and shell respectively.

2.3. Physicochemical and proximate characterization of macuácua pulps

After homogenization of the pulp, different parameters were measured, namely pH (pH meter, Crison micro pH 2002), total soluble sugars refractometer (PR 32α, Atago Co., Ltd., Japan), and color (colorimeter Chroma Meter CR-400, Konica Minolta, Sensing, Inc., Japan). The determination of moisture, protein, fat, and ash followed the procedures described in the Association of Official Analytical Chemists (AOAC, 2005), methods 925.09, 920.39, 992.15 and 923.03, respectively (AOAC, 2005). Total dietary fiber content was determined by the enzymatic-gravimetric method based on AACC method 32-05.01 and AOAC method 985.29, according to Martins et al. (2017). Carbohydrates were calculated by the differential method.

2.4. Mineral content determination

Mineral analysis was performed after wet digestion according to Pinto et al., (2020). Briefly, approximately 0.25 g of each sample was weighed, then 3 mL of nitric acid (65%) and 1 mL of hydrogen peroxide were added, and the sample was digested in microwave An MLS-1200 Mega high-performance microwave digestion (Milestone, Sorisole, Italy) unit equipped with an HPR-1000/10 S rotor was used. After digestion, the necessary dilutions were made. The mineral content of calcium, magnesium, potassium, and sodium minerals were determined using the flame atomic absorption spectrophotometer (FAAS), using a PerkinElmer (Überlingen, Germany) Analyst 200 instrument. Analyst 200 and for other minerals it was used inductively coupled plasma mass spectrometer (ICP-MS) ICAP^{Tm} Q (Thermo Fisher Scientific, Bremen, Germany). Details and figure of merit, namely limits of detection (LOD) and limits of quantification (LOQ) were presented in Chapter 4.

2.5. Vitamins E and A determination

Extraction of vitamins A and E was carried out according to the HPLC method. Approximately 200 mg of samples were mixed to 20 µL of tocol with a concentration of 100 µg/mL + 1.5 mL of 2-propanol + 2.0 mL of hexane were added to each sample. The mixture was stirred for 1 hour in a shaker after the solution was in the refrigerator during the night. Then, 2 mL of the cycle hexane were added, and the mixture was shacked vigorously for 15 minutes and centrifuged at 3,500 rpm. The supernatant material was collected and put to 4 mL valets. Then 2.25 mL of sodium chloride were added, Shaked, and centrifuged at 3,500 rpm. The supernatant was collected again into the 4 mL valets, allowed to evaporate in the vaporizer. Two mL of hexane + anhydrous sulphate was added and vortexed, centrifuge at 3,500 rpm for 5 minutes, and then filtered and injected on the in HPLC.

Vitamin E contents were determined by HPLC with fluorescence detection (Jasco, Japan) according to the international standard method ISO 9936, with slight modifications. An accurate solution was quickly prepared by diluting flour in n-hexane; an appropriate amount of the internal standard solution (tocol) was added and homogenized by stirring. For the separation of different vitamin E compounds, 20 µL sample was injected into a normal phase silica column (Supelcosil TM LC-SI; 7.5 cm × 3 mm; 3µm) (Supelco, USA), conditioned at 25 °C (ECOM, ECO 2000, Czech Republic) and eluted with a mobile phase of 1,4-dioxane in- hexane (2.5%, v/v), at a flow rate of 0.75 mL/min. Detection was programmed for excitation at 290 nm and emission at 330 nm. The different compounds of vitamin E (α-tocopherols) were identified by comparing the retention times with authentic standards and quantified by individual calibration curves.

Total Carotenoid Content (TCC) estimation was based on the method described *(*Cruz and Casal, 2018*)*. The adequate pulp samples mass was dissolved in an acetone-hexane mixture (4:6, v/v) and the absorbance readings were performed with a SPECTRO Star Nano equipment (BMG LABTECH GmbH, Germany) at the wavelengths 663, 645, 505, and 453 nm, following the calculations given by the authors. Total carotenoids were expressed in mg/100 g of pulp.

2.6. Phenolic and Antioxidant activity determination

Samples were previously prepared for the extraction process. Five grams of pulp were mixed with 50 mL of methanol: water solution (80:20). The mixture was homogenized in an Ultra-Turrax (T18 Basic; IKA Works, Inc., Wilmington, NC, USA) at 24000 rpm for 30 seconds, then put under agitation (100 rpm) for 1 hour at 20°C, away from the light. Samples were then centrifuged at 4000 rpm, 4°C, for 10 minutes, and the supernatant was filtered with 0.45 µm pore size filters (Chromafil® PET - 45/25, Macherey-Nagel, Germany).

Total phenolic content was estimated as the concentration of gallic acid equivalents (GAE, mg/L), according to the Folin-Ciocalteau method (Re et al., 1999). Fifty µL of the sample (supernatant), 50 µL of Folin-Ciocalteau reagent, 1 mL of 75 g/L sodium carbonate, and 1.4 mL of deionized water were sequentially added; the samples were agitated using a vortex and kept away from light for 1 hour. The absorbances were read at 750 nm in triplicate.

Antioxidant activity was determined using ABTS (2,2-Azinobis, 3-ethylbenzthiazoline-6-sulphonic acid radical) and DPPH (diphenyl-1-picrylhydrazyl radical) methods. However, both methods are based on an electron transfer and involve oxide-reduction reactions. DPPH, insoluble in water, is more efficient for measuring less polar compounds. In contrast, ABTS, soluble in both water and organic (alcoholic) solvents, is more suitable for measuring water-soluble compounds. The ABTS procedure was based on the methodology proposed by (Gião et al., 2007) and, later, modified (Bondet et al., 1997) 20 µL of each sample (previously prepared) was added to 1 mL of ABTS diluted solution, and the absorbance was read at 734 nm for 6 min. Each sample was read in triplicate. Total oxidant capacity was expressed as a percentage of inhibition (PI), as shown below:

$$PI = \frac{Abs_{ABTS}^{\bullet +} - Abs_{sample}}{Abs_{ABTS}^{\bullet +}} \times 100,$$

Where $Abs_{ABTS}^{\bullet +}$ corresponds to the initial absorbance of diluted $ABTS^{\bullet +}$ and Abs_{sample} corresponds to the absorbance of the sample after 6 min of reaction. Quantitative results were obtained by correlating PI of standard solutions and relative absorbance; quantitative results were obtained. Ascorbic acid was used as standard. The DPPH procedure followed the method proposed (Bondet et al., 1997), with few modifications: 250 µL of the sample was added to 1.75 mL of methanolic DPPH• solution (freshly prepared) and allowed to react for 30 minutes. The absorbance was read at 515 nm. All readings were in triplicate. Total oxidant capacity was expressed as a percentage of inhibition (PI), as shown below:

$$PI = \frac{Abs_{DPPH}^{\bullet} - Abs_{sample}}{Abs_{DPPH}^{\bullet}} \times 100,$$

where $Abs_{DPPH}^{\bullet}$ corresponds to the initial absorbance of DPPH$^{\bullet}$ solution and Abs_{sample} corresponds to the absorbance of the sample after 30 min. Trolox was used as the standard. By the correlation of PI of standard solutions and relative absorbance, quantitative results were obtained.

2.7. Statistical Analysis

Results were expressed as the mean ± standard deviation. The results were analyzed statistically by one-way analysis of variance (ANOVA), followed by Tukey post hoc test considering a p-value < 0.05 to denote statistically significant differences. The Statistical analyses, including the Principal Component Analysis, were performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, La Jolla, California USA).

3. Results and Discussion

3.1. Physicochemical and proximate characterization

In Table 1, color, pH, and % of total soluble solids (TSS) as principal physicochemical ripening characteristics of macuácua pulps indicate some differences ($p < 0.05$). The CIE L*a*b coordinates that only the Chicualacuala sample differed with Lower green-red (a*) and blue-yellow (b*) indicating an opaque yellowish color compared to the other samples. pH as an acidity indicator pointed the Chókwè pulp to own an inferior value, though all the samples were around 6. Likewise, the latter pulp presented the higher %TSS and the Chicualacuala the Lower (19.1 ± 0.72 and 10.9 ± 0.6, respectively).

The presented values agree with those reported for ripped macuácua pulp collected from Marracuene District (Khan et al., 2019). In general, *Strychnos* spp, including macuácua, is commonly harvested in a ripen state, and its physicochemical, sensory characteristics are also dependent on environmental factors like soil, geographical location, and climatic differences (Ngadze et al., 2017a). It was reviewed that the presence of tannins, carotenoids, and a variety of phenolic compounds in the pulp can affect some sensory attributes, including color (Ngadze et al., 2017a). Likewise, the accumulation of organic acids, sugars and the breakdown of superior sugars into the smallest (sucrose to glucose and fructose) occur along with pulp ripening (Ngadze et al., 2017a). Therefore, the high %TSS also indicates a lower pH value observed among Chókwè and Chicualacuala pulps. A high pH value (around 6) is characteristic of *S. madagascariensis*, compared to low (2 - 4) of other *Strychnos* spp (Khan et al., 2019; Omotayo and Aremu, 2021).

Macuácua fruits are essential indigenous products to improve food security thus are highly appreciated due to their nutrient composition (Khan et al., 2016). In the proximate composition of the pulps from the four provinces (see Table 1), moisture % was observed in an interval from 61 to 78% following the sequence: Chókwè< Manhiça< Marracuene< Chicualacuala, with significant differences among the samples. Similarly, carbohydrates were the primary nutrient in all the samples ($p < 0.05$); being higher % in Manhiça pulp (16.04 ± 0.1) and lower in Marracuene (10.4 ± 0.1) pulp. Contrariwise, the Chókwè pulp was statistically superior in fiber % (9.6 ± 0.8) and Chicualacuala pulp the inferior (3.7 ± 0.28); the remaining two samples were around 6% ($p < 0.05$). The fat % was also higher in the Chókwè pulp ($p < 0.05$) (12.5 ± 0.2) followed by Manhiça (8.4 ± 0.80), Marracuene and Chicualacuala where inferior in fat (~4%) and did not statistically differ. Regarding protein, the percent of Marracuene and Manhiça presented a higher content (1.5%) ($p > 0.05$), statistically superior to Chókwè and Chicualacuala (~1.1) ($p > 0.05$). Despite that ash % was higher in Chókwè pulp (2.1 ± 0.34), no statistical differences were found among the samples (around 1.5%).

Previously, it was reported moisture values around 60-80%, carbohydrates 15-61%, fiber 3-9%, fat 20- 64%, protein 3-5%, and ashes 0.5-5% for *Strychnos* spp including macuácua (Hassan et al., 2014; Khan et al., 2016, 2019; Omotayo and Aremu, 2021; Ngadze et al., 2017a). Therefore, the values agree with the previously cited reports, with variations related to the fruit origin.

Table 1. Characterization physicochemical the fresh fruit of macuácua (*Strychnos madagascariensis)* from four districts in southern Mozambique.

Sample origin	L	a*	b*	pH	Total soluble solids (TSS) %	Moisture %	carbohydrates %	Fiber %	Fat %	Proteins %	Ash %
Marracuene	32.37 ± 6.7^{a}	10.00 ± 1.1^{a}	36.70 ± 4.0^{a}	6.39 ± 0.44^{a}	12.30 ± 0.31^{a}	76.01 ± 0.2^{a}	10.41 ± 0.1^{a}	6.7 2 ± 0.06^{a}	4.01 ± 0.1^{a}	1.51 ± 0.03^{a}	1.50 ± 0.34^{a}
Manhiça	30.20 ± 0.9^{a}	9.90 ± 0.7^{a}	38.51 ± 2.8^{a}	6.26 ± 0.01^{a}	17.22 ± 0.21^{b}	67.02 ± 0.1^{c}	16.42 ± 0.1^{c}	5.81 ± 0.54^{c}	8.40 ± 0.2^{b}	1.50 ± 0.01^{a}	1.31 ± 0.19^{a}
Chókwè	29.40 ± 7.4^{a}	9.70 ± 1.1^{a}	42.5 2± 5.0^{a}	6.17 ± 0.06^{b}	19.1 1 ± 0.72^{c}	61.01 ± 0.2^{d}	13.40 ± 0.3^{d}	9.6 1 ± 0.80^{d}	12.5 0 ± 0.2^{c}	1.10 ± 0.03^{b}	2.1 1 ± 0.34^{b}
Chicualacuala	30.8 0 ± 1.6^{a}	8.10 ± 0.4^{b}	32.71 ± 2.3^{b}	6.31 ± 0.01^{a}	10.92 ± 0.6^{d}	78.03 ± 0.1^{b}	11.9 1 ± 0.1^{b}	3.71 ± 0.28^{b}	4.70 ± 0.1^{a}	1.10 ± 0.01^{b}	1.1 0 ± 0.02^{a}

Results are presented as the mean ± standard error of the mean, n =6. Means with different letters in the same column represent values significantly different (P-value < 0.05). L a* b* represents CIELAB color space, where L represents lightness from black to white on a scale of zero to 100, while a* and b* represent chromaticity with no specific numeric limits. Negative a* corresponds with green, positive a* corresponds with red, negative b* corresponds with blue and positive b* corresponds with yellow..

3.2. Determination of minerals

Table 2 shows the mineral composition of fresh *S. madagascariensis* fruit pulp. The prevailing elements in all four districts are potassium, magnesium, and calcium. The results showed that the *S. madagascariensis* is rich in potassium with values ranging from 506.4 ± 41.5 mg/100g (Manhiça) as the lowest, and 621.6 ± 41.5 mg/100g (Chicualacuala) as the highest. There was a significant difference ($p < 0.05$) between all districts except Marracuene and Chókwè, which did not differ significantly. Compared to the common potassium food source like peeled bananas (241 mg/100 g) (Wolmarans et al., 2010) and avocado (345 mg/100 g) (Dreher and Davenport, 2013), macuácua fruit has a substantially high amount. In general, the *Strychnos* species fruits are very rich in potassium as reviewed by Ngadze et al. (2017a), for *S. spinosa* (1341.4 mg/100 g*)*, *S. innocua* (1561.7 mg/100 g), and *S. pungens* (1718 mg/100 g), although expressed as dry. Potassium is an essential mineral of great importance for health. It brings several benefits, plays a fundamental role in the heart, bones, and kidneys, and improves muscle, nervous, and cardiac function (Magaia et al., 2013).

In general, the Chicualacuala district showed significantly ($p < 0.05$) higher amounts of almost all micronutrients detected in the Marracuene district. The reason for the wide variations in mineral content between samples of different districts might be due to differences in the topography of the soil and agro-climatic condition (Ministério da Administração Estatal, 2005).

Table 2. Mineral content mg per 100 g of fresh macuácua pulp from the southern provinces of Mozambique.

Element	Marracuene	Manhiça	Chókwè	Chicualacuala
Essential macrominerals (mg/100g)				
Ca	11.5 ± 0.3^{a}	9.1 ± 0.5^{b}	10.6 ± 0.6^{a}	15.4 ± 1.0^{c}
Mg	27.8 ± 1.8^{a}	29.9 ± 2.2^{b}	34.8 ± 2.6^{b}	33.5 ± 0.6^{b}
K	557.7 ± 2.7^{a}	506.4 ± 41.5^{b}	588.8 ± 48.3^{a}	621.6 ± 41.5^{c}
Na	1.7 ± 0.0^{a}	1.5 ± 0.0^{b}	1.7 ± 0.0^{a}	2.3 ± 0.1^{c}
Essential trace elements (µg/100g)				
Fe	216.0 ± 11.0^{a}	ND	ND	181.1 ± 13.0^{b}
Zn	99.0 ± 2.0^{a}	44.0 ± 1.0^{b}	51.0 ± 1.0^{b}	93.0 ± 5.0^{a}
Mn	666.0 ± 65.0^{a}	525.0 ± 12.0^{b}	611.0 ± 14.0^{a}	961 ± 30.0^{c}
Cu	195.0 ± 16.0^{a}	67.0 ± 2.0^{b}	78.0 ± 2.0^{b}	216.0 ± 2.0^{a}
Cr	14.0 ± 3.0^{a}	26.0 ± 1.0^{b}	31.0 ± 1.0^{c}	13.0 ± 1.0^{a}
Co	1.0 ± 0.0^{a}	0.4 ± 0.1^{b}	0.5 ± 0.1^{ab}	0.7 ± 0.1^{c}
Non-essential trace elements (µg/100g)				
Al	105.0 ± 4.0^{a}	55.0 ± 5.0^{b}	64.0 ± 6.0^{b}	46.0 ± 5.00^{c}
Rb	844.0 ± 63.0^{a}	747.0 ± 13.0^{b}	868.0 ± 15.0^{a}	767.0 ± 6.0^{b}
Ni	60.0 ± 2.0^{a}	10.0 ± 0.1^{b}	12.0 ± 0.1^{b}	63.0 ± 1.0^{a}
Sr	66.0 ± 1.0^{a}	105.0 ± 3.0^{c}	122.0 ± 4.0^{b}	101.0 ± 3.0^{c}
Ba	6.0 ± 0.0^{a}	11.0 ± 1.0^{b}	13.0 ± 1.0^{c}	12.0 ± 0.2^{c}
Cd	0.6 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	1.2 ± 0.0^{c}

Results are presented as the mean ± standard error of the mean, n = 6. Means and standard deviation with different superscript letters in the same row represent values significantly different (P-value < 0.5). Not detected (ND).

3.3. Vitamins and Antioxidant assessment

Fruits are highly appreciated as sources of vitamins and phytochemicals with health-promoting properties. Vitamin A is commonly associated with undernourishment in developing countries; its deficiency causes the fatality of viral infections, especially those associated with the respiratory system in children (Trujillo-Mayol et al., 2021). β-Carotene is the principal precursor of vitamin A in food (Dias et al., 2021). Consequently, the concentration (mg) of β-carotene and other carotenoids was considered to calculate the vitamin A per 100 g of pulp (see Table 3).

Table 3. Content of Vitamin E, Carotenes and Phenolic Content, and Antioxidant activity of macuácua pulp from four districts of southern Mozambique

mg/ 100g	Marracuene	Manhiça	Chókwè	Chicualacuala
Vitamin E	3.31 ± 0.01^{a}	4.32 ± 0.01^{b}	3.60 ± 0.00^{c}	8.43 ± 0.08^{d}
β-Carotene	1.48 ± 0.01^{a}	3.20 ± 0.09^{b}	4.82 ± 0.04^{c}	1.65 ± 0.03^{d}
TCC	5.86 ± 0.12^{a}	7.60 ± 0.36^{b}	11.36 ± 0.03^{c}	5.81 ± 0.12^{a}
Vitamin A (RE)	0.61 ± 0.01^{a}	0.90 ± 0.04^{b}	1.35 ± 0.01^{c}	0.63 ± 0.01^{a}
TPC (GAE)	208.24 ± 3.27^{a}	168.157 ± 7.07^{b}	256.65 ± 26.78^{c}	129.94 ± 6.50^{d}
DPPH (TE)	46.00 ± 1.34^{a}	47.51 ± 4.08^{a}	44.61 ± 2.95^{a}	41.88 ± 2.06^{a}
ABTS+ (AcE)	5.74 ± 0.31^{a}	5.32 ± 0.31^{a}	6.18 ± 0.72^{b}	4.73 ± 0.11^{c}

Mean and standard deviations with different superscript letters in the same row represent values significantly different (P-value < 0.05). The different vitamin E compounds were expressed as mg of tocopherol/100 g of fruit. Total Carotenoid Content (TCC) expressed as β-carotene equivalents. Vitamin A was calculated based on the conversion of β-carotene and other carotenes to retinol equivalents (1 mg RE = 6 mg of β-carotene = 12 mg of other carotenoids) according with EFSA (2019). Total Phenolic Content (TPC) expressed as mg of gallic acid equivalent (GAE). 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical expressed in mg Trolox equivalent (TE) and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) radical expressed in mg of ascorbic acid equivalent (AcE).

In Table 3 it is possible observe that vitamin A content is in the range from 0.61 ± 0.01 to 1.35 ± 0.01 mg/100g of sample in the sequence: Chókwè > Manhiça > Chicualacuala > Marracuene. In general significant differences were observed among communities ($p < 0.05$), except for Chicualacuala and Marracuene. Together with vitamin A, the vitamin E is a precise indicator of the antioxidant balance of the organism (Márquez et al., 2002). Tocopherols and tocotrienols are generically described as vitamin E (Alissa and Ferns, 2012; Gowele et al., 2019). This liposoluble vitamin is of great interest to the consumer due to its beneficial health effects and as an alternative as a natural antioxidant for the preservation of food products (Fearne et al., 2012). Vitamin E is easily found in foods with high levels of lipids. Evidence suggests that it prevents or minimizes the damage caused by free radicals associated with some specific diseases, such as cancer, arthritis, cataracts, and other aging-related affections such as dementia and Alzheimer's (Alissa and Ferns, 2012; Lewis et al., 2019). The content of vitamin E (expressed as mg of tocopherol/100 g of pulp) differed statistically in all the samples (see Table 3). The higher content was observed in Chicualacuala pulp (8.43 ± 0.08 mg of tocopherol/100 g of pulp) and the lower in Marracuene (3.31 ± 0.01 mg of tocopherol/100 g of pulp), Manhiça and Chókwè displayed middle values (3.60 and 4.32, respectively). With the presence of vitamins, PCs also synergistically provide antioxidant activity.

Considered naturally occurring antioxidants, carotenoids and PCs are presented in fruits in high concentrations (Alissa and Ferns, 2012). In developing countries, the consumption of fruits rich in antioxidants decreases with the increase of economic income of the population; thus, the risk of non-transmissible diseases and the fatality of the transmissible illnesses increase (Kucich and Wicht, 2016; Trujillo-Mayol et al., 2021). Specifically, indigenous fruits such as macuácua have been related to prevent and cure illnesses due to the antioxidant composition; then, its consumption shall be promoted (Aparicio et al., 2021; Kucich and Wicht, 2016; Ngadze et al., 2017a; Oboh et al., 2020). In this sense, liposoluble pigments, namely carotenoids, are potent antioxidants with health-promoting properties (Gowele et al., 2019). TCC (expressed as mg of β-carotene/100g) displayed in Table 3, was found ($p < 0.05$) superior in Chókwè pulp (11.36 ± 0.03 mg of β-carotene equivalents/ 100 g), followed by Manhiça (), and lower in Marracuene and Chicualacuala that did not statistically differ (~5.8 mg of β-carotene/100g), although some variability can be derived from different storage conditions after picking, or environmental conditions (Boukandoul et al., 2017), the amount of carotene follows the same trend of total fat. The presence of other polar and less polar PCs in the assessed pulps expressed generally as TPC in mg GAE in fruits (Almeida et al., 2011), varied ($p < 0.05$) in all the samples (see Table 3); it was found higher in Chókwè (256.65 ± 26.78), then in Marracuene (208.24 ± 3.27), Manhiça

(168.15 ± 37.07) and finally, in Chicualacuala (129.93 ± 6.50) per 100 g of pulp. The radical scavenging capacity expressed by DPPH (mg Trolox/100 g) and ABTS (mg of ascorbic acid/100 g) indicators showed similar antioxidant activity against the first radical in all the assessed samples ($p > 0.05$) (~45 mg Trolox/100 g). Nevertheless, the latter radical was ($p < 0.05$) strongly reduced by Chókwè (6.18 ± 0.71), and weaker by Chicualacuala (4.71 ± 0.11). In comparison, Manhiça and Marracuene extract presented an average anti-ABTS activity (~5.5) ($p > 0.05$). Interestingly, the results indicated a correlation ($p<0.05$) in TPC and ABTS, also observed in other tropical fruits (Almeida et al., 2011).

The literature provides very little information about bioactive compounds in macuácua (*S. madagascariensis*) and other orange monkey fruits. Khan et al., 2016 reported β-carotene content of macuácua pulp (3.83 mg/100g) in agreement with our results (1.40 to 4.82 mg/100g) and observed that *S. madagascarienis* presented higher β-carotene content compared to masala pulp (*S. spinosa*), although there is no information about the vitamin E. Compared with other fruits and vegetables, the total carotene content of macuácua fruit (5.8 to 11.4 mg/100g) is outstanding. Raw carrots, sweet-potato and spinach presents carotene content of 5.6, 3.9, 3.3 mg of carotenes/100g, respectively. Considering other fruits: fresh mango, persimmon, and papaya presents 1.8, 1.1 and 0.8 mg of carotenes/100g, respectively and dried apricot presents 2.5 mg of carotenes/100g.

Aldough the vitamin C were not evaluated in the present study, high content have been described in *Strychnos* spp (88-268.8 mg/100 g), similar to other tropical fruits (Khan et al., 2016). Recently, it was reported that the aqueous extracts of *S. madagascariensis* pulp displayed inhibitory activity against DPPH and ABTS radicals IC50: 0.012 and 0.06 mg/mL, respectively. Besides, it inhibited α-amylase, α-glucosidase, and pancreatic lipase, so it was concluded that this fruit could be used to control diabetic Mellitus (Oboh et al., 2020). Also, the incorporation of *S. cocculoides* juice improved the maize diet characteristic of some southern African countries attributable to the high nutritional factors and phenolic acids of this fruit (Ngadze et al., 2019).

Comparing the samples, the high antioxidant activity of Chókwè fruit is probably given by the high content of vitamin A, TCC, and TPC compared to the other pulps. Even though there is no data about the phytochemical composition of those fruits related to the origin, Magaia et al., 2013, did not find differences in the dietary fiber, organic acids, and mineral wild fruits from Mozambique (*Adansonia digitata*, *Landolphia* *kirkii, Salacia kraussii, Sclerocarya birrea, and Vangueria infausta*). Contrarywise, this work observed differences among the macuácua pulps related to the origin.

3.4. Principal Components analysis

Figure 1 summarizes the overall statistical treatment of the data obtained for the four assessed macuácua pulps represented by principal component analysis (PCA). The associations between the pulp of macuácua from the four provinces of Mozambique (Marracuene, Manhiça, Chókwè, and Chicualacuala) are highlighted, in terms of physicochemical parameters, proximate composition, mineral content, and antioxidants.

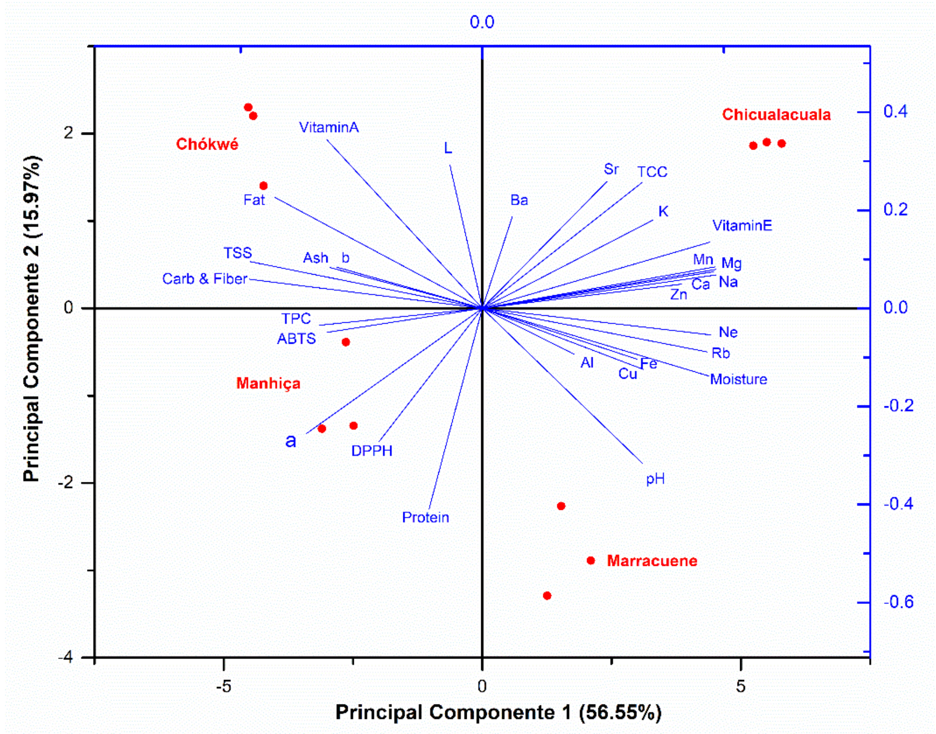

Figure 1. Principal Component Analysis of Physicochemical parameters (colour [L a* b*]) pH, Total soluble solids [TSS]), proximate composition (moisture, Carbohydrates and fibber, Fat, Proteins, ash), minerals (calcium [Ca], Magnesium[Mg], sodium [Na], potassium [K], iron [Fe], Zinc [Zn], aluminium [Al], manganese [Mn], nickel [Ne], strontium [Sr], rubidium [Rb], barium [Ba]), vitamin A, vitamin E, Total Carotenoid Content (TCC), Total Phenolic Content (TPC), antioxidant activity expressed as DPPH and ABTS scavenging capacity of the four assessed macuácua pulps.

According to the PCA results, two principal components explained 72.52% of the data variance; the first component was 56.55%, while the second component was 15.97%. It is possible to associate two groups; the first on the left side, Chókwè and Manhiça sharing the antioxidant activity, carbohydrates and fiber, fat, and protein principally, and the second on the right side, Marracuene and Chicualacuala sharing mainly the mineral content, however, the last sample associated most of the minerals and vitamin E. Likely, Chókwè pulp gathered primarily the antioxidant activity, fat, protein and vitamin A, linked to the color coordinates and TSS.

It has been reported that in the pulp of the fruits, the color intensity is associated with high antioxidant capacity given by the phenolic compounds and other associated molecules such as sugars (Ngadze et al., 2017a). This is common in fruits under biotic and abiotic adverse conditions, including geographical, soil and climatic conditions (Trujillo-Mayol et al., 2020)**.** Marracuene district has colic sandy highlands (to the west and along the coast) with siliceous areas. It has a plain along the Incomáti river and clayey, stratified, and tufted soils. The district of Manhiça has sandy soils (west along the coast) and an area of coastal dunes, an alluvial plain, with less than 100 m along the Incomáti river, with clayey soils with an esterified or peat texture. The Chókwè is plain with less than 100 m of altitude made up of alluviums along the Limpopo River that crosses the entire district. In some areas there is red clay and finally, the district of Chicualacuala, which has soils (sandy, clayey, and hydromorphic). Those features could influence the macuácua pulp composition.

4. Conclusions

Macuácua (*Strychnos madagascariensis*) is an essential indigenous food to support food security in southern Mozambique, where it principally grows. The macuácua pulp from Marracuene, Manhiça, Chókwè, and Chicualacuala districts were evaluated to define if there are differences among the physicochemical parameters, proximate composition, mineral content, and antioxidants. In fact, it was observed that the pulp from Chókwè displayed the higher antioxidant content, fat, protein, and carotenes, while the sample from Chicualacuala had the higher mineral content and vitamin E. Thus, here it was demonstrated the origin influence on the nutritional and antioxidant aspects of the fruits.

CHAPTER 4 - NUTRITIONAL CHARACTERIZATION OF *STRYCHNOS MADAGASCARIENSIS* FRUIT FLOUR PRODUCED BY MOZAMBICAN COMMUNITIES AND EVALUATION OF ITS CONTRIBUTION TO NUTRITIOENT ADEQUACY

In this chapter macro and micronutrients of *nfuma* are presented, as well as the evaluation of nutritional adequacy in terms of nutrients considering current nutritional recommendations.

ABSTRACT

The indigenous fruit *Strychnos madagascariensis* is usually processed to flour, called *nfuma,* being highly consumed during staple food shortage. This study aimed to evaluate the nutritional composition of *nfuma* and its nutrient adequacy. Flours from four districts of Mozambique were analyzed using AOAC methods for proximate composition, HPLC for sugar, amino acids (AA), vitamin E and carotenoids and ICP-MS and FAAS for minerals. Results showed that *nfuma* stands out for its high content of fat (26.3-27.8%), mainly oleic acid, fiber (> 6%), vitamin E (6.7 to 8.0 mg/100g) and carotenes (2.2 to 2.6 mg/100g). The main AA of *nfuma* protein were Arg, Asp and Glu, and Lys was the limiting AA. The mineral composition reveals K (~1200 to 1700 mg/100g) as the main macro-mineral followed by Mg > Ca > Na. The main trace element was Mn (~4 mg/ 100g) followed by Fe > Zn > Cu > Cr > Co. Aluminium (~3 mg/ 100 g) was the main non-essential element and Rb, Ni, Sr, Ba, V, Cd were also quantified. Assuming the daily consumption of 50 g, *nfuma* provides 82% of Vitamin A DRV for toddlers, while the consumption of 100 g contributes to 132% and 60% of Mn and vitamin A DRV for adults, respectively. Despite the nutritional advantages of *nfuma*, this flour can be a relevant source of Ni, highlighting the importance of the study of good practices in its preparation to decrease the exposure to non-essential elements. the most abundant element (~3000 µg/100g) and Cd was the less abundant (~2 µg/100 g).

KEYWORDS: Monkey orange, Fruit flour, Macronutrients, Micronutrients, Indigenous fruits, Estimated daily intake

1. Introduction

In recent years, the increased knowledge about the protective role of fruits and vegetables, has led to an increase in campaigns promoting their consumption for better health (Rekhy and McConchie, 2014). Although fruits and vegetables can be eaten *in natura*, since fresh products are highly perishable, they can be processed to increase their shelf life and maintain (or even improve) their nutritional quality and sensory characteristics (Brito et al., 2017).

Indigenous fruits have been receiving considerable attention from the scientific community as they can be important contributors to the diet of people in developing countries, reducing nutritional deficiencies and food insecurity, as well as improving the health and economic status of those populations (Nyanga et al. 2013; Stadlmayr et al. 2013). In addition, they can be exploited by the agro-industry and become a source of income for local communities in the future (Magaia et al. 2013; Van Wyk 2011). Indigenous fruits are easily accessible to the most vulnerable people because fruit trees are not farmed and often grow in forests and around homes and fields. However, in African countries, indigenous fruits are still underutilized, while several communities are food insecure and, consequently, malnourished (Bvenura and Sivakumar, 2017; Kuhnlein and Johns, 2003; Van Wyk, 2011).

Strychnos spp (monkey orange) is an indigenous fruit tree known for its edible fruits and drought tolerance. However, it has been labeled as a “lost fruit” (National Research Council, 2008) and little attention was given to its potential commercialization due to limited knowledge and disseminated information compared to many other exotic fruits (Ngadze et al., 2017a).

Despite some imprecision in species differentiation, five species are prevalent and consumed in Southern Africa (*S. innocua*, *S. cocculoides, S. pungens* and *S. spinosa, S. madagascariensis*). Due to their seasonality and high perishability, traditional fruit processing is a very common practice in addition to their immediate consumption as fresh fruits, with *S. madagascariensis* and *S. innocua* being processed preferably into dry products (Ngadze et al., 2017b). Significant variations in the nutritional composition of these fruits have been reported in the literature, and scarce information is available concerning their processed products (Ngadze et al., 2017a).

Mozambique has numerous native and exotic fruit species that are important for rural communities' survival in times of food shortages. The *S. madagascariensis* fruit, known in southern Mozambique as macuácua, can be consumed immediately in natura, but it is usually processed into flour by local communities to increase the stability and shelf life of the fruit. Once harvested, the fruit pulp is first dried under the sun and roasted over a fire, and then ground to produce *nfuma* flour, which is consumed by local communities as a snack or as a complement of staple foods in times of food scarcity. However, there is practically no data on its nutritional value. Thus, this study aimed to evaluate the nutritional composition of *nfuma*, the fruit flour of *S. madagascariensis*, and its adequacy in terms of nutrients in light of current recommendations.

2. Materials and methods

2.1. Fruit collection and Flour Preparation

Fruit samples were collected by residents of four different districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique. A total of 360 fresh fruits were harvested per region (eight randomly selected trees; 15 fruits per tree; three different times), during the summer.

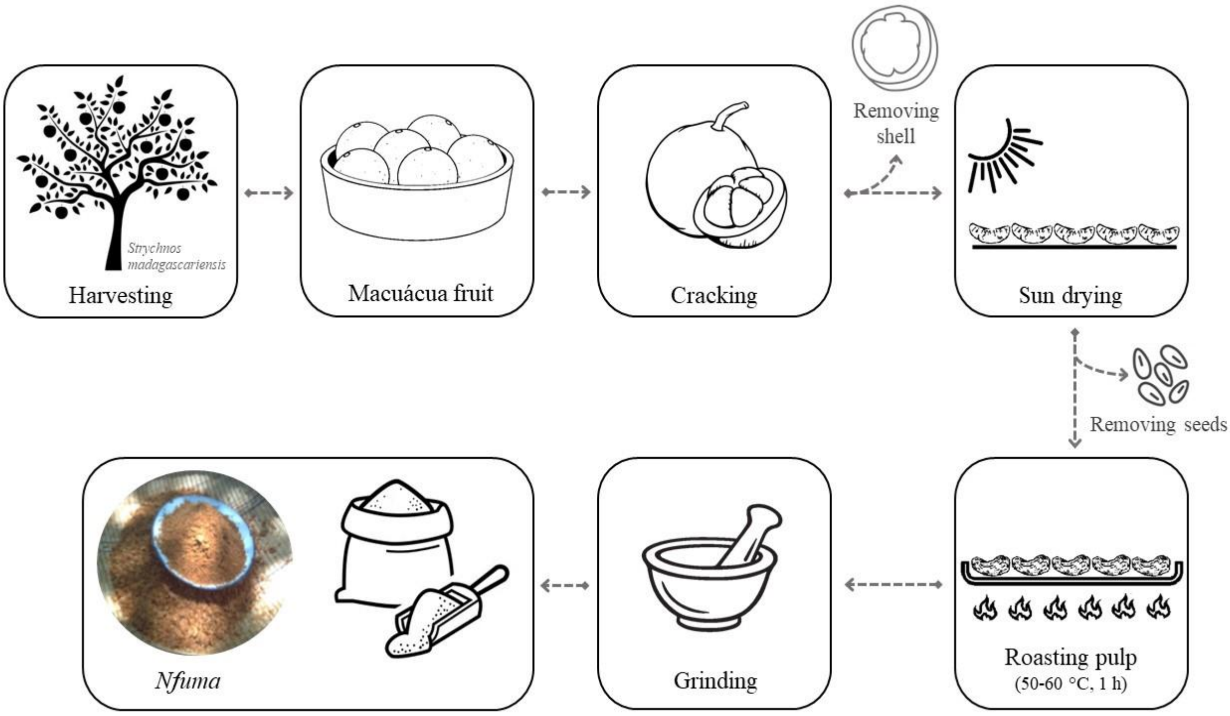

Figure 1. Scheme of *nfuma* preparation by Mozambican communities: from *Strychnos madagascariensis* fruit to flour.

Nfuma was traditionally prepared by local residents. Briefly, 120 ripe fruits were broken and the orange pulp with seeds was left to dry in the sun (2 to 4 days), to facilitate the removal of the seeds. Then, the pulp was roasted at about 50-60 °C (temperature measured using a digital thermocouple with a surface probe), on a metal plate under fire, for about 1 hour.

The dried pulp was then ground into flour using a pestle and mortar, producing about 5 kg of flour. The process was repeated three independent times per region. A schematic description of the sampling methodology is provided in Figure 1.

2.2. Chemical Analysis

2.2.1 Proximate composition

Moisture, crude fat, protein and ash contents were determined according to the Association of Official Analytical Chemists (AOAC) methods 925.09, 920.39, 992.15 and 923.03, respectively (AOAC 2005). Total dietary fiber was determined by the enzymatic-gravimetric method based on American Association of Cereal Chemists 32-05.01 method and AOAC 985.29 method, according to Martins et al. (2017). Carbohydrates were calculated by the differential method and the energy value was determined based on Regulation (EU) No 1169/2011 (European Commission, 2011). That is, the energy content was calculated from the amount of protein, fat, available carbohydrates and fiber using the factors 17, 37, 17 and 8 kJ per gram (4, 9, 4 and 2 kcal per gram), respectively.

2.2.2. Low weight carbohydrates determination by HPLC-RI

The low molecular weight carbohydrates (mono and disaccharides) were deter-mined by high-performance liquid chromatography with refractive index detection (HPLC-RI), as described by Santos et al. (2016) with some modifications. Briefly, five hundred milligrams of flour were accurately weighed into a centrifuge tube. Prior to sugar extraction, the oil was removed from the flour with the aid of three 5 mL portions of hexane, discarded after centrifugation, and the solid residue was left under a nitrogen stream to evaporate the solvent. The defatted flour was then mixed with 5 mL of ethanol (50% v/v), for sugar extraction. The suspension was stirred for 30 s and the extraction was carried out in an ultrasonic bath (FungiLab, Barcelona, Spain) for 30 min at 50 °C. Thereafter, the mixture was centrifuged at 5000g at 4 °C for 10 min and 2 mL of the supernatant were left under a nitrogen stream to reduce the ethanol fraction. The final volume was then rigorously adjusted to 2 mL with acetonitrile and allowed to stand for 20 min. The final solution was centrifuged at 5000g for 10 min at 4 °C and filtered through a 0.22 µm PTFE filter prior to injection.

2.2.3. Fatty acids composition by GC-FID

The fatty acid composition of extractable lipids was evaluated as methyl esters derivatives by gas chromatography with flame ionization detection (GC-FID), using alkaline trans-esterification with methanolic potassium hydroxide, as detailed in Regulation EEC 2568/91 (European Commission, 1991). The analysis was performed using a Chrompack CP 9001 gas chromatograph (Middelburg, the Netherlands), equipped with a split-splitless injector, a flame ionization detector, an autosampler (Chompack CP-9050) and a 50m x 0.25mm id fused silica capillary column coated with Select-FAME. Helium was used as carrier gas at an internal pressure of 120 kPa. The detector and injector temperatures were 250 and 230°C, respectively. The results were initially expressed as a relative percentage of each fatty acid methyl ester, without discriminating positional and geometric isomers, calculated by internal normalization of the chromatographic peak areas after standardization of the detector response with the certified reference standard, and converted to the flour mass based on the determined fat content.

2.2.3.1. Atherogenicity and thrombogenicity indexes

The nutritional quality parameters, atherogenic (AI) and thrombogenic indexes (TI), were calculated according to Ulbricht and Southgate, 1991:

$$\mathrm{AI} = \frac{\mathrm{C12{:}0} + 4 \times \mathrm{C14{:}0} + \mathrm{C16{:}0}}{\mathrm{MUFA} + \mathrm{n3\ PUFA} + \mathrm{n6\ PUFA}}$$

$$\mathrm{TI} = \frac{\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}}{0.5 \times \mathrm{MUFA} + 0.5 \times \mathrm{n6\ PUFA} + 3 \times \mathrm{n3\ PUFA} + \frac{\mathrm{n3\ PUFA}}{\mathrm{n6\ PUFA}}}$$

where MUFA and PUFA correspond to monounsaturated and polyunsaturated fatty acids, respectively.

2.2.4. Analysis of vitamin E and Carotenoids by HPLC-DAD/FLD

The vitamin E and total carotene contents of the lipid extract were determined by normal-phase high-performance liquid chromatography with diode-array and fluorescence detection (HPLC-DAD/FLD), as described by Cruz and Casal (2018). An exact amount of fat was dissolved in hexane, an appropriate volume of the internal standard solution (tocol) was added, and the mixture was homogenized by stirring. A normal phase silica column (Supelcosil TM LC-SI; 7.5cm × 3mm; 3µm) (Supelco, Bellefonte, PA), conditioned at 25°C and eluted with a gradient of 1,4-dioxane in hexane at a flow rate of 0.75 mL/min was used. Detection was programmed for excitation at 290 nm and emission at 330 nm for tocols and 450 nm for carotenes. The different vitamin E compounds were identified by comparing retention times with standards, and quantified through individual calibration curves, being expressed in mg of tocopherol/ 100g of flour. β-carotene was also quantified based on a calibration curve.

2.2.5. Amino Acid Composition by HPLC-FLD

Amino acids (Asp, Glu, Ser, His, Gly, Thr, Arg, Ala, Tyr, Val, Met, Phe, Ile, Leu, Lys, Pro, Trp, Cys, where Asp means aspartic acid/asparagine and Glu means glutamic acid/ glutamine) were analyzed by HPLC-FLD, after hydrolysis and derivatization with 9-fluorenylmethyl chloroformate and O-phthaldialdehyde, according to Benhammouche et al. (2021). Briefly, about 100 mg of flour (± 3.5 mg of protein) was weighed into a glass crimp vial. 10 mL of hydrochloric acid solution HCl 6 M containing 0.5% (w/v) phenol were added, sealed and the acid hydrolysis was performed at 110 °C for 24 h. 1 mL from the resulting hydrolysate was taken and neutralized with NaOH 6 N, and the final volume was made up to 10 mL with borate buffer (0.1 M). 32 µL of the neutralized solution was mixed with 8 µL of internal standard 250 µM (Norvaline) and 40 µl O-phthaldialdehyde and 20 µL 9-fluorenylmethyl chloroformate were added. Trp was determined separately using alkaline hydrolysis (NaOH 4.2 N, for 18 h at 110 °C). The resulting derivatization products were then subjected to HPLC analysis under the conditions detailed in Benhammouche et al. (2021). Amino acid (AA) content was reported as mg of AA/ g protein.

2.2.5.1. Assessment of Protein Quality

In order to evaluate the quality of protein in *nfuma*, the essential amino acid profile (EAA) scores (EAAS) and EAA index (EAAI) were calculated using the following equations (Friedman, 1996):

$$\mathrm{EAAS} = \frac{\mathrm{EAA}_{\text{test protein (mg/g)}}}{\mathrm{EAA}_{\text{reference protein (mg/g)}}}$$

$$\mathrm{EAAI} = \sqrt[n]{\mathrm{EAAS}\ 1 \times \mathrm{EAAS}\ 2 \times \mathrm{EAAS}\ 3 \times \mathrm{EAAS}\ n} \times 100$$

where n is the number of amino acids included into the calculation. The reference protein used was the FAO/WHO EAAS pattern from the joint FAO/WHO/UNU (2007).

2.2.6. Mineral analysis

Mineral analysis was performed according to (Pinto et al., 2020). Sample mineralization was performed by microwave-assisted closed-vessel acid diges-tion using an MLS-1200 Mega high-performance microwave digestion unit (Milestone, Sorisole, Italy) equipped with an HPR-1000/10 S rotor. Microminerals determination was performed by inductively coupled plasma-mass spectrometry (ICP-MS) using an iCAP™ Q instrument (Thermo Fisher Scientific, Bremen, Germany) and measuring the following elemental isotopes (m/z ratios): ^{7}Li, ^{9}Be, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{85}Rb, ^{88}Sr, ^{114}Cd, ^{133}Cs, ^{137}Ba, ^{205}Tl, ^{208}Pb and ^{209}Bi. The determination of Ca, Mg, Fe, Na and K was performed by flame atomic absorption spectroscopy (FAAS) using a PerkinElmer (Überlingen, Germany) AAnalyst 200 instruments. Limits of detection (LOD) and limits of quantification (LOQ) were estimated from the analysis of 10 digestion blanks. The results are presented in Supplementary material (Table S1). For quality control purposes, the certified reference material (CRM) IRMM 807 (rice flour, supplied by EC Institute for Reference Materials and Measurements, Geel, Belgium) and BCR 679 (white cabbage, supplied by EC Institute for Reference Materials and Measurements, Geel, Belgium) were analyzed under the same conditions as the samples. The results obtained were in good agreement with the certified values (recoveries ranging from 95.7% to 108.6%), proving that the method accuracy was adequate.

2.2.7. Estimated Intake of nutrients and non-essential elements

2.2.7.1. Evaluation of Nutrient Adequacy

The estimated daily intake (EDI) of nutrients and energy, as % of EFSA dietary reference values (DRVs) (EFSA, 2019), was calculated assuming an average daily consumption of 100 g of *nfuma*. DRVs include a set of nutrient reference values: Population Reference Intakes (PRIs), Average Requirements (ARs), Adequate Intakes (AIs) and Reference Intake (RIs). For nutrients that have both PRI and AR, the EDI was calculated based on PRI, since it corresponds to the intake level that meets the needs of all people in a population (EFSA, 2019). Regarding energy, the AR for adults with a physical activity level (PAL) between 1.4 and 2.0 was calculated as the mean value of AR for all age groups between 18 and 79 years. The AI and AR (g/day) for macronutrients were calculated based on the lower and upper limit of AR range for energy.

2.2.7.2. Estimated Intake of Non-Essential Elements

The estimated intake (EI) was calculated based on the elemental content ($C_{element}$: µg/g), the average per capita consumption of *nfuma* (C_{nfuma}: g) and the adult and toddlers standard human body weight (bw) of 70 and 12 kg (EFSA, 2012), respectively, according to the following formula:

$$\mathrm{EWI} = \frac{\mathrm{Celement} \times \mathrm{WCnfuma}}{\mathrm{bw}}$$

The estimated daily intake (EDI: µg/day/kg bw) of Ni, weekly intake (EWI: µg/week/kg bw) of Al and monthly intake (EMI: µg/month/kg bw) of Cd were calculated assuming a C*nfuma* of 100 g, 700 g and 3000 g, respectively.

The obtained EI were expressed as % of the toxicological guidance levels for exposure assessment, namely the tolerable daily intake (TDI) (EFSA, 2020), the Provisional Tolerable Weekly Intake (PTWI) and Provisional Tolerable Monthly Intake (PTMI) (JECFA, 2021), for Ni, Al, Cd, respectively.

2.2.8. Statistical analysis

All samples were analyzed in triplicate. Data were tested for normal distribution of the residuals with Shapiro-Wilk test. The existence of statistically significant differ-ences between means was studied using one-way analysis of variance (ANOVA) when the normal distribution of residuals was confirmed. Welch correction was applied when the homogeneity of variances was not verified. Whenever statistical significance was found, Tukey's or Tamhane's T2 posthoc tests were applied to compare the means, depending, respectively, on equal variance or not. All these statistical analyses were conducted with the XLSTAT for Windows version 2014.5 (Addinsoft, Paris, France) at the 0.05 significance level.

3. Results and Discussion

3.1. Proximate composition, Energy value and Sugar profile

The macronutrient composition of *S. madagascariensis* fruit flour (*nfuma*) is presented in Table 1. The main component was available carbohydrates (49.7-54.9%), fol-lowed by fat (26.3-27.8 %), total fiber (5.8-10.8%), ash (4.8-6.0%), moisture (~4%) and protein (~3%). *Nfuma* provides an energy value of approximately 475-490 kcal/100 g of flour.

Nfuma has available carbohydrates ranging from 49.7 to 54.9%, with the lowest content found in Chókwè and the highest in Marracuene flour. Regarding the free sugars, which together represent ~10% of flour mass, fructose and glucose are the ones present in higher levels, ranging between 3.3-4.3% and 3.7-5.0%, respectively, while sucrose represents only 1.4-1.9% of the flour. No significant differences ($p < 0.05$) were observed between sugars profiles of flours from different communities. The total fiber content of *nfuma* varies significantly between communities, with Marracuene flour having the lowest content of total fiber (5.8%) and Chókwè flour the highest one (10.8%). In fact, the highest soluble fiber content in Chókwè (3.9% vs 0.6-1.4 %) is responsible for the higher total fiber content of these flour since the insoluble fiber value is similar in all flours (5.2-6.9%). Although Ngadze et al. (2017a) in their review reported lower values of total carbohydrates for *Strychnos* spp (*S. innocua*, *S. spinosa*, *S. cocculoides* and *S. pungens*). Kalenga Saka and Msonthi (1994) and Lockett et al. (2000) described a total carbohydrate value of ~60 % for *S. innocua* and *S. spinosa*, respectively. Regarding the sugar profile, the higher ratio of monosaccharides/ sucrose agrees with Wilson and Downs (2012) study where most of the indigenous South

African fruits were monosaccharides dominant. This pattern was also observed in several dried fruits (Donno et al. 2019).

Table 1. Proximate composition, energy value, sugar and fiber profiles (%) of *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

	Marracuene	**Chókwè**	**Chicualacuala**	**Manhiça**	*p*
Components (unit/100 g)					
Moisture (g)	4.4 ± 0.2	4.6 ± 0.0	4.3 ± 0.1	4.4 ± 0.1	ns
Protein (g)	3.1 ± 0.0^{b}	3.2 ± 0.1^{b}	3.0 ± 0.0^{b}	3.5 ± 0.1^{a}	0.003^{**}
Fat (g)	26.3 ± 0.2^{b}	27.0 ± 0.0^{b}	26.9 ± 0.1^{b}	27.8 ± 0.0^{a}	0.016^{**}
Ash (g)	5.5 ± 0.1^{b}	4.8 ± 0.0^{c}	6.0 ± 0.1^{a}	5.0 ± 0.1^{c}	0.020^{**}
Total Carbohydrates$^{\#}$ (g)	60.7 ± 0.1^{a}	$60.5 \pm 0.1^{a,b}$	$59.8 \pm 0.2^{a,b}$	59.3 ± 0.1^{b}	0.026^{**}
Available Carbohydrates$^{\#}$ (g)	54.9 ± 0.1^{a}	49.7 ± 0.1^{d}	52.4 ± 0.0^{c}	53.1 ± 0.1^{b}	0.006**
Sugars					
Fructose (g)	4.0 ± 0.0	3.5 ± 0.1	3.3 ± 0.2	4.3 ± 0.5	ns
Glucose (g)	4.4 ± 0.2	3.7 ± 0.0	3.7 ± 0.1	5.0 ± 1.0	ns
Sucrose (g)	1.4 ± 0.2	1.8 ± 0.4	1.9 ± 0.4	1.8 ± 0.4	ns
Total dietary fiber (g)	5.8 ± 0.1^{c}	10.8 ± 0.0^{a}	7.4 ± 0.2^{b}	6.2 ± 0.1^{c}	0.001^{**}
Insoluble (g)	5.2 ± 0.1	6.9 ± 0.4	6.0 ± 0.2	5.5 ± 0.2	ns
Soluble (g)	0.6 ± 0.1^{c}	3.9 ± 0.4^{a}	1.4 ± 0.01^{b}	0.6 ± 0.1^{c}	0.022^{**}
Energy (kJ)	$2005 \pm 8^{a,b}$	$1982 \pm 1^{b,c}$	19959 ± 1^{a}	2039 ± 3^{a}	0.012^{**}
(kcal)	$480 \pm 2^{a,b}$	$476 \pm 1^{b,c}$	478 ± 1^{a}	489 ± 1^{a}	0.014^{**}

Data expressed as mean ± standard deviation; ns, not significant. Different letters for each district in a row indicate statistically significant differences ($p < 0.05$) between means. ∗ p Values from one-way ANOVA. Means were compared by Tukey's since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). ∗∗ p Values from one-way Welch ANOVA. Means were compared by Tamhane's T2 test since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

Given the total fiber content (ranging between 6 and 11%), *nfuma* can be considered as a food "high in fiber", according to European Commission Regulation No. 1924/2006 (European Commission, 2006), a claim which "may only be made where the product contains at least 6 g of fiber per 100 g". Compared with cassava four (1.6%) and corn flour (2.6%) (INSA, 2007), *nfuma* stands out for its high fiber content. In the study by

Carli et al. (Carli et al., 2016), only coconut (9.4%) and orange (7.6%) flours had similarly high fiber contents. The other fruit flours studied had fiber contents between 2.0-4.9%. Ngadze et al. (2017a) describe a fiber content of 2.5-22.2% dry weight basis (dw) for other *Strychnos* spp, with the mean fiber content of *S. innocua* (9.4%) the most similar to *nfuma*, the *S. madagascariensis* flour.

Nfuma has a high-fat content (26.3-27.8%) (Table 1), with the flour originating in Manhiça having the highest value ($p < 0.05$), with no significant differences between flour from other communities. The fat content of *nfuma* is higher than the values reported by Ngadze et al. (2017a) for all *Strychnos* spp (0.3-20%), except for *S. spinosa*, where a content of 31.2% dw was reported (Kalenga Saka and Msonthi, 1994), which was considered an outlier (Ngadze et al. 2017a). This flour has an unusual high fat content, even when compared with flours from fat-rich fruits, such as coconut and nuts, because those are by-products of oil/ vegetable milk extraction process (Raczyk et al., 2021)

Protein (3.0-3.5%) was the macronutrient found in the lowest content, similar to flours from other fruits such as green bananas (3.4%) (Brito et al. 2017) and other common fruits, as described by Carli et al. (2016) for 10 different commercial fruit flours (1.59-6.59 %). Regarding *Strychnos* spp, Ngadze et al. (2017a) reported a low protein content for monkey orange, especially for *S. innocua* (0.3-11.5%). Amarteifio and Mosase (2009) also found low protein contents in indigenous fruits (1.3-3.7% dw), reporting 3.3% for *S. spinosa*.

The fixed residue (ash) in *nfuma* ranged from 4.8 to 6.0%. The values obtained are similar to those described for *S. innocua* (4.7%) (Bello et al. 2007). Compared to commercial fruit flours studied by Carli et al. (2016), passion fruits, papaya and açai presented values (4.9-6.5%) in the same range of *nfuma*.

The moisture content of *nfuma* ranged from 4.4 to 4.6%, with no significant differences between the different origins (districts). *Nfuma* showed a low moisture content when compared to cereal flours such as maize and wheat (~13.4) (INSA, 2007). Compared to commercial fruit flours, it presented a higher content than coconut flour (3.8 %), but lower than grape (5%) and other fruit flours (up to 12.2%). The low moisture content of *nfuma* together with proper packaging minimizes the risk of microbiological contamination as well as product deterioration during storage and improving shelf life.

3.2. Fatty acids composition and atherogenic and thrombogenic indices

Nfuma has a high percentage of monounsaturated fatty acids-MUFA (~17 g/100g of flour; ~65% of total fatty acids), followed by saturated fatty acids-SFA (~7 g/100g; ~25% of total fatty acids) and polyunsaturated fatty acids-PUFA (~2.5 g/100g; ~9% of total fatty acids) (Table 2).

Table 2. Fatty acids composition (mg/100 g of flour) and atherogenic (AI) and thrombogenic (TI) indices of *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

Fatty acid	Marracuene	Chókwè	Chicualacuala	Manhiça	*P*
C12:0	3.4 ± 0.2	3.5 ± 0.9	3.8 ± 0.4	3.7 ± 0.5	ns
C14:0	34.1 ± 1.9	35.4 ± 1.0	35.3 ± 1.2	36.1 ± 0.5	ns
C16:0	5229.9 ± 118.3^{a,b}	5354.5 ± 58.6^{a}	5359.1 ± 67.2^{a,}	5528.3 ± 52.6^{b,c}	0.033*
C16:1-n9	421.4 ± 13.5	428.7 ± 3.1	428.5 ± 8.9	439.1 ± 1.8	ns
C18:0	1198.7 ± 13.2	1201.6 ± 23.3	1213.6 ± 15.8	1258.0 ± 24.5	ns
C18:1-n9	16464.1 ± 298.7^{a,b}	16905.2 ± 3.3^{c}	16812.7 ± 147.7^{a,c}	17388.1 ± 25.0^{b,c}	<0.001**
C18:2-n6	1791.6 ± 55.4	1832.8 ± 17.6	1839.6 ± 36.2	1884.9 ± 13.0	ns
C20:0	161.0 ± 2.8	165.7 ± 2.5	168.2 ± 5.4	171.8 ± 2.8	ns
C18:3-n3	457.5 ± 21.1	460.8 ± 9.9	466.0 ± 14.1	472.4 ± 9.6	ns
C20:1-n9	78.1 ± 1.4	82.4 ± 2.7	83.3 ± 3.9	84.8 ± 3.7	ns
SFA	6926 ± 138^{a}	7068 ± 67^{a,b}	7084 ± 76^{a,b}	7314 ± 80^{a,b}	0.021*
MUFA	17013 ± 313^{a,b}	17469^{a} ± 4	17390 ± 160^{a,c}	17965 ± 17^{b,c}	<0.001**
PUFA	2304 ± 79	2345 ± 27	2361 ± 51	2408 ± 17	ns
AI$^{\#}$	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	-
TI$^{\#}$	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	-

Data expressed as mean ± standard deviation; ns, not significant. Different letters for each district in a row show statistically significant differences ($p < 0.05$) between means. * *p* Values from one-way ANOVA. Means were compared by Tukey's since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). ** *p* Values from one-way Welch ANOVA. Means were compared by Tamhane's T2 test since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

$^{\#}$AI and TI were calculated according to Ulbricht and Southgate (1991).

The fatty acid profile of *nfuma* is similar to that of high-fat fruits and their oils, namely olives, avocados and nuts (Berasategi et al. 2012; Venkatachalam and Sathe 2006), but with a slightly higher saturated content. Although *nfuma* has a higher fat content than cereal flour, it presents high amounts of oleic acid (~16 g/100 g of flour), some linoleic (1.8 g/ 100 g) and alpha-linolenic (0.5 g/100 g) acids and low amounts of SFA, which means it can play a protective role in health. The SFA fraction was mainly palmitic acid (~5 g/100 g), known for its controversial association with detrimental health effects, however, an optimal intake of palmitic acid, in an adequate ratio to unsaturated fatty acids may be crucial to maintain membrane phospholipids balance (Carta et al., 2017).

Regarding the health-related lipid indices of *nfuma*, the atherogenic index (AI) and thrombogenic index (TI) were 0.28 and 0.60, respectively, and did not differ between flour origins. Lower AI and TI values (close to zero) translate into lower atherogenic and thrombogenic potential. Thus, the consumption of *nfuma* can contribute to the prevention of cardiovascular diseases, since this flour, like olive oil (AI = 0.14; TI = 0.32), has AI and TI values below 1 (Ulbricht and Southgate, 1991). Higher indices were reported for coconut (AI = 13.63; TI = 6.18) and palm (AI = 2.03; TI = 2.07) lipids, which are mainly characterized by SFA and are associated with cardiovascular diseases (Elson and Alfin-Slater, 1992; Ulbricht and Southgate, 1991).

3.3. Vitamin E and Carotenoids

Unlike cereal flours, the high-fat content of *nfuma* is also responsible for its high content of liposoluble bioactive compounds, namely vitamin E and β-carotene (provitamin A activity).

Table 3. Vitamin E and β-carotene contents (mg/100 g) of *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) of southern Mozambique.

	Vitamin E	**β-carotene**
Marracuene	6.73 ± 0.74	2.56 ± 0.08
Chókwè	7.97 ± 0.72	2.45 ± 0.34
Chicualacuala	6.88 ± 0.13	2.19 ± 0.11
Manhiça	7.44 ± 0.4	2.64 ± 0.13
p Value	Ns	Ns

Data expressed as mean ± standard deviation; ns, not significant

Vitamin E and β-carotene contents ranged between 6.73-7.97 and 2.19-2.64 mg/100 g, respectively. Although no statistically significant differences were observed between communities, Chókwè and Manhiça presented the highest content of vitamin E and β-carotene, respectively. *Nfuma* has a higher β-carotene content than yellow sweet potato flour (0.6 mg/100 g) which is commonly used by local communities in Mozambique to make bread, cakes and porridges (Nogueira et al., 2018). Regarding vitamin E, *nfuma* was shown to have a significant amount, similar to peanuts (9.9 mg/100 g) and some vegetable oils, such as palm oil (9.5 mg/100 g) (INSA, 2007).

3.4. Amino acids and Protein nutritional quality

The amino acid (AA) composition (mg/g protein) of *nfuma* from the different districts is shown in Table 4. Of the 18 amino acids analyzed, 17 were identified in the flour. Arg was the amino acid with the highest amount (148-156 mg/g protein), followed by Asp (106-121 mg/g), Glu (92-103 mg/g) and Ser (93-97 mg/g), Val (73-76 mg/g) and Leu (73-75 mg/g), Thr (58-61 mg/g) and Phe (59-62 mg/g) and Cys (50-56 mg/g), with significant differences between communities ($p < 0.05$) for Arg, Asp and Ser. Protein from Marracuene flour presented the higher Asp and Ser content, and lower of Arg. The lowest AA amounts were found for Met, Trp and Lys (up to 17 mg/g protein), being significantly different between communities, and Ala was not detected. Lys and Met were significantly higher in protein from Chicualacuala flour (16.9 mg/g protein) and Chókwè (15.6 mg/g protein), respectively, and Trp was significantly lower in protein from Marracuene flour (10.3 mg/g protein).

Studies on the AA composition of indigenous fruits is scarce in the literature (Sibiya et al., 2021), and no information is available for fruits or products of *Strychnos* spp (Ngadze et al., 2017a). Arg, the main AA in *nfuma*, was also found as the main AA of *Dovyalis longispina* and is generally abundant in other indigenous fruits (Sibiya et al., 2021). Glu and Asp, the second and third most abundant AA in *nfuma*, have been described in relatively high amounts in other indigenous fruits (Sibiya et al., 2021). These three AA have been described as major amino acids in nut seeds (Venkatachalam and Sathe, 2006).

Table 4. Amino acids composition of protein (mg/g protein) from the *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

Amino acid	Marracuene	Chókwè	Chicualacuala	Manhiça	*p* Value
Asp	121 ± 1^{b}	106 ± 3^{a}	114 ± 2^{a,b}	116 ± 4^{b}	0.004
Glu	101 ± 5	103 ± 17	92.0 ± 2.6	103 ± 18	ns
Ser	97.2 ± 0.4^{b}	93.2 ± 1.5^{a,b}	96.9 ± 0.5^{a,b}	92.7 ± 2.2^{a}	0.020
His	25.0 ± 2.4	22.6 ± 0.3	24.1 ± 0.6	22.6 ± 0.7	ns
Gly	27.3 ± 0.4	27.3 ± 0.4	27.8 ± 0.2	27.7 ± 0.5	ns
Thr	59.5 ± 0.8	59.1 ± 0.7	61.1 ± 1.7	58.4 ± 1.5	ns
Arg	148 ± 1^{b,c}	153 ± 2^{a,c}	156 ± 1^{a}	153 ± 3^{a,c}	0.041
Ala	ND	ND	ND	ND	
Tyr	42.6 ± 0.3	40.7 ± 0.6	42.7 ± 0.1	40.3 ± 1.5	ns
Val	74.6 ± 0.3	73.1 ± 0.9	75.6 ± 0.3	73.1 ± 2.3	ns
Met	12.0 ± 0.0^{b}	15.6 ± 0.3^{a}	11.5 ± 0.3^{b,c}	10.9 ± 0.2^{c}	<0.001
Phe	59.0 ± 0.2	59.0 ± 0.8	62.4 ± 0.2	58.9 ± 2.4	ns
Ile	43.3 ± 0.6	43.1 ± 1.1	43.8 ± 0.3	42.6 ± 1.1	ns
Leu	72.8 ± 0.3	73.6 ± 1.2	75 ± 0.3	72.7 ± 2.2	ns
Lys	14.9 ± 0.1^{b}	15.6 ± 0.2^{b}	16.9 ± 0.3^{a}	15.4 ± 0.5^{b}	0.002
Pro	41.1 ± 1.5^{a,b}	49 ± 5.2^{b}	30.1 ± 3.1^{a}	43.1 ± 3.6^{b}	0.005
Trp	10.3 ± 0.5^{b}	14.9 ± 0.3^{a}	14.0 ± 0.6^{a}	14.0 ± 0.3^{a}	<0.001
Cys	49.9 ± 1.6	50.4 ± 4.3	56.4 ± 1.3	55.5 ± 1.0	ns
∑AAA[1]	102 ± 0	100 ± 1	105 ± 0	99.2 ± 3.9	-
∑SAA[2]	61.9 ± 0.0	66.8 ± 0.3	68.4 ± 0.3	66.1 ± 0.2	-
∑EAA[3]	464 ± 3	469 ± 6	484 ± 1	464 ± 12	-

Data expressed as mean ± standard deviation; ND, below limit of detection (4.06 µM for Ala); ns, not significant. Different letters for each district in a row show statistically significant differences (*p* < 0.05) between means. *p* Values from one-way ANOVA analysis. Means were compared by Tukey's. since homogeneity of variances was confirmed by Levene's test (*p* > 0.05).

[1] aromatic amino acids: Phe+Tyr; [2] sulfur amino acids: Met+Cys;

[3] Sum of essential amino acids Thr+Val+Met (+Cys)+Ile+Leu+Phe (+Tyr)+ His+Lys+Trp used for daily requirements and protein value as suggested by FAO/WHO/UNU (2007).

Met and Trp were the least abundant AA in *nfuma* (up to 15.6 mg/g protein), which is a common finding for proteins of plant origin (Friedman 1996). Met was also found to be the least abundant AA by Sibiya et al. (Sibiya et al., 2021) in almost all indigenous fruits, however, different results were found for Lys, one of the least abundant AA in *nfuma* (up to 16.9 mg/g protein), but with relatively high or intermediate levels in some indigenous fruits (Sibiya et al., 2021). Lys exhibits significant thermal instability even at low temperatures (Friedman, 1996); therefore, some losses may have occurred during the drying and roasting processes and its concentration may be higher in *S. madagascariensis* fresh fruits.

Although in low amounts, *nfuma* presents all nine essential AA, which is in agreement with the findings of Sibiya et al. (2021) who found 8 essential AA (Trp was not evaluated) in 14 indigenous fruits.

The nutritional quality of the protein of *nfuma*, expressed as essential amino acid scores (EAAS) (FAO/WHO/UNU, 2007), is presented in Figure 2.

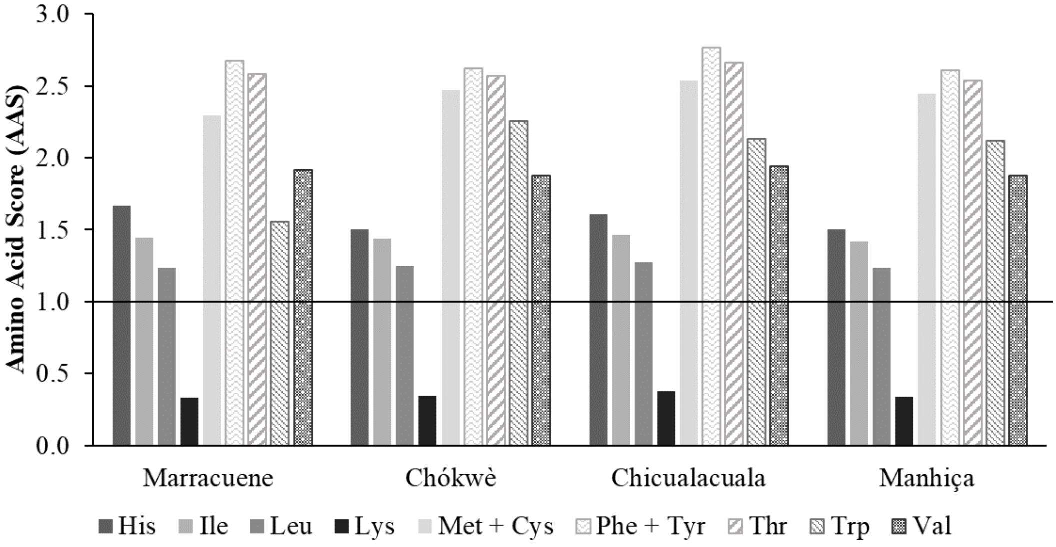

Figure 2. Essential amino acid scores (EAAS) of *S. madagascariensis* fruit flour (*nfuma*) from 4 districts of Mozambique. WHO/FAO/UNU (2007) adult maintenance patterns are expressed as mg AA/g protein: His. 15; Ile. 30; Leu. 59; Lys. 45; Met + Cys. 27; Phe + Tyr. 38; Thr. 23; Trp. 6.6; Val. 39.

When all nine individual scores are greater than or equal to 1, the protein is considered complete. Despite having all essential AA, according to the results obtained, the protein of *nfuma* is incomplete, presenting Lys as the limiting AA (AAS < 1), which is in agreement with the results available for the protein of other flours, namely those derived from cereals, e.g. wheat and corn (Friedman, 1996), or nut seeds (Venkatachalam and Sathe, 2006)

Although the protein of *nfuma* is incomplete, it can balance other amino acid deficiencies. *Nfuma* is a relatively important source of Met+Cys, Phe+Tyr and Thr (AAS $\geq$ 1). To achieve the recommended daily allowances of all EAA, local communities should combine *nfuma* with other protein sources, namely legumes such as beans, as they are a rich source of Lys and a poor source of Met (Friedman, 1996). Beans are mainly grown in rural areas of Mozambique and can help to alleviate malnutrition (Baptista et al., 2017). The EAA with the highest ratio to daily requirements was Phe+Tyr. These AA are precursors of the physiologically active molecules catecholamines, which act as both neurotransmitters and hormones (Fernstrom and Fernstrom, 2007). Although the three most abundant AA in *nfuma* are considered non-essential, it has been shown that Arg, Asp and Glu (Table 4) can act as regulators of key metabolic pathways, leading to a new concept of functional AA (Wu, 2010).

3.5. Mineral elements

Table 5 presents the results of mineral content in *nfuma* from different origins (districts). Of the 25 elements analyzed, the content of eight of them (Li, Be, As, Se, Cs, Pb, Tl and Bi) was below the limits of detection. The most abundant macromineral was K (ranging from approximately 1200 to 1700 mg/100g) and the less abundant was Na (4.0 - 6.6 mg/100g).

This trend was also observed in other fruit flours(Brito et al. 2017; Carli et al., 2016), dried fruits (Alasalvar and Shahidi, 2013) and indigenous fruits (Amarteifio and Mosase, 2009; Sibiya et al., 2020). Brito et al. (2017) found similar values for green banana flour, with mean value of 1100, 88, and 45 mg/100 g for K, Mg and Ca, respectively. Commercial fruit flours (up to 952 mg/100g) (Carli et al., 2016) and dried fruit products (up to 1162 mg/100g), have been described as essential sources of K (Alasalvar and Shahidi, 2013), however, *nfuma* presents even higher values. Among the 14 wild fruits native to southern African studied by Sibiya et al. (Sibiya et al., 2020), higher K levels were found in *Carissa macrocarpa* and *Syzygium cordatum* (1312.3 and

1427.1 mg/100g dw, respectively). Regarding *Strychnos* spp, our results for *S. madagascariensis* flour are in the range of those observed by Kalenga Saka and Msonthi (1994) and Amarteifio and Mosase (2009) for *S. spinosa* fruit (1968) and 1370 mg/100g dw, respectively) for K, and higher for Mg (43 and 49 mg/100g dw, respectively).

Table 5. Mineral composition of the *S. madagascariensis* fruit flour (*nfuma*) from four districts (Marracuene, Manhiça, Chókwè and Chicualacuala) in southern Mozambique.

Element	**Marracuene**	**Chókwè**	**Chicualacuala**	**Manhiça**	*p* Value
Essential macrominerals (mg/100g)					
Ca	24.7 ± 0.2	31.3 ± 6.3	26.2 ± 0.0	29.9 ± 2.5	ns
Mg	85.6 ± 2.8^{a}	80.8 ± 3.4^{ab}	75.3 ± 4.7^{ab}	69.4 ± 4.3^{b}	0.025*
K	1654 ± 102^{a}	1399 ± 71^{b}	1303 ± 15^{b}	1204 ± 80^{b}	0.002*
Na	4.9 ± 0.2^{b}	4.0 ± 0.1^{b}	6.6 ± 0.1^{a}	4.9 ± 0.8^{b}	0.002*
Essential trace elements (µg/100g)					
Fe	1683 ± 71	1620 ± 56	1476 ± 73	1706 ± 122	ns
Zn	261.4 ± 11.2	216.5 ± 12.5	205 ± 13.4	228.6 ± 20.8	ns
Mn	4017 ± 141	3885 ± 71	3874 ± 21	4098 ± 181	ns
Cu	215.2 ± 1.0^{a}	200.3 ± 5.9^{ab}	193.9 ± 7.7^{b}	208.5 ± 5.1^{ab}	0.021*
Cr	58.1 ± 1.4	55.2 ± 2.7	57.5 ± 1.9	58.1 ± 0.5	ns
Co	7.6 ± 0.3	7.3 ± 0.2	6.8 ± 0.3	7.6 ± 0.0	ns
Non-essential trace elements (µg/100g)					
Al	2631 ± 210^{a}	3042 ± 221^{a,b}	2995 ± 73^{a}	3331 ± 26^{b}	0.026**
Rb	1100 ± 19^{a}	980 ± 29^{b}	968 ± 15^{b}	968 ± 31^{b}	0.002*
Ni	463.8 ± 3.8	482.6 ± 20.5	458.8 ± 12.6	478.0 ± 20.0	ns
Sr	258.2 ± 2.6	240.9 ± 5.5	250.2 ± 14.4	256.2 ± 4.8	ns
Ba	222.3 ± 5.1^{b}	252.2 ± 8.6^{a}	249.7 ± 9.5^{a}	257.0 ± 4.6^{a}	0.006*
V	17.9 ± 0.4^{a}	17.6 ± 0.5^{a}	17 ± 0.6^{a}	21.9^{b} ± 0.3	0.002*
Cd	2.4 ± 0.0	2.1 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	ns

Data expressed as mean ± standard deviation; ns, not significant. Different letters for each district in a column show statistically significant differences ($p < 0.05$) between means. * *p* Values from one-way ANOVA. Means were compared by Tukey's since homogeneity of variances was confirmed by Levene's test ($p > 0.05$). ** *p* Values from one-way Welch ANOVA. Means were compared by Tamhane's T2 test since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

For essential macrominerals, statistically significant differences were observed between the content of K, Mg and Na in the flours of the different communities (Marracuene, Chókwè, Chicualacuala and Manhiça). A high K and Mg content was observed for Marracuene flour (1654 ± 102 and 85.6 ± 2.8 mg/100g, respectively) and a high Na content was observed for Chicualacuala flour (6.6 ± 0.1 mg/100g).

Regarding essential trace elements, the following trend was observed: Mn (~4000 µg/100g) > Fe > Zn > Cu > Cr > Co (6.8-7.6 µg/100g). Significant differences ($p < 0.05$) were observed only for Cu (higher Cu content in Marracuene flour compared to Chicualacuala flour). *Nfuma* can be considered an interesting source of Mn when compared to dried fruits (~300 µg/100g: apricot, dates, peach, poir, runes, raisins) (Alasalvar and Shahidi, 2013). Of the commercial fruit flours studied by Carli et al. (2016), only coconut showed a Mn concentration similar to *nfuma*. Hassan et al. (Hassan, 2014) found a Mn content of 2500 µg/100g dw for *S. innocua* fruit. For Fe (~1600 µg/100g) and Zn (~250 µg/100g) in *nfuma*, lower levels were found when compared to cashew flours (~5000 and 4000 µg/100g, respectively) (Xavier et al., 2019). Carli et al. (2016) reported higher Fe values in 8 out 10 fruit flours (~3000-11,000 µg/100g: plum, coconut, orange, papaya, apple, passion fruit, green banana flours, in ascending order) and all flours had higher Zn values (500 to 4000 µg/100g) than *nfuma*. Amarteifio and Mosase (2009) reported a similar Zn content (220 µg/100g dw) for *S. spinosa* fruit, and a lower value for Fe (~110 µg/100g dw), proposing the supplementation of these fruits to meet Fe requirements. Interestingly, several studies report the ability of *Strychnos* spp to improve nutrition based on their high Fe and Zn contents (Ngadze et al., 2017a,b; Omotayo and Aremu, 2021), despite the wide variation between and within the *Strychnos* spp (other than *S. madagascariensis*), as reviewed by Ngadze et al. (2017a). The Cu content (200 µg/100g) of *nfuma* was in agreement with the data for some fruit flours, as reported by Carli et al. (2016) (~170-210 µg/100g for açai, orange and lemon flours) and Brito et al. (Brito et al. 2017) (up to 300 µg/100g for apple and green banana). Regarding Cr, *nfuma* has contents (~58 µg/100g) higher than those reported by Brito et al.(2017) for apple and green banana flours (up to 20 µg/100g). The minor essential element found in *nfuma* was Co (~7 µg/100g), a constituent of vitamin B12. *Nfuma* presents higher Co levels than grain flours (up to 1.2 µg/100g) (Ertl and Goessler, 2018) and within the range of dried sweet cherries (~ 0.6 to 14 µg/100g; mean value of 3 µg/100g dw) (Gonçalves et al., 2022).

For *S. innocua*, Hassan et al. (2014) reported quite different Co levels compared to *nfuma*, reaching 1200 µg/100g dw.

Some non-essential elements were also quantified in *nfuma*. Among those, Al was the most abundant (~2600-3300 µg/100 g) and Cd the least (~2.1-2.4 µg/100 g). Statistically significant ($p < 0.05$) were observed for Al, Rb and Ba, with a higher Rb content and a lower Ba content in the Marracuene flour. For Al, a higher content was observed for Manhiça flour compared to Marracuene flour (3331 ± 26 vs. 2631 ± 210 µg/100g). Aluminum content can vary significantly in food, depending on the food composition itself as well as on "external" factors (e.g., soil contamination, culinary practices). Our results are in close agreement with Brito et al. (2017), who studied two fruit flours (apple and green banana) and found Al levels ranging from 190 to 4,900 µg/100 g.

3.6. Nutrient adequacy of *nfuma*

The estimated daily intake of nutrients and energy, expressed as % of DRV, was calculated assuming an average per capita consumption of *nfuma* of 100 g per day and is presented in Table 6a. For macronutrients, except protein and total dietary fiber, the EDI was calculated based on the energy value. Thus, macronutrients DRVs depend on each individual's energy needs. Overall, consumption of 100 g of *nfuma* contributes to 15-27% of daily energy needs, depending on the physical activity level. EDI values show that *nfuma* is an important source of fiber and lipids, namely alpha-linolenic acid, contributing to 30%, 22-69% and 27-48% of DRVs, respectively. Regarding liposoluble vitamins, *nfuma* provides 55-63% and 56-66% of vitamins A and E, respectively. In Mozambique, 69% of children under 5 years of age are deficient in vitamin A (Amaro, 2019; WHO, 2006). Considering a daily consumption of 50 g of *nfuma*, children aged 1-3 and 4-6 years can obtain, respectively, 82 and 68% (Table 6b) of vitamin A EDIs (EFSA, 2019). Thus, the consumption of *nfuma* by children in Mozambique may alleviate vitamin A deficiency due to its high β-carotene content. As mentioned above, flour is used during times of food shortage as a supplement to staple foods, such as maize and cassava flour. Maize flour is often boiled in water to make a maize-meal porridge that is consumed for breakfast by communities in sub-Saharan Africa (Ngadze et al., 2017b).

Table 6a. Estimated daily intake (EDI), expressed as % of the dietary reference value (DRV), of energy, macronutrients, vitamins and essential elements for adults considering an average per capita *nfuma* consumption of 100 g/day.

	DRV[¥] (AI[a]/AR[b]/PRI[c]/RI[e]/SAI[f])		**EDI (% DRV)**	
	Male	**Female**	**Male**	**Female**
Energy (MJ/day)				
Energy	9.1-13.0[b*]	7.4-10.5[b*]	15 - 22	19 – 27
Macronutrients (g/day)				
Protein	53.1[c#]	45.6[c#]	6.0	7.0
Fat	48.3-120.8[d]	38.9-97.3[d]	22 - 56	28 – 69
Alpha-linolenic acid	1.2-1.7[a]	1.0-1.3[a]	27 - 38	33 – 48
Linoleic acid	9.7-13.8[a]	7.8-11.1[a]	13 - 19	17 – 24
Carbohydrates	244.5-465.8[e]	197.1-375.5[e]	11 - 22	14 – 27
Total Dietary Fiber	25[a]		30	
Vitamins				
Vitamin A (µg RE/day)	750[c]	650[c§]	55	63
Vitamin E (mg/day)	13[a]	11[a]	56	66
Minerals (mg/day)				
Ca	950[c]		3	
Mg	350[a]	300[a]	22	26
K	3500[a]		40	
Na	2000[f]		0.3	
Fe	11[c]	16[c§]	15	10
Zn	9.4-16.3[c†]	7.5-12.7[c†]	1.4 - 2.4	1.8 - 3.0
Mn	3[a]		132	
Cu	1.6[a]	1.3[a]	12.8	15.7

***Average Requirement for adults (18-79 years) with a physical activity level (PAL) between 1.4 and 2.0.**

[#] Population Reference intake for men and women with a reference body weight of 64 and 55 kg, respectively, based on IMC of 22 kg/m^2.

[§] Population Reference intake for premenopausal women.

[†] Population Reference intake for adults (≥ 18 years) with a phytate intake level between 300 and 1200 mg/day.

DRV: Dietary Reference Value; AI: Adequate Intake; AR: Average Requirement; PRI: Population Reference Intake; RI: reference intake; SAI: Safe and adequate intake.

EDI of vitamin A was calculated based on the conversion of β-carotene content (expressed as mg/100 g) to retinol equivalent (1 µg RE = 6 µg of β-carotene).

According to the FAO Food Balance Sheets, Mozambican communities consume 192 g of maize and 285 g of cassava per day (FAOSTAT, 2019). Maize and cassava flour have a higher carbohydrate content (75% and 85%, respectively) (INSA, 2007), while *nfuma* has more fiber, fat and liposoluble vitamins. On the other hand, *nfuma* has a low protein content, providing only 6% (male) and 7% (female) of DRVs. Therefore, as noted above, *nfuma* must be combined with other protein sources to achieve DRVs.

Considering the EDI of essential elements (expressed as % of DRV), *nfuma* contributes significantly to the daily intake of Mg and K, representing 22-26% and 40% of DRVs, respectively. In addition, daily consumption of 100 g of *nfuma* is sufficient to meet Mn requirements, since it provides more than 100% of Mn DRV. *Nfuma* has a higher content of specific minerals, especially K and Mg, compared to common staple flours in Mozambique, such as maize (120 mg/100 g of K and 46 mg/100 g of Mg) and cassava flours (20 mg/100 of K and 2 mg/100 g Mg) (INSA, 2007). On the other hand, 100g of *nfuma* provides only 10% (female) to 15% (male) of Fe DRV, although it contains twice the Fe content of maize flour (800 µg/100 g). For children (1-6 years), 50g of *nfuma* provides 12% of Fe DRV (Table 6b).

Table 6b. Estimated daily intake (EDI), expressed as % of the dietary reference value (DRV), of vitamin A and Fe for children (1-6 years) considering an average per capita *nfuma* consumption of 100 g/day.

	DRV$^{\gamma}$ (PRI)		**EDI (% DRV)**	
	1 - 3 years	**4 - 6 years**	**1 - 3 years**	**4 - 6 years**
Vitamin A (µg RE/day)	250	300	82	68
Fe (mg/day)	7		12	

DRV: Dietary Reference Value; Population Reference Intake. EDI of vitamin A was calculated based on the conversion of β-carotene content (expressed as mg/100 g) to retinol equivalent (1 µg RE = 6 µg of β-carotene).

Consequently, the combination of maize flour with *nfuma* in porridges, together with consumption of *nfuma* as a daytime snack, as reported by people from Mozambican communities, may increase Fe intake, which is of great importance given the high prevalence (64%) of anemia in children in Mozambique (Amaro, 2019). However, it is still necessary to study the bioaccessibility and bioavailability of *nfuma* minerals to define its real potential in human nutrition.

3.7. Exposure assessment to non-essential trace elements

Considering the non-essential trace elements for which a tolerable intake is established, the amount of Ni, Al and Cd in *nfuma* contributes, respectively, to 52%, 15% and 9% of the corresponding TDI, PTWI and PTMI for adults (Table 7). Thus, the consumption of *nfuma* (100 g) alone is not likely to be considered a relevant source of Al and Cd. The same is not true for Ni since the average content found contributed to ~50% of the TDI established by the European Food Safety Authority (13 µg/kg bw) (EFSA, 2019).

Table 7. Estimated daily (EDI), weekly (EWI) and monthly (EMI) intake, expressed as % of toxicological guidance values of Ni, Al and Cd, considering the consumption of 100 g/ day (adults) or 50 g/ day (toddlers) of *nfuma*.

Element	Reference Value	Estimated intake of non-essential elements	
	TDI (µg/day/kg bw)	EDI (% TDI)	
Ni	13	Toddlers	Adult
		151	52
	PTWI (µg/week/kg bw)	EWI (% PTWI)	
Al	2000	Toddlers	Adult
		44	15
	PTMI (µg/month/kg bw)	EMI (% PTMI)	
Cd	25	Toddlers	Adult
		11	4

TDI: Tolerable Daily Intake (EFSA, 2020); PTWI: Provisional Tolerable Weekly Intake and PTMI: Provisional Tolerable Monthly Intake (JEFCA, 2021).

When looking at the exposure of toddlers, the consumption of 50 g of *nfuma* can contribute to 151%, 44% and 11% of the TDI, PTWI and PTMI of Ni, Al and Cd, respectively. These results indicate that young age groups can be at high risk of health complications due to Ni exposure. Since the fruits used to prepare the flour had low levels of Al and Ni (data not shown), the selection of appropriate materials and the use of good practices in the preparation of *nfuma* should be evaluated in order to mitigate the presence of these non-essential elements, while maintaining the nutritional advantages of the food discussed above.

3. Conclusions

This research aimed to determine the nutritional composition of *nfuma*, a flour from S. madagascariensis pulp fruit, prepared by local communities in Mozambique and evaluate its adequacy in terms of nutrient recommendations. This fruit flour stands out for its high fat content, mainly composed by MUFA, delivering vitamin E and carotenes, together with naturally occurring sugars and high fiber content. *Nfuma* is also a good source of Mn and K and, despite being a poor source of Fe, *nfuma* contains twice the Fe content of maize flour. However, its Ni content should be addressed with caution and mitigation strategies are required in order to guarantee its safety. Although *nfuma* bioaccessibility evaluation is still needed, its consumption seems to be a promising food-based strategy to alleviate the high prevalence of anemia and vitamin A deficiency in children of Mozambique. Its local use in the "enrichment" of maize-based porridges or as ingredient for pastry and snacks for the development of healthier new food products deserves to be technologically approached for wider valorization.

Supplementary Material

Table S1: Limits of detection (LOD) for the elements analyzed by ICP-MS.

Element	LOD (µg/g)
Al	0.928
As	0.127
Ba	0.091
Be	0.011
Bi	0.009
Cd	0.024
Co	0.020
Cr	0.033
Cs	0.012
Cu	0.233
Li	0.013
Mn	0.041
Ni	0.335
Pb	0.018
Rb	0.021
Se	0.181
Sr	0.036
Tl	0.006
V	0.041
Zn	0.529

CHAPTER 5-CHEMICAL CHARACTERIZATION OF THE OIL SEPARATED BY MECHANICAL PRESSING FROM *STRYCHNOS MADAGASCARIENSIS* DRIED FRUIT PULP FLOUR

After the production of flour in the four communities and in the Laboratory, the oil was extracted to materialize the objectives of this chapter. Fatty acid profiles, analyses of TG, DG, FFA, acidity, vitamins E and finally sterol carotenoids were made.

ABSTRACT

In Mozambique, rural communities produce flours from the dried pulp of *Strychnos madagascariensis* fruits. Owing to its high-lipid content, the oil from this flour is frequently separated by pressing to be used as seasoning and medicine. Aiming to characterize this oil, flour samples (n=24), dried at two different temperatures (55ºC and 65ºC), were collected from four local communities, together with a control sample prepared in the lab (50ºC). The resulting oil is fluid at room temperature, deep-orange, and characterized by a high content of oleic acid (62-63%), followed by palmitic (20%), and linoleic (7%), highly consistent between samples. It contains considerable amounts of tocols (25-34 mg/100g), carotenoids (8-10 mg/100g), as well as sterols (431±10 mg/100g) and triterpenic alcohols (191±4 mg/100g mg/100g). Small statistically differences ($p<0.05$) between the community dried flours and control group, as well as between temperatures, were observed. However, its high free fatty acid content (22-25%) reveals intensive enzymatic hydrolysis during the drying/fermentation step, whose extension might be potentially reduced by optimizing its technological process. Its chemical profile supports some of its folklore uses and reveals a promising source of edible oil, with health and technological potential that is worth optimizing and exploring.

Keywords: Native fruits; *Strychnos madagascariensis*; high-oleic oil; monounsaturated oil; monkey fruit; sterols

1. Introduction

The *Strychnos* genus belongs to the Loganiaceae family, indigenous from tropical and subtropical Africa. The most consumed species, which are prevalent in woodlands of Southern Africa, are *S. innocua*, *S. madagascariensis*, *S. cocculoides*, *S. pungens* and *S. spinosa*. *S. madagascariensis* fruits, known as monkey orange, are usually eaten raw, or fermented and dried by sun exposure and used to make alcoholic beverages, while the pulp produces an appreciated sweet when mixed with honey or in the form of tea (Ngadze et al., 2017a; Van Wyk and Gericke, 2000; Van Rayne et al., 2021). Due to their abundance, the preservation of *Strychnos* spp fruits is recognized as an important strategy to enhance food availability in times of shortage, reducing losses and underutilization (Ngadze et al., 2017a,b) Local populations also report its use as a multipurpose folk medicine (Ngemakwe et al., 2017) including the folklore use in the management of diabetes mellitus and hypertension (Oboh et al., 2020). In the southern part of Mozambique, where the fruit is called macuácua, it is usually eaten after being transformed into flour-like product obtained from the dried pulp *nfuma* especially in times of scarcity of basic foods. Some communities extract a liquid oil with intense orange-brown color from *nfuma* with several applications, including cooking purposes.

Edible vegetable oils are essential in people's daily diet and can be produced from seeds, nuts, kernels, beans, cereals and fruits pulp (mesocarp). The most widely consumed oil worldwide is palm oil, also obtained from a fruit pulp, followed by other important sources such as soybean, corn, sunflower, peanut, rapeseed and olives, among others (Absalomé et al., 2020). Oils extracted from plant sources have a successful history of use by rural communities as a food ingredient, as well as in medicine, cosmetic and fuel applications. The continuing seek for innovation in ingredient sources and the demand for natural alternatives increases the opportunity for indigenous African vegetable oils. Some oils pressed from seeds of African trees have become popular ingredients in cosmetic formulations (Vermaak et al., 2011), or for food, as the oil from seeds of *Moringa oleifera* (Boukandoul et al., 2017) or avocado pulp (Berasategi et al., 2012) relatively new in culinary circles. As far as the authors are aware, no detailed characterization of the oil extracted from the *nfuma* flour was reported so far.

This research aimed to determine the compositional basis of the oils physically extracted from the dry pulp of *S. madagascariensis* fruits produced following traditional procedures, while evaluating its nutritional and technological potential. Gathering local communities’ knowledge is important and useful to determine the true value of their practices and will lead to more rational decisions about its sustainable utilization and valorization.

2. Materials and Methods

2.1 Reagents and standards

The reagents used in this work were of analytical or chromatographic grade, obtained from several suppliers. A certified fatty acids methyl ester (FAME) standard mixture (Supelco 37 Component FAME Mix, TraceCERT, Bellefonte, PA, USA) was used. All sterols’ standards (cholesterol, cholestanol, campesterol, stigmasterol, ß-sitosterol, and stigmasterol) were purchased from Sigma–Aldrich as were the mono, di, and triglycerides reference standards and ß-carotene. Tocopherols and tocotrienols standards were acquired from Supelco (USA) and Larodan (Sweden) while the internal standard tocol (2-Methyl-2-(4,8,12-trimethyltridecyl)-chroman-6-ol) was purchased from (Matreya, Inc., USA). The HPLC solvents n-hexane, dioxane, and tetrahydrofuran were from Merck (Germany).

2.2. Sampling and oil extraction

Oil was extracted from flours produced in four districts in southern Mozambique - Marracuene, Manhiça, Chókwè and Chicualacuala. The flour was obtained from pulp of ripe fruits collected in the summer season, sun-dried for 2 to 4 days to ease seeds separation, and then slightly toasted in a metallic tray at two temperature ranges (50-60ºC and 60-70ºC) for about one hour. The process was performed in triplicate on different days, for both temperatures, on each community, resulting in six independent samples of 5 kg per community, in a total of 24 independent samples. The seeds were separated from the dried pulp which was further crushed manually using a mortar pestle until the flour was obtained. All processed flours were stored at room temperature in closed containers for later extraction of the oil in a central laboratory. Additionally, three fresh fruit batches collected in Marracuene were totally processed in the laboratory, being dried at 50ºC ±2 for two days, and the oil obtained was used as a control oil for process variability.

For oil extraction purposes, the flour (500g portions) was warmed using an oven (Memmert UN 110) at 40°C ± 2 for 30 minutes, and oil extraction was performed by using a hydraulic hand press machine (Carver Menomonee Falls, WIS 53051 USA Serie 24000-103). The oils were filtered with Whatman 40 paper and anhydrous sodium, to remove all moisture and solid impurities, placed in glass containers, closed, protected with aluminum foil and stored at 4°C until analysis. Oil extraction yield was not evaluated.

2.3. Fatty acids composition

Esterified fatty acids were evaluated as methyl esters, after alkaline trans-esterification with a 2M methanolic solution of potassium hydroxide (Regulation (EEC) No. 2568/91, 1991). Fatty acid separation was carried out on a Select FAME (50 m × 0.25 mm x 0.25 μm) column (Agilent, USA) using helium as carrier gas (pressure of 140 kPa), and temperature of injector and detector set at 250 and 270 °C, respectively. Data were processed by the CP Maitre chromatography data system program (Chrompack International B. V., Middelburg, Netherlands, version 2.5). The results were expressed in relative percentage of each fatty acid methyl ester in the total fatty acids methyl esters, calculated by internal normalization of the chromatographic peak areas after standardization of the detector response with the certified reference standard.

2.4. Triglycerides, diglycerides and free fatty acids

Total triglycerides (TG), diglycerides (DG), and free fatty acids (FFA) contents were assayed following ISO 18395:2005 by size-exclusion high-performance liquid chromatography (HPSEC) (Jasco, Japan) with refractive index detection (132 RI Detector, Gilson, France). Separation was achieved by size exclusion chromatography on a Phenogel 5µm 100Å column (600×7.8mm; Phenomenex, USA), eluted with tetrahydrofuran at a flow rate of 1mL/min. An accurate oil mass was diluted in THF and homogenized by stirring. The results were expressed in relative percentage, after calibration of the detector responses with adequate standards.

2.5. Phytosterols

The quantification of phytosterols was made by gas chromatography with FID detection (TRACE GC; Thermo Finnigan, Italy) of their trimethylsilyl esters, after separation of the sterol and triterpenic alcohol fractions from the unsaponifiable matter using saponification and thin-layer chromatography (TLC) (Regulation (EEC) No. 2568/91, 1991). Separation was accomplished with a temperature gradient from 250 to 280°C and an Agilent JandW GC DB-5MS column (30m×0.250mm, 0.25µm) (USA), with a helium flow of 1.0 mL/min. Injection (split ratio 1:10; 280°C) was performed with an automatic injector (Thermo Scientific Al 1310, Italy). Identification of sterols was based on retention time comparisons with commercial standards available and by computer matching with the reference mass spectra of the MS NIST Library using GC-MS using an Agilent GC 6890N chromatograph equipped with a mass selective detector (5977B MSD), with electron ionization of 70 eV set in the full scan mode (m/z 50−650). Temperatures of interphase, ionization source, and quadrupole were: 300, 230 and 150°C respectively. The compounds were identified by spectra comparison with authentic standards, using the NIST 11 mass spectra libraries, and literature data. Results for individual sterols were reported in relative percentage while total sterols were reported in mg/100g, using betulin as an internal standard.

2.6. Tocopherols and carotenes

Tocopherols and carotenes were analyzed by HPLC with fluorescence and diode-array detection, respectively (Jasco, Japan) (Cruz and Casal, 2018). An accurate solution was prepared in n-hexane, an appropriate amount of the internal standard solution was added and homogenized by stirring. For the separation of different compounds, 20µL sample was injected into a normal phase silica column (Supelcosil TM LC-SI; 7.5 cm×3 mm; 3 µm) (Supelco, USA), conditioned at 25°C (ECOM, ECO 2000, Czech Republic) and eluted with a gradient of 1,4-dioxane and hexane at a flow rate of 0.75 mL/min. Detection was programmed for excitation at 290 nm and emission at 330 nm for tocols, and at 446 nm for carotenes. The different compounds were identified by comparing the retention times with authentic standards and quantified by individual calibration curves, being expressed in mg/100g of oil. GC-MS was used further used for the tentative confirmation of the main tocol-like compound detected in the oil samples, after silylation with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), using an HP5-MS column (30 m × 0.25 mm I.D. × 0.25 μm film thickness, Agilent JandW), on the same GC-MS equipment described above.

2.7. Statistical analysis

The results derived from the chemical analysis are presented as average values and standard deviation of each community/temperature sample group, with duplicate chemical analysis of each sample. To compare the lipid profile of the flour oil from the different communities, normal distribution of the residuals and the homogeneity of variances were evaluated through the Shapiro−Wilk test (sample size <50) and the Levene's test, respectively. Afterwards, all dependent variables were studied using a one-way analysis of variance (ANOVA), subjected or not to Welch correction, followed by Duncan's or Dunnett's T3 test, depending on if the requirement of the homogeneity of variances was verified or not, respectively.

Principal component analysis (PCA) was applied to emphasize variation and bring out strong patterns in a data set according to the different lipid main constituents. For this purpose, data were previously standardized (z-scores) and oblimin with Kaiser normalization was selected as the rotation method considering that dependent variables were highly correlated. Statistical analyses were performed at a 5% significance level using SPSS software (version 27.0, IBM Corporation, New York).

3. Results and discussion

The oils obtained from the *S. madagascariensis* fruit flours prepared from the four communities (Marracuene, Manhiça, Chókwè and Chicualacuala) were characterized for fatty acids composition, glycerides, phytosterols, tocols and carotenes.

3.1. Fatty acid composition

The representative chromatogram shown in Figure 1 highlights the clear dominance of oleic acid.

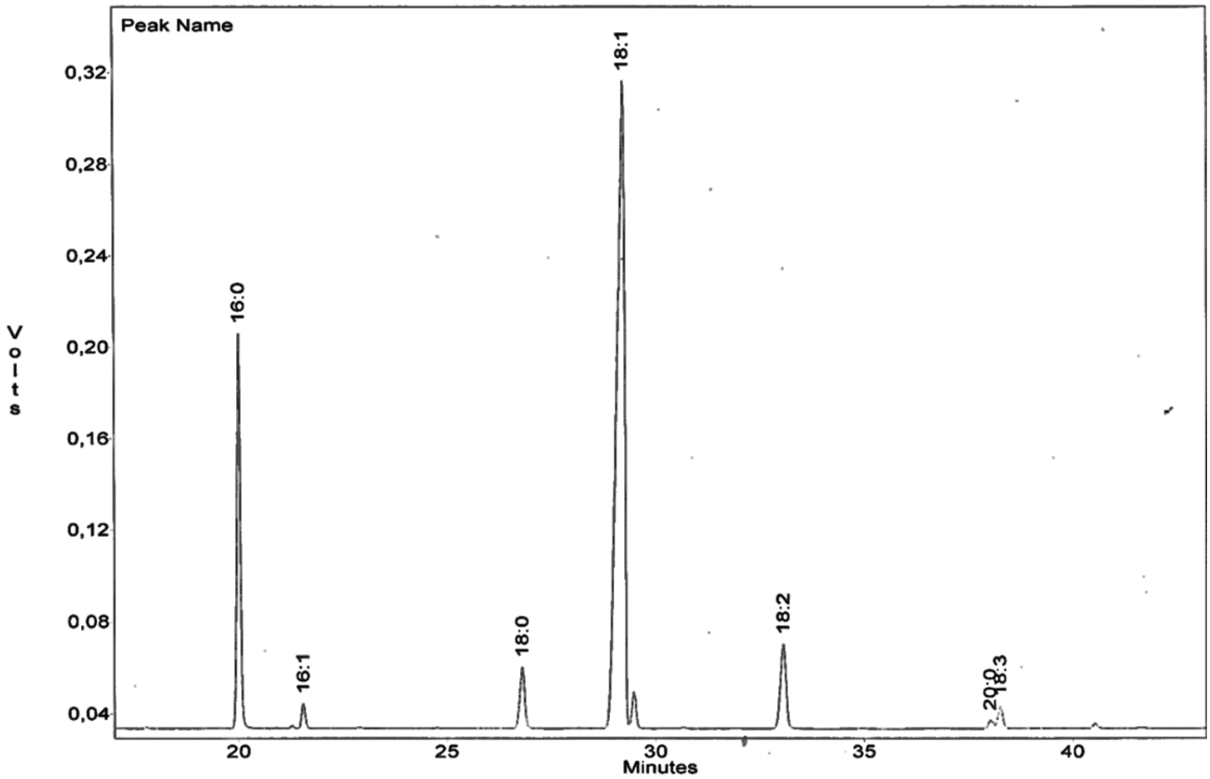

Figure 1. Fatty acid profile of oleic-rich macuácua oil.

The fatty acids composition, detailed in Table 1, shows a high-oleic oil, with a very consistent content of oleic acid, ranging from 62.4 to 63.7%, followed by the saturated fatty acids palmitic (19.4 to 20.1%) and stearic acids (4.5 to 4.6%). The only polyunsaturated fatty acids detected were linoleic acid (6.8%) and linolenic (1.7 %). The amount of short-chain saturated fatty acids is reduced (<0.3%), as is the sum of long-chain ones (arachidic, behenic and lignoceric, <1.2%).

The fatty acid profile is very similar to that of other high-oleic crude oils, such as olive oil, or avocado pulp oil, with oleic acid as major fatty acid, being this fatty acid largely responsible for the unique nutritional impact of these oils and their great stability to oxidation. High-oleic oils have also shown to have beneficial impacts on serum cholesterol and low-density lipoproteins, without affecting the high-density ones, thus minimizing the risk of cardiovascular disease, already with approved health claims on cardiovascular health (Riley et al., 2022). The low amount of saturated fatty acids is interesting from a nutritional point of view, while contributing to the liquid appearance of this oil at room temperature.

In comparison with the most common edible oils, its profile is similar to that of almond, hazelnut, pistachio, avocado and olive oils (Berasategi et al., 2012; Codex Alimentarius, 2015).

In comparison with other oils of African origin, macuácua oil has a higher content of oleic acid than *Adansonia digitata* (baobab) (30 to 42%), *Citrullis lantus* (13 to 17%), *Schinziophyton rautanenii* (15 to 19%), or *Trichilia emética* (15%), but is similar to *Simenia africana* (54 to 72%) and *Sclerocarya birrea* seed oil (70 to 78%). These oils are used in dermatological issues, among others (Wren and Stucki, 2003; Ojewole et al., 2010; Saeed and Bashier, 2010).

In terms of variability between communities, the results indicate a high consistency between all the communities studied. When the two temperature range (50-60 ºC and 60-70 ºC) used in the communities are compared, only minor differences are observed within each community, and only in the most sensible fatty acids were found, namely in linolenic acid, with significantly lower amounts in the samples processed at higher temperatures in both Marracuene and Chicualacuala, but not in the other two communities. The *trans* fatty acid content, known to increase with temperature, was significantly higher in the Chóckwé samples processed at higher temperatures against the lower processed ones but no significant differences were observed in the other communities. However, significant differences between communities and the control samples were verified (p <0 .05).

Table 1. Fatty acid composition relative percentage, %) (mean ± standard deviation, n = 3) of oil extracted from the flour obtained at different temperatures from *Strychnos madagascariensis* fruits harvested by the communities of Chóckwé, Manhiça, Chicualacuala and Marracuene, in southern Mozambique.

	Chókwè		**Manhiça**		**Chicualacuala**		**Marracuene**		
	50-60 °C	**60-70 °C**	**50-60 °C**	**60-70 °C**	**50-60 °C**	**60-70 °C**	**50-60 °C**	**60-70 °C**	**50°C***
C6:0	0.22 ± 0.09	0.28 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.23 ± 0.02	0.21 ± 0.04	0.24 ± 0.03	0.25 ± 0.02	0.23 ± 0.01
C8:0	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.11 ± 0.01
C14:0	0.13 ± 0.01	0.15 ± 0.02	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.00	0.15 ± 0.01
C15:0	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.09 ± 0.00
C16:0	19.9 ± 0.24	20.1 ± 0.34	19.9 ± 0.22	20.0 ± 0.17	19.9 ± 0.27	19.9 ± 0.10	19.9 ± 0.19	20.0 ± 0.04	19.5 ± 0.08
C17:0	0.09 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
C18:0	4.46 ± 0.09	4.45 ± 0.18	4.53 ± 0.09	4.57 ± 0.05	4.51 ± 0.07	4.61 ± 0.06	4.56 ± 0.11	4.54 ± 0.02	4.33 ± 0.04
C20:0	0.61 ± 0.01	0.64 ± 0.03	0.62 ± 0.01	0.62 ± 0.01	0.63 ± 0.02	0.63 ± 0.01	0.61 ± 0.01	0.62 ± 0.02	0.65 ± 0.01
C22:0	0.31 ± 0.01	0.30 ± 0.03	0.30 ± 0.01	0.30 ± 0.02	0.31 ± 0.01	0.32 ± 0.03	0.31 ± 0.02	0.32 ± 0.02	0.35 ± 0.01
C24:0	0.18 ± 0.01	0.18 ± 0.02	0.18 ± 0.02	0.18 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
Total SFA	**26.2 ± 0.3^{ab}**	**26.6 ± 0.5^{ab}**	**26.4 ± 0.3^{ab}**	**26.5 ± 0.2^{a}**	**26.4 ± 0.3^{a}**	**26.5 ± 0.1^{ab}**	**26.4 ± 0.2^{a}**	**26.5 ± 0.1^{a}**	**25.9 ± 0.1^{b}**
C16:1	1.59 ± 0.02	1.60 ± 0.04	1.58 ± 0.03	1.61 ± 0.02	1.59 ± 0.04	1.60 ± 0.02	1.60 ± 0.03	1.61 ± 0.01	1.55 ± 0.02
C18:1	62.74 ± 0.17	62.36 ± 0.17	62.67 ± 0.18	62.58 ± 0.14	62.53 ± 0.13	62.50 ± 0.09	62.61 ± 0.26	62.49 ± 0.06	62.66 ± 0.11
C20:1	0.30 ± 0.02	0.30 ± 0.03	0.31 ± 0.02	0.30 ± 0.02	0.31 ± 0.03	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.34 ± 0.02
Total MUFA	**64.9 ± 0.1^{b}**	**64.5 ± 0.2^{a}**	**64.7 ± 0.2^{ab}**	**64.7 ± 0.1^{ab}**	**64.7 ± 0.1^{ab}**	**64.6 ± 0.1^{ab}**	**64.7 ± 0.2^{ab}**	**64.6 ± 0.1^{ab}**	**64.8 ± 0.1^{b}**
C18:2	6.80 ± 0.08^{A}	6.85 ± 0.24^{AB}	6.79 ± 0.07^{A}	6.76 ± 0.03^{A}	6.84 ± 0.13^{A}	6.78 ± 0.03^{A}	6.81 ± 0.1^{A}	6.75 ± 0.02^{A}	7.14 ± 0.06^{B}
C18:3	1.71 ± 0.04^{A}	1.71 ± 0.07^{AB}	1.70 ± 0.04^{A}	1.70 ± 0.01^{A}	1.73 ± 0.06^{B}	1.72 ± 0.02^{A}	1.74 ± 0.05^{B}	1.70 ± 0.03^{A}	1.83 ± 0.02^{B}
Total PUFA	**8.7 ± 0.1^{a}**	**8.7 ± 0.3^{ab}**	**8.7 ± 0.1^{a}**	**8.7 ± 0.1^{a}**	**8.8 ± 0.2^{a}**	**8.7 ± 0.1^{a}**	**8.8 ± 0.2^{a}**	**8.7 ± 0.1^{a}**	**9.2 ± 0.1^{b}**
Total *trans*	**0.24 ± 0.01^{b}**	**0.28 ± 0.02^{d}**	**0.22 ± 0.02^{abc}**	**0.23 ± 0.02^{bc}**	**0.20 ± 0.03^{ab}**	**0.21 ± 0.02^{abc}**	**0.21 ± 0.03^{abc}**	**0.21 ± 0.01^{abc}**	**0.18 ± 0.01^{a}**

The control group revealed a higher content of both linoleic and linolenic acid ($p < 0.05$) and long-chain SFA, while showing lower contents of palmitic and stearic as well as reduced content of total trans fatty acids. All together, this highlights a lower fatty acid oxidation and a less aggressive procedure, as expected from the more controlled drying process performed in the lab, as well as lower temperature.

3.2. Triglycerides, diglycerides and free fatty acids

The results of Table 2 illustrate that triglycerides are the most important glyceride forms in the oil, ranging from 65 to 75%, while the diglycerides vary from 8 to 10 %. However, the oil has a high content of free fatty acids, ranging from 17 to 26%. Although typical in crude, non-refined oils (Lee and Balick, 2008), it reveals an intense lipolytic activity during the preparation of the flour, with glycerides hydrolysis, most likely induced by natural lipases in the pulp, although some microbial activity cannot be excluded. A similar situation is described for palm oil, with a rapid lipolytic activity after picking, particularly in ripe fruits. In palm oil, the free fatty acid content is a determinant for its quality and economic revenue since these free fatty acids should be removed by refining, with a limit of edibility of only 5% in this oil. To reduce this enzymatic activity, a rapid enzyme inactivation after fruit picking could be considered as a practice to be tested, as already performed with palm fruit, with sterilization of the fruits immediately after, but a recommendation to pick the fruits before being fully ripe can also potentially contribute for hydrolysis reduction (Morcillo et al., 2013). Both procedures could contribute to enhancing the quality of this oil and deserve further testing.

Overall, there are no significant differences ($p > 0.05$) in the results of all communities samples, even with different drying temperatures, except in Manhiça for the samples processed at the higher temperatures. Interestingly, these samples presented significantly lower amounts of FFA, and consequently higher triglycerides, with percentages close to the ones obtained in the laboratory samples. Therefore, the temperature was not the determinant factor in this hydrolysis extent, because lab samples were the ones processed at the lower temperatures. The sun-drying step extension is influenced by weather conditions thus likely being the main determinant of this hydrolysis extension, with probable enzymatic inactivation at the roasting step, for all the temperatures tested. The time taken to inactivate the enzymes is probably the main determinant for glyceride hydrolysis. Since the time between fruits picking and its processing in the lab was also not under full control, hydrolysis still occurred in the control samples. The high consistency in the hydrolysis values between all the samples is indicative that time/temperature controls will have minor effects on hydrolysis extension and that more drastic measures should be implemented.

3.3. Minor bioactive constituents

Carotenoids are responsible for the oil typical orange color but are also known to protect the lipids from oxidation because of their ability to act as effective quenchers of single oxygen (Young and Lowe, 2018). The vitamin A profile in the oil was dominated by carotenes, with minor non-identified compounds quantified as β-carotene equivalents (Table 2). The analytical method used does not distinguish α and β-carotenes, so the carotenes are expressed on a β-carotene basis but correspond to the α+β-carotenes. The total amounts ranged from 8 to 13 mg / 100g. These carotene amounts are superior to other crude vegetable oils (Franke et al. 2010), supporting its orange color, despite being lower than typical palm oil amounts (50-70 mg/100g) (Absalomé et al., 2020), recognized as one of the highest food sources for carotenoids.

Apparent variability was observed between communities but of no statistical significance. As to differences imposed by the processing temperatures, again no differences were observed, except in Manhiça, with significantly higher carotene amounts in the samples processed at the higher temperatures, even higher than the control one. This is indicative of higher carotene protection, being consistent with the lower lipolysis already discussed above.

Vitamin E includes tocopherols and tocotrienols. Although tocopherols are most frequently found in vegetable oils, with α-tocopherol being generally the most abundant in foods, tocotrienols are also typical in some vegetables. Macuácua oil is characterized by an atypical profile, with a single main tocol compound, with values between 24 to 35 mg / 100g (Table 2). This compound elutes in our normal-phase chromatographic system between β- and γ-tocotrienols and has a maximum absorbance at 296 nm, as typical from these tocols but no true identification was possible. Knowing that palm oil is also rich in tocotrienols, and due to its chromatographic behavior, we have quantified it as β-tocotrienol equivalents. Several studies have demonstrated that tocotrienols have highly interesting activities in health and disease that are distinct from that of tocopherols (Birringer et al., 2018) representing also an interesting potential health feature for this oil. Even without a clarification on the tocol identity, the amounts are within the amounts reported for crude vegetable oils (Codex Alimentarius, 2015).

When the communities are compared, the amounts were highly consistent, except again Manhiça samples processes at the higher temperatures, with significantly higher amounts, equivalent to those prepared in the laboratory. Again, higher temperatures were not responsible for tocol losses.

The composition of the phytosterols in the *S. madagascariensis* oil was highly consistent between all samples, with an average of 431±10 mg/100g for total desmethylsterols, characterized by relative percentages of 30.5±0.4% for β-sitosterol, 30.4±0.2% of campesterol, 21.4±0.2% of Δ5- avenasterol and 17.7±0.7% of stigmsterol. Additionally, two triterpene alcohols (4,4-dimethylsterols) were present in high amounts, identified by GC-MS after conversion to trimethylsilyl ether as cycloartenol and 24-methylenecycloartanol (Zhang et al., 2020), summing up 191±4 mg/100g, and with a very consistent proportion between them of 2.4-2.6 to 1. The global amounts of phytosterols can be considered high within the most common vegetable oil, being higher than soybean, sunflower, peanut or olive oils, and similar to sesame and flaxseed oils, but still lower then corn, rapeseed or rice bran oils. Their relative profile is distinct from the most common vegetable oils, with flaxseed as the most similar one (Codex Alimentarius, 2015; Yang et al., 2019).

Vestigial amounts of β-amyrin and squalene were also detected but not quantified.

The presence of these compounds is interesting from a health perspective. While phytosterols in general are considered relevant to reduce atherogenesis-related risks, the triterpene alcohols have been identified as having antidiabetic properties (Nair et al., 2020), health effects reported for this fruit.

Table 2. Glycerides profile (%TG, DG, FFA), carotenes (mg/100g) and tocols (mg/100g) of the oil extracted from the flours obtained at different temperatures from *Strychnos madagascariensis* fruits (mean ± standard deviation, n = 3).

	Chókwè		**Manhiça**		**Chicualacuala**		**Marracuene**		
	50-60 °C	**60-70 °C**	**50-60 °C**	**60-70 °C**	**50-60 °C**	**60-70 °C**	**50-60 °C**	**60-70 °C**	**50 °C***
Triglycerides (%)	66 ± 1^{a}	69 ± 5^{b}	66 ± 1^{a}	75 ± 1^{b}	67 ± 3^{a}	67 ± 3^{a}	65 ± 0^{a}	66 ± 1^{a}	74 ± 0^{b}
Diglycerides (%)	9 ± 1^{b}	9 ± 1^{ab}	9 ± 0^{ab}	8 ± 0^{a}	9 ± 0^{ab}	9 ± 0^{ab}	10 ± 1^{b}	9 ± 1^{ab}	9 ± 1^{ab}
Free fatty acids (%)	25 ± 1^{a}	22 ± 5^{ab}	25 ± 1^{a}	17 ± 1^{b}	24 ± 2^{a}	24 ± 3^{ab}	26 ± 1^{a}	25 ± 0^{a}	17 ± 1b
Carotenes$^{\#}$ (mg/100g)	9 ± 2^{a}	8 ± 1^{a}	10 ± 1^{a}	14 ± 1^{c}	8 ± 1^{a}	8 ± 1^{a}	10 ± 1^{a}	10 ± 1^{a}	12 ± 1^{b}
Tocols$^{\#\#}$ (mg/100g)	30 ± 3^{ab}	24 ± 2^{a}	27 ± 2^{a}	34 ± 0^{b}	26 ± 1^{a}	26 ± 2^{a}	26 ± 3^{a}	26 ± 1^{a}	35 ± 2^{b}

*** Prepared at the laboratory. Quantified in $^{\#}$β-carotene and $^{\#\#}$β-tocotrienol equivalents. Different letters in a row correspond to statistically significant ($p < 0.05$) differences between means.**

3.4. Product classification

With the data acquired from oil samples, a PCA test was performed to classify the communities and production processes based on their lipid composition. This analysis allowed explaining 82.5% of the total data variance using two principal components, as represented in Figure 2, whose variable communities were all higher than 0.709. Herein, three distinct clusters are identified, corresponding to the lab, Manhiça (high temperature), and the remaining groups. The first principal component (PC1) factor, which comprises 54.0% of the total variance, can separate the two groups: the field samples located in the neutral and negative region and the control positioned in the positive region. This finding may be correlated with the fact that the control samples exhibited the highest values for linoleic acid (PC1 loading = 0.946) and linolenic acid (PC1 loading = 0.949) and a lower content of SFA (PC1 loading = −0.915). The second principal component (PC2) factor, which accounts for 28.5% of the total variance observed, was also able to separate two sample groups, probably due to the higher content of tocols (PC2 loading = 0.894) and carotenes (PC2 loading = 0.890) and a lower content of FFA(PC2 loading = -0.828) of the control and Manhiça (high temperature) with respect to the remaining samples.

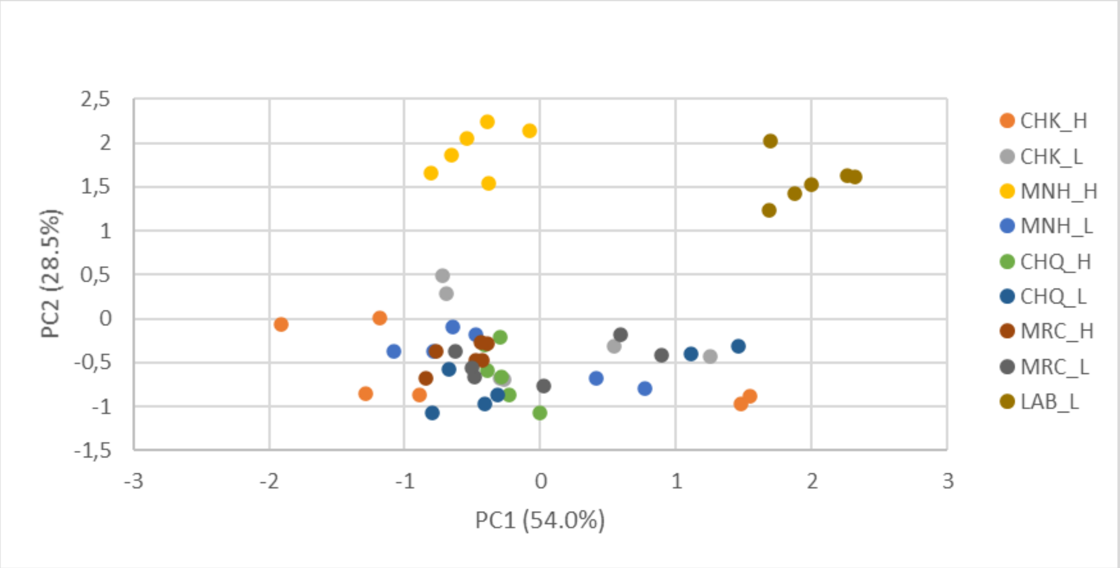

Figure 2: Principal component analysis of flour oils produced at Marracuene (MRC), Manhiça (MNH), Chokwé (CHK), Chicualacuala (CHQ) and lab (control) at low (L) and high (H) temperatures.

4. Conclusions

This research aimed to determine the composition and quality of *S. madagascariensis* pulp oil, obtained in local communities in Mozambique by a traditional process from the dried and grinded pulp flour, called *nfuma*. The analysis revealed a high-oleic oil, with high amounts of carotenoids and tocotrienols, both important from a health and technological perspective. In addition, this study provides scientific evidence that the method of drying and extraction performed by the communities does not seem to impose variations in its composition, being also very closed to the one obtained in the samples prepared in the laboratory. Its high content of non-sterified fatty acids, probably derived from lipases activity during drying, deserve to be explored, either by thermal inactivation or shorter drying periods.

Fats are an integral part of a balanced and complete diet. Macuácua oil has a long tradition of consumption by local communities, and seems to be suitable for consumption because of its nutritional attributes, namely its monounsaturated profile and richness in characteristics antioxidants and health-promoting substances, constituting a fat that is worth to be explored. Furthermore, technological solutions to improve the oil quality and shelf life of these not-yet-fully-exploited resource must be developed and could be exploited by agro-industry and become a source of income for poor rural areas in the future.

CHAPTER 6- DISCUSSION AND CONCLUSIONS

In this chapter, an overall discussion of the knowledge acquired during this work is depicted in line with the objectives proposed. Conclusions of this thesis as well as further research directions are also presented.

6.1. Overall Discussion

Africa has made a major contribution to the world's main food crops, not only in the total number of species used in international trade, but also in the importance of products. In 1992, there were 2155 African species used as food, representing 4.3% of African flora. Van Wyk (2005) in his Global Review of Food Plants showed that Africa made a substantial contribution with 119 African products marketed. The commercial factor of indigenous plants is relevant considering the potentialities of the native fruits for the development of Africa. In relation to *Strychnos* spp, people from rural communities in southern Africa have attributed nutritional benefits and used it in folk health, however few research work exists. Despite the few mentions, significant variations in the nutritional composition of fresh monkey orange fruits have been reported in the literature. Regarding *S. madagascariensis* the identification and characterization of the fruit is even more scarce.

This fruit grows mainly in the different districts of the south of Mozambique, namely Marracuene and Manhiça at Maputo province, and districts of Chókwè and Chicualacuala at Gaza province. Thus, in Chapter 3, the pulp of fruits from these 4 districts from the south of Mozambique were evaluated in terms physicochemical properties (pH, total soluble solids, color), proximate composition, minerals, and antioxidant compounds given by vitamins A (by means of carotenoids content) and vitamin E, phenolic contents and its antioxidant capacity. Macuácua fruit has yellow-orange color, the pH is around 6 and presents a high fat content. The fruit of *S. madagascariensis* presents interesting minerals amounts important for health, such as Ca, Mg, Fe and Zn. It has high potassium content and low sodium, meaning that there is a good relationship between potassium and sodium, which is good for cardiovascular diseases. It also has a considerable vitamin E content. In relation to carotene, this fruit is outstanding in its content, as expected from its color (positive in a*-yellowish and b*-reddish, according to CIELAB space color). Its profile is dominated by β-carotene, the carotenoid with the highest theoretical vitamin A activity and probably the most ubiquitous in foods (Dias et al., 2021).

Comparing the samples, high antioxidant activity of Chókwè fruit was observed, likely due to the higher content of carotenes and total phenolic content compared to the other pulps. Additionally, the pulp from Chókwè displayed higher fat and protein contents.

On the other side, pulp from Chicualacuala had the higher mineral and vitamin E contents. Thus, here it was demonstrated the origin influence of the nutritional and antioxidant aspects of the fruits. The nutritional composition and antioxidant activity revealed to be similar to well-known antioxidant fruits, giving it added nutrition–health-promoting benefits. Since these fruits are wasted because of their seasonality and high perishability, rural communities apply traditional processing techniques in order to improve its availability and shelf-life. In Mozambique, local communities dry and ground the pulp, making *nfuma*, a flour consumed during staple food shortage, and from this flour also an oil can be obtained.

To produce *nfuma* flour, once harvested, the fruit pulp is first dried under the sun (2-4 days) and roasted in metallic tray over fire (~1 hour), and then ground. It is mainly consumed by local communities as a snack or as a complement of staple foods in times of food scarcity. However, there is practically no data on its nutritional value, and in Chapter 4 this information was exploited.

As described in Chapter 4, *nfuma* stands out for its high fat content (near 30%), mainly composed by monounsaturated fatty acids, carrying interesting amounts of vitamin E and carotenes. Given the total fiber content (ranging between 6 and 11%), *nfuma* can be considered as a food "high in fiber", according to Regulation (EC) No. 1924/2006," and compared with cassava and corn flour, consumed as a staple in the African region, *nfuma* stands out for its higher fiber content. In relation to the current dietary recommendations (EFSA, 2019), assuming consumption of 100 g of *nfuma,* it provides 30% of fiber and 27-48% of alpha-linolenic acid of the adult dietary reference value (DRV). Regarding liposoluble vitamins, *nfuma* provides 55-63% and 56-66% of vitamins A and E, respectively.

In relation to protein, *nfuma* provides only 6% (male) and 7% (female) of DRVs (assuming 100 g of daily consumption). Therefore, *nfuma* must be combined with other protein sources to achieve DRVs. Despite some thermal modification, in this thesis it was revealed for the first time the amino acid profile of a *Strychnos* spp fruit. The main amino acids of *nfuma* protein were Arg, Asp and Glu. Considering the essential amino acids, Lys was the limiting one.

The mineral composition of *nfuma* reveals K as the main macromineral followed by Mg > Ca > Na, and Mn as the main trace element followed by Fe > Zn > Cu > Cr > Co), hence *nfuma* contributes significantly to the estimated daily intake of K and Mn (assuming consumption of 100 g), representing 40% and more than 100% of DRVs, respectively. Despite being a poor source of Fe, *nfuma* contains twice the Fe content of maize flour. The combination of maize flour with *nfuma* in porridges, together with the consumption of *nfuma* as a daytime snack, as reported by people from Mozambican communities, can increase Fe intake. Despite the nutritional advantages of *nfuma*, this flour also carries some non-essential minerals, from which Ni content should be addressed with caution and mitigation strategies are required to guarantee its safety.

Owing to *nfuma* high lipid content, some communities separate, by pressing, a liquid oil with several applications, including cooking purposes. The characterization of this oil was revealed in Chapter 5. For that, oils extracted mechanically from the flours produced in the four districts by local communities, were characterized for fatty acids composition, glycerides, phytosterols, tocols and carotenes. After the sun dry step, two different temperatures were applied during the slight toast of the pulp fruit: 50–60 °C (as in the flour described in Chapter 4) and 60–70 °C. Additionally, fresh fruits from Marracuene were processed in the laboratory, being dried at 50 ºC for two days, and its oil was used as group control.

In general, the oil from pulp fruit of *S. madagascariensis* was fluid at room temperature, deep orange, and characterized by a high and very consistent content of oleic acid (62–63%), followed by saturated palmitic acid (20%) and linoleic acid (7%). The only polyunsaturated fatty acids detected were linoleic acid (6.8–7.1%) and α-linolenic (1.7–1.8%). The amount of short-chain saturated fatty acids was low (<0.3%), as was the sum of long-chain ones (arachidic, behenic, and lignoceric, <1.2%). Like other natural high-oleic crude oils, such as nuts and olive oil, it presents great stability to oxidation and contribute to the liquid state at room temperature. Furthermore, high-oleic oils have also been shown to have beneficial impacts on serum cholesterol and low-density lipoproteins without affecting the high-density ones, thus minimizing the risk of cardiovascular disease (Riley et al., 2022). Although the amount of saturated fatty acids of macuácua oil is higher than the upper mentioned oils, its well below the tropical edible fats, palm or coconut. The health-related lipid indices of the fat of this fruit and its products, the atherogenic and thrombogenic indices, discussed in Chapter 4, presented values below 1, which translate into lower atherogenic and thrombogenic potential.

Among communities, a high consistency on the fatty acids profile was observed. In general, few impact of temperature was observed, with only some communities presenting minor differences in the most heat sensitive fatty acids and *trans* fatty acid content. However, control group (50 °C) revealed significantly ($p < 0.05$) higher content of both linoleic and linolenic acids, and long-chain SFA, while showing lower contents of palmitic and stearic fatty acids, as well as reduced content of total *trans* fatty acids when compared with oils from communities. This highlights a lower fatty acid oxidation and a less aggressive procedure, as expected from the more controlled drying and lower temperature.

Minor bioactive compounds of macuácua oil were accessed, as the liposoluble vitamins A and E, already evident in fruit (Chapter 3) and flour (Chapter 4). The vitamin A profile in the oil was dominated by carotenes, especially β-carotene, and highlights from other crude vegetable oils due its high content (8 to 13 mg β-carotene equivalents/ 100 g), responsible for the oil's typical orange color but are also known to protect lipids from oxidation. Vitamin E in oils generally includes tocopherols and tocotrienols, however this oil is characterized by an atypical profile of tocols presenting only vestigial amounts of α-tocopherol and tocopherol esters and dominated by a single main unknown tocol. Considerable amounts of phytosterols (431 ± 10 mg/100 g) and triterpenic alcohols (823 ± 4 mg/100 g) were found in macuácua oil. These compounds are considered relevant to reduce atherogenesis-related risks, and triterpene alcohols have been identified as having antidiabetic properties, health effects already reported for this fruit. The overall composition of these minor bioactives was highly consistent among origins and temperatures.

However, this oil presents high free fatty acid content (22–25%) which reveals intensive lipolytic activity during the preparation of the flour, or already in the mature fruit, with glyceride hydrolysis, most likely induced by natural lipases in the pulp, although some microbial activity cannot be excluded. The sun-drying step extension may influence the hydrolysis extension, more than the roasting temperature. This high free fatty acid content raises doubts regarding its edibility, however the hydrolysis extension can be reduced by picking the fruits before they are fully ripe and also by optimizing its technological process (as in palm oil). Both procedures could contribute to enhance the quality and edibility of this oil and deserve further testing in order to allow wide consumption.

As referred in Introduction and Chapter 4, in Mozambique, about two thirds of children 6–59 months of age are affected by vitamin A deficiency and anemia. *Nfuma* is mainly used during times of staple foods shortage, such as maize and cassava flour. In Mozambique, porridge is a typical first food for young children made from local cereal or tuber flours prepared with water. However, is not sufficient to meet the nutritional needs of infants. Thus, the promotion of dietary diversity for Mozambican children has been centered on “papas enriquecidas” or enriched porridges, achieved by adding locally available nutritious foods, as recommended by Ministry of Health. Food-based strategies that promote dietary diversity, such as through complementary feeding recipes and cooking demonstrations are a key feature of health programming (Picolo et al., 2019).

Despite the carotene conversion into vitamin A activity being an issue without scientific consensus, provitamin A carotenoids are particularly important for populations with limited availability of animal foods, for those who do not eat them by choice (Dias et al., 2021) or in populations where deficiency of vitamin A is a public health concern, as is the case of children from Mozambique. Macuácua fruit *per si* provides 0.6 to 1.35 mg RE/100g (Chapter 3). Therefore, according to the DRV for children (0.250 and 0.300 mg RE/day for 1-3 and 4-6 years old, respectively) (EFSA, 2019), the consumption of 50 g of the fruits provides at least 100% of the DRV.

In Chapter 4, it was also demonstrated that considering a daily consumption of 50 g of *nfuma*, children aged 1-3 and 4-6 years can obtain, respectively, 82 and 68% of vitamin A EDIs. Thus, the daily consumption of *nfuma* by children in Mozambique may alleviate vitamin A deficiency due to its high β-carotene content. In relation to Fe, 50 g of *nfuma* children (1–6 years) only provide 12% of DRV, however its use in “enrichment” of maize-based porridges together with consumption as a daytime snack, may help to increase the daily intake of Fe. Despite the nutritional advantages of *nfuma*, some constraints were also found, such as the non-essential minerals, especially the Ni content, that deserve caution before being recommended as food-based strategies.

Additionally, the addition of a spoon of oil to increase the energy density of the porridge has been also recommended by Ministry of Health (Picolo et al., 2019). The high-oleic content of macuácua oil, carries relevant carotene amounts (Chapter 5) and considering a daily consumption of 10 g of this oil, the children aged 1-3 and 4-6 years can obtain, respectively, 67 and 56 % of vitamin A EDIs. This could be a sustainable and promising strategy.

Although high content of carotenes were found in flour and oil, losses from fruit clearly occurred. The processing of fruit to flour implies that macuácua pulp contacts with oxygen and light. The sun-drying step extension and elevation of temperature during the thermal treatment dramatically increase the rates of degradation reactions of the carotenes. Total carotene content of *nfuma* varied from 2.2 to 2.6 mg β-carotene equivalent/ 100 g, which is very low compared to the total carotene content found in pulp, expressed as dry weigh basis, 23 to 29 mg of β-carotene equivalent/ 100g dw (data not shown). Furthermore, 8 to 10 mg of carotenes/ 100 g of oil were found, and considering the fat amount of the fruit, this value can be potentially higher. Thus, the selection of appropriate maturation stage of the fruit together with post-harvest treatments, processing, and storage conditions would improve the carotenoid content, and its vitamin A potential, in the products of macuácua fruit.

6.2. Main Conclusions

The present thesis brings scientific information concerning three major issues related with *S. madagascariensis* fruit: 1) the nutritional and antioxidant properties of this indigenous fruit; 2) the detailed nutritional composition of the flour from the pulp fruit, *nfuma*, including its nutritional impact in terms of nutrient adequacy; and 3) characterization of the oils physically extracted from *nfuma*, together with the evaluation of its nutritional and technological potential.

1) Macuácua fruit reveals an orange and acid-neutral pulp with high lipid content dragging vitamin E and carotenes. High antioxidant activity was reported and probably given by the high content of vitamin E and carotenes and also from the phenolic compounds. Furthermore, the results indicated pulp differences according to the origin of the fruits. Samples from Chókwè and Manhiça presented higher fat content, antioxidants and carotenes. Samples from Chicualacuala presented the most outstanding mineral composition and vitamin E.

2) This fruit flour, *nfuma*, stands out for its high fat content, mainly composed by monounsaturated fatty acids, delivering vitamin E and carotenes, together with naturally occurring sugars and high fiber content. *Nfuma* is also a good source of

Mn and K and, despite being a poor source of Fe, *nfuma* contains twice the Fe content of maize flour. However, its Ni content should be addressed with caution and mitigation strategies are required in order to guarantee its safety. Its local use in the "enrichment" of maize-based porridges or as an ingredient for pastry and snacks for developing healthier new food products deserves to be technologically approached for wider valorization.

3) The deep orange oil of macuácua shows a high content of oleic acid and it contains considerable amounts of tocols and carotenes, as well as sterols and triterpenic alcohols. However, its high free fatty acid content (22–25%) reveals intensive enzymatic hydrolysis during the drying/fermentation steps, whose extension can be reduced by optimizing the technological process. Its chemical profile supports some of its folklore uses, revealing that it can be a promising source of edible oil, with health and technological potential that is worth optimizing and exploring.

The data in this thesis provide valuable information about the fruit of *Strychnos madagascariensis*, its *nfuma* flour and oil. Their chemical and nutritional profiles support some of its folklore uses, filling the gap between the historically conserved communities' knowledge and scientific recognition.

At the end, the present thesis and its results contribute for the achievement of goals for sustainable development set by the UN (2030 agenda), namely to the goal 1: no poverty, goal 2: zero hunger, goal 3: good health and well-being and goal 15: life on land.

6.3. Future Perspectives

Further studies are needed regarding the best practices in selecting mature fruits and its storage, as well as in the production of *nfuma* and its oil, from a safety, nutritional and technological approach, in order for it to be used as a nutrition-health strategy to address the micronutrient deficiencies in children and be used as an ingredient for new food products and wider valorization of the *S. madagascariensis* fruit.

With potential improvement of macuácua oil nutritional and technological properties, the resulting pressed cake would be richer in minerals and fiber, therefore also deserves to be exploited in further studies.

CHAPTER 7- REFERENCES

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SEDE ADMINISTRATIVA

FACULDADE DE **CIÊNCIAS DA NUTRIÇÃO E ALIMENTAÇÃO**

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