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Nutritional content of Zambian underutilized species: The case of mumosomoso (*Vangueriopsis lanciflora* Hiern)

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled "Nutritional content of Zambian underutilized species: The case of mumosomoso (*Vangueriopsis lanciflora* Hiern)" independently in Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 16th April, 2021

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Aneta Taušnerová

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Abstract

Vangueriopsis lanciflora is a fruit tree species native to Southern Africa. In Zambia, the most valued product of the tree are the fruits, which are usually collected from natural stands. Despite its importance especially among the rural communities, the species remain neglected from the scientific point of view. Therefore, the objective of this study was to summarize information on *V. lanciflora* with a focus on its current as well as and potential uses and to evaluate the nutritional content of the fruit pulp from lyophilizate. The fruit pulp was subjected to nutritional analyses of: dry weight (in drying oven), ash (in muffle furnace), crude fat (according the Soxhlet method), fibre (using Filter Bag Technology on ANKOM Fiber Analyzer), protein (using Kjeltec apparatus), NFE content and vitamin C along with B-group vitamins (HPLC). The nutritional values were determined as follows: dry weight - 90.76 %, ash - 2.55 %, crude fat - 1.74 %, crude fibre - 5.02 %, crude protein - 4.8 % and NFE - 76.65 %. As for vitamins, the content of vitamin C proved to be in really high concentration of 1148.26 mg/100 g, suggesting that only 6.53 g of lyophilized fruit pulp is needed to cover the recommended daily allowance. Based on the results we can conclude that *V. lanciflora* is an important source of both macro and micronutrients, showing that its consumption may enhance the food security and contribute to the balanced diets in rural areas of Zambia. However, the fruits are predominantly eaten and sold in a fresh form, thus food processing methods such as lyophilization may increase their value and create an interesting opportunity for export of *V. lanciflora* fruits to other countries.

Key words: domestication, food security, lyophilizate, neglected crops, nutrition, Southern Africa, wild foods, vitamins

Abstrakt

Vangueriopsis lanciflora je ovocný druh pocházející z jižní Afriky. V Zambii jsou nejcennějším produktem stromu plody, které se obvykle sbírají z přírodních porostů. Navzdory svému významu zejména mezi místními obyvateli, zůstává tento druh z vědeckého hlediska opomenut. Proto bylo cílem této studie shrnout informace o druhu *V. lanciflora*, se zaměřením na jeho současné i potenciální využití, a vyhodnotit nutriční obsah ovocné dužiny z lyofilizátu. Ovocná dřeň byla podrobena nutričním analýzám a to na: sušinu (v sušárně), popela (v muflové peci), hrubého tuku (dle Soxhletovy metody), vlákniny (pomocí technologie Filter Bag na analyzátoru ANKOM Fiber Analyzer), bílkovin (pomocí Kjeltec zařízení), obsah NFE a vitamín C spolu s vitamíny skupiny B (HPLC). Nutriční hodnoty vyšly následovně: sušina - 90,76 %, popel - 2,55 %, hrubý tuk - 1,74 %, hrubá vláknina - 5,02 %, protein - 4,8 % a obsah bezdusíkatých látek - 76,65 %. Pokud jde o vitamíny, obsah vitamínu C vyšel v opravdu vysoké koncentraci 1148,26 mg/100 g. K pokrytí doporučené denní dávky tak stačí pouze 6,53 g lyofilizovaného ovoce. Na základě výsledků můžeme dojít k závěru, že *V. lanciflora* je důležitým zdrojem jak makroživin, tak mikroživin, což ukazuje, že její konzumace může zlepšit zabezpečení potravin a přispět k vyvážené stravě ve venkovských oblastech Zambie. Plody se však převážně konzumují a prodávají v čerstvé formě, takže způsoby zpracování potravin, jako je lyofilizace, mohou zvýšit jejich hodnotu a vytvořit zajímavou příležitost pro vývoz ovoce jako je právě *V. lanciflora* do jiných zemí.

Klíčová slova: domestikace, potravinová bezpečnost, lyofilizát, opomíjené plodiny, výživa, jižní Afrika, divoké plodiny, vitamíny

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List of the abbreviations used in the thesis

AOAC – Association of Analytical Chemists

DW – dry weight

HPLC - High performance liquid chromatography

ITCZ - Inter-tropical convergence zone

NFE - Nitrogen-free extracts

NTFPs – Non-timber forest products

NUS – neglected underutilized species

RDA - Recommended Dietary Allowance

SD – standard deviation

1. Introduction

Thousands of edible plant species have been cultivated by humans throughout history, but modern agriculture is dominated by just three species: rice, maize and wheat, supporting the extension of homogenous farming landscapes (Padulosi 2013). It is estimated that only 30 crops provide 95 percent of human food energy needs (FAO 2010). In stark contrast, at least 20,000 species of edible plants in the world have been compiled as edible and provided food needs (Plants For A Future 2021). There are still many countries with hundreds of crops suitable for agronomic purposes, which are not yet used. Neglected or underutilized species (NUS) represent an immense wealth of agrobiodiversity, containing highly nutritious species, which can both contribute to healthier diets worldwide as well as to fight malnutrition in developing countries (IPGRI 2002).

NUS are species which are of secondary priority in terms of research and development. Most of the plants used grow indigenously in the wild or are cultivated on a very small scale. Hence, production as well as availability is limited. Typically, NUS are not traded as commodities at the international level. They are wild or semi-domesticated varieties and non-timber forest species adapted to particular, often quite local, environments (Padulosi 2013). To exploit the full potential of these indigenous neglected plants for human nutrition and health, we have to understand how the plants are used in different communities as well as to determine which substances contribute to the associated health benefits (Muhanji et al. 2011; Tumwet et al. 2014; Aworh 2015).

Agriculture is the main source of livelihood for almost 60 % of Zambia's population. Dependence on the rainy season, which affected by climate change, is growing very unpredictably, is increasing the risk of low production, malnutrition and poverty. In addition, the poor infrastructure and the remoteness of rural areas makes it difficult for local people to access the market and health services. The nutrition situation in Zambia continues to deteriorate due to many reasons (Nyirenda et al. 2007). Hunger, undernourishment, and malnutrition rates in Zambia have been reported as extremely high and among highest rates in the world. The most recent statistics on undernourishment by

Food and Agriculture Organisation (FAO), International Fund for Agricultural Development (IFAD) and World Food Programme (2014) ranked Zambia as having the highest levels of malnutrition in Africa and second from the bottom in the world at 48% (Mukuka & Mofu 2016). Therefore, NUS may hide an unexploited options how to deal with such trends (Padulosi et al. 2013).

An important issue is the storage and post-harvest processing of NUS, which are the factors that play a role in the breakdown of nutrient such vitamins and minerals (Ball 2005). Freeze-drying, also known as lyophilisation could be the answer to this problem. It is a modern method which found many applications for the production of high-quality food and pharmaceuticals. Long shelf-life as well as lower risk of pathogenic infestation and low weight of lyophilised products are one of the major advantages of this method (Fernandes 2015).

Therefore, this thesis focuses on *Vangueriopsis lanciflora* Hiern, an underutilized fruit tree species, indigenous to Zambia. Even though the tree has been used by local communities for decades, its exact nutritional values remain scientifically unknown. Therefore, the main objective of this thesis was to analyse the dietetic content of *V. lanciflora* fruits from lyophilizate and to summarize the available information on this neglected species.

2. Literature Review

2.1. Zambia

Zambia is large, resource-rich country in South Africa. It is situated on a high plateau and takes its name from the Zambezi River. It shares its borders with eight countries (Angola, Botswana, Democratic Republic of Congo, Malawi, Mozambique, Namibia, Tanzania, and Zimbabwe) that serve as an expanded market for its goods. Large parts of the country are sparsely populated. Much of the population is concentrated in the country's most developed area known as the Line of Rail. This area is between the railway connecting Copperbelt with the capital Lusaka and the border town Livingstone (Williams 2021). Zambia is experiencing a large demographic shift and is one of the world's youngest countries by median age. Its population, much of it urban, is estimated at about 17.9 million and grows rapidly at 2.8 % per year, partly because of high birthrate, resulting in the population doubling close to every 25 years (World Bank 2020).

2.1.1. Agriculture

Arable land (% of land area) in Zambia was reported to be 5.12 % in 2016 (FAO 2018). Zambia has the potential for significant increases in agricultural output. Currently, less than 30 % of potentially arable land is cultivated. In general, the agricultural sector in Zambia supports livelihoods of 85 % of the population. Maize is the main staple crop, taking more than >65 % of cropped land (Chikowo 2016). Per capita consumption of maize is estimated at 105 kilograms annually, making Zambia the third largest consumer of maize in the world after Lesotho and Malawi (Ranum 2014). Apart from the maize the domestic production comprises of crops such as rice, sorghum, millet, and cassava while sugar, soybeans, coffee, cashew, groundnuts and cotton are mainly meant for export (ITA 2020).

Unlike elsewhere in sub-Saharan Africa, agriculture is relatively unimportant in Zambia's economy compared to mining industry (primarily copper) (Chikowo 2016). It contributes to approximately 20 % of GDP (Gross Domestic Product) and employs three quarters of the population (Chikowo 2016; ITA 2020). Agricultural cultivation in Zambia is mostly non-mechanized and most agricultural production on the continent is rain dependent (Jain 2007). Opportunities include large-scale farming, farm input and equipment supply, irrigation systems, agro-processing, and commodity trading. The sector is in dire need for mechanization (ITA 2020).

2.1.2. Population

The population comprises of approximately 73 ethnic groups. Almost 90 % of Zambians belong to the nine main ethnolinguistic groups : the Nyanja-Chewa, Bemba, Tonga, Tumbuka, Lunda, Luvale, Kaonde, Nkoya and Lozi (Figure 1) (Williams 2021).

"One Zambia - one nation" is the motto of the national emblem and is intended to unite the multicultural Zambian society, as more than 70 languages are spoken there. However, the only official language is English. Mixing indigenous languages with English creates new languages, such as the Copperbelt mining language. Literacy reaches 79 %. Christianity predominates in religion, Islam and Hinduism are also represented and about 1 % of the population are animists (Olša & Hulec 2008).

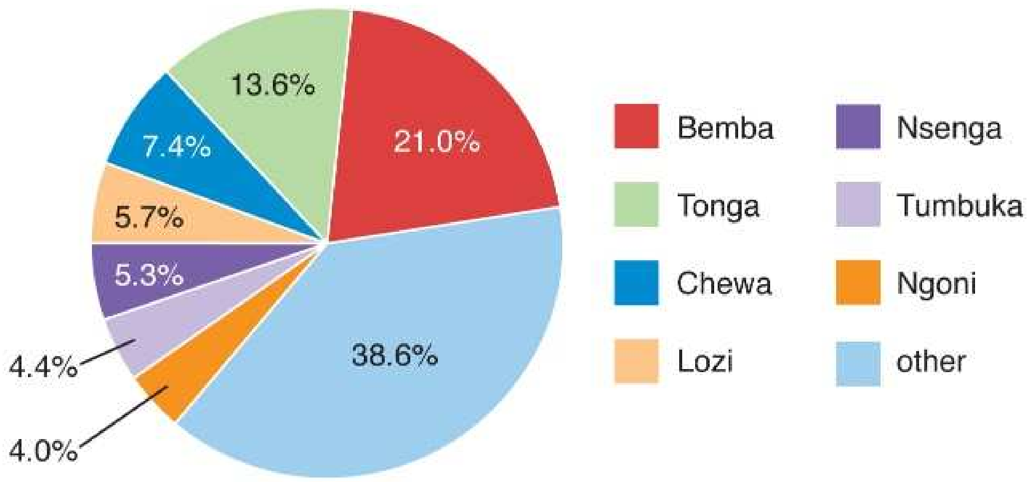

Figure 1. Ethnic groups composition in Zambia 2010 (Source: Encyclopedia Britannica, 2010)

2.1.3. Climate and agroecological zones

There are three main climatic seasons in Zambia – cool and dry from May to August, hot and dry from September to November, and warm and wet from December to April. The climate is mostly affected by the movement of the inter-tropical convergence zone (ITCZ), which is the meeting place of the subtropical high-pressure areas of the northern and southern hemispheres (Zambia Tourism 2021). In January the ITCZ is in its southernmost position, and the rainy season is at its peak. Precipitation reaches up to 200 mm per month. By June it moves north, resulting in a dry weather. Most months by this period are absolutely without rain. Precipitation (concentrated in just five months) varies according to agro-ecological regions but generally comes in storms with heavy raindrops leading to vast soil erosion. Temperature is modified by altitude. However, average monthly temperatures remain above 20 °Cover most of the country for eight or more months of the year. The average annual temperature is 20.4 °C. The average temperature in Zambia in the summer (November to April) is 30°C. Summer rains reduce the high temperature (Williams 2021; Climate data 2020; Musonda 2013).

Zambia has three major Agro-Ecological Regions: Regions I, II and III (Figure 2). These regions are distinguished and classified based on climatic, geo-physical, soil types, land-use, farming systems and socio-economic parameters (Phiri et al. 2013).

Semi-arid Region I includes areas of Southern, Eastern and Western Zambia: Zambia's valleys at 300-800 m altitude mostly lie in Region I. The growing season is relatively short (80-120 days) and risky for crop production, as poorly distributed rains result in enduring frequent dry periods. This region is the driest and most prone to drought, and its soils contain low levels of organic matter, low nutrient reserves, and high acidity levels (Chikowo 2016).

The second region spans the central part of the country and is divided into two subregions: the first subregion is plateau of the Southeast and the Kalahari desert and the second is Zambezi floodplain in the West (Williams 2021). The region has the most fertile soils and has good plant growing season range of 90- 150 days which is good and capable of supporting growth of almost all crops grown in Zambia. Much of the country's agriculture activities take place in this region and comprises of both mixed cropping subsistence to commercial farming (Phiri et al. 2013).

Region III, the high-rainfall area, is situated in the northern part of the country and is the largest AER covering 50% of Zambia's land area with average temperatures of 30-33 °C (Phiri et al. 2013). The soils are nutrient poor but high in exchangeable aluminium and manganese, both of which are toxic to most of the crops. Therefore, soils must be often limed here to increase pH. The region has the longest plant growing season of 140-200 days (Chikowo 2016).

Figure 2. Zambia's agro-ecological regions and rainfall distribution (mm) (Source: Thurlow et al. 2008).

2.2. Tropical fruit species

The edible fruits of the tropics are many in number, dozens of species of tropical fruits, including bananas, dates, figs, mangoes and pineapples, have been popular outside of the tropics for a long time now (Patterson & Gardener 2021).

People in many rural African communities are not aware of the nutritional value of the fruits. For example, in the case of marula (*Sclerocarya birrea*) and baobab (*Adansonia digitata*), people often eat only the pulp of the fruits, while throwing out the seeds, which are of a higher protein and fat content than peanuts are. The long-term goal should be to raise awareness and increase the use and consumption of these indigenous fruits. These fruits are popular in Zambia and play an important role in the diet, especially in arid areas (Magaia et al. 2013).

However, in recent years, many new species like longan (*Dimocarpus longan*) and lichi (*Litchi sinensis*) have grown in popularity in the temperate regions as well. Even so, there are still many lesser-known fruits that are rarely exported and used almost exclusively in tropical countries. The mumosomoso (*Vangueriopsis lanciflora)* is a good example. Although its fruit is tasty, soft and sweet, little is known about the species itself and market chain has not yet been developed. Hence, *V. lanciflora* cannot be transported and traded over long distances. However, introducing new fruits into a country is often difficult and must be done legally. Obtaining information on the fruits and their sources is a first step (Martin et al. 1987; Blancke 2016).

2.3. Neglected and underutilized species (NUS) and their potential

Crops that have been forgotten over the last century are being rediscovered. Scientists and policymakers begin to recognize the value of so-called 'neglected and underutilized' crops, affirming what local communities have known for generations. Although not traded internationally, the species are uniquely adapted to their local environments and play a vital role in supporting diverse diets in Sub-Saharan Africa (FAO 2017).

It is difficult to precisely define which attributes make a crop "underutilized", but often they have the following features (adopted from Hammer et. al 2001):

- linkage with the cultural heritage of their places of origin
- local and traditional crops whose distribution, biology, cultivation and uses are poorly documented
- adaptation to specific agro-ecological niches and marginal land
- weak or no formal seed supply systems
- traditional uses in localized areas
- they are produced in traditional cultivation systems with little or no external input and without chemical treatment

- they receive little attention from research, extension services and policy
- they may be highly nutritious and have medicinal properties or other multiple uses

Staple crops face major challenges in the near future and a diversification from our over-reliance on staples will be important as part of the progress towards securing the food production. Neglected and underutilized crops are often indigenous ancient species which are still used at some level within the local, national or even international communities, but have the potential to contribute both to the planet and people much more than they currently do (Mayes 2012).

Here are some examples of the world's most successful NUS. Baobab (*Adansonia*), mangosteen (*Garcinia mangostana)*, chia (*Salvia hispanica),* dragon fruit (*Hylocereus spp*), noni (*Morinda citrifolia*) and maca (*Lepidium meyenii*) (Padulosi 2013). The maca plantis sometimes referred to as Peruvian ginsengand is commonly available in powder form as a supplement. The maca plant has exploded in popularity in recent years. Maca root has traditionally been used to enhance fertility and sex drive. It is also claimed to improve energy and stamina (Palsdottir 2016).

2.4. Vitamins

Vitamins are organic compounds that people need in small quantities. Vitamins and minerals can be divided into four categories: water-soluble vitamins, fat-soluble vitamins, macrominerals and trace minerals. Most vitamins dissolve in water and are known as water-soluble. Even each water-soluble vitamin has a unique role, their functions are related (Table 1). For example, most B vitamins act as coenzymes and help trigger important chemical reactions. A lot of these reactions are necessary for energy production (Streit 2018). Their solubility in water is based on polar and ionizable groups (phosphate, carboxyl, hydroxyl, etc.) (Combs 2008). Mild vitamin deficiency (hypovitaminosis) or complete deficiency (avitaminosis) can cause dysfunctions in organism resulting in serious diseases.

2.4.1. Vitamin C

Long before the discovery of vitamin C (ascorbic acid) in 1932, nutrition experts recognized that something in citrus fruits could prevent scurvy, a disease that killed as many as 2 million sailors between the 15th and 18th century. Vitamin C is a white crystalline compound highly soluble in water (Roberts & Caserio 1977). According to Teucher et al. (2004), vitamin C helps to enhance availability and absorption of iron from non-heme sources. Vitamin C also has antioxidant properties since it can easily lose the electron to neutralize and inhibit free radicals from being oxidized in preventing cell damage (Whitney & Rolfes 2008). The vitamin plays a role in controlling infections and the body's response to stress. It is also commonly used as food additive which acts as an antioxidant and can neutralize harmful free radicals. It helps to make collagen, a tissue needed for healthy bones, teeth, gums and blood vessels (Carr & Frei 1999; Hwang 1999). Many methods can be used for determination of vitamin C such as spectrophotometry, electrophoresis, titration, and highperformance liquid chromatography (HPLC) (Tang and Wu 2005; Dong et al. 2007; Spinola et al. 2012). RDA for vitamin C is 75 mg per day for adults (Combs 2008).

2.4.2. B vitamins

The discoverer of vitamin B was the Polish biochemist Kazimierz Funk in 1912 in rice bran. B-complex is a collective term for B vitamins, sometimes it is a trade name for a mixture of different B vitamins, but it usually does not contain all of them (Velíšek 1997).

Table 1. Overview of hydrophilic vitamins that have been tested in the study and their basic functions (Source: Combs 2008)

Vitamin	Trivial name	Basic functions
B1	Thiamine	Essential for the proper functioning of the heart, nervous system and carbohydrate metabolism.
B2	Riboflavin	Necessary for obtaining energy from proteins, fats and carbohydrates. It is also necessary for function of eyes, skin, nerves, immune system.
B3	Nicotinic acid	Controls the production of energy from food.
B5	Panthothenic acid	Necessary for fatty acid synthesis
B6	Pyridoxine	Helps release sugar from stored carbohydrates for energy and create red blood cells. Plays an important role in homocysteine metabolism.
B9	Folic acid	Important for proper cell division

Vitamin B_6 is a general term for three closely related derivatives: pyridoxal, pyridoxol and pyridoxamine and their corresponding phosphates (Figure 3) (Slíva & Votava 2011). The most stable compound is pyridoxol.

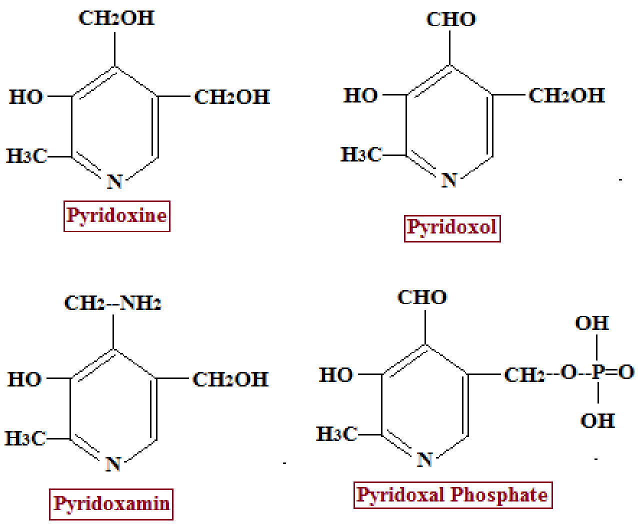

Figure 3. Pyridoxine occurs in three forms (Source: Vrolijk et al. 2017)

2.5. Mumosomoso (*Vangueriopsis lanciflora* Hiern)

In Zambia, *Vangueriopsis lanciflora* is recognised for its ability to 'make visible' (ku-solola), revealing animals to hunters or used to treat barren women to 'make children visible'. In this way, plant species richness is linked to a lush cultural tradition which provides a social link (Campbell 1996). The English common name is crooked false medlar, while in Tanzania the species is known as msambalawe-lulenga and mumosomoso in Zambia (ICRAF 2018).

2.5.1. Botanical description

Vangueriopsis lanciflora Hiern (Rubiaceae) is a deciduous shrub or small tree usually reaching from 1.5 - 6 metres in height, occasionally up to 13 metres (Tropical Plants Database 2020). The trunk is straight and cylindrical, with smooth bark, which is grey, flaking off to expose a brownish-pink or rusty-red underbark. Branches are thick and stiff but young branches are densely grey-pubescent. Young branchlets are rusty-red, often powdery and knobbly.

Leaves are elliptic oblong to broadly elliptic, leathery, dark green above, yellowish-white or yellowish-green below and densely downy (Figure 4). Margin is usually undulate, stipules joined at the base, triangular and is about 10 mm long. Petiole is robust and up to 10 mm long elliptic and densely tomentose. Stipule is deltoid with long tapering apices: opposite, blades 5.3-21.5 × 1.6-12.5 cm, which are elliptic or oblong-elliptic, ± rounded or subacute at the apex, markedly discolorous, velvety grey tomentose or pubescent beneath and very rarely glabrous.

Inflorescences is densely yellowish velvety pubescent, simply cymose or branched and axillary on the leafless nodes of older branches. Cymes is several-flowered. Bracts reach length of 4–6 mm. Peduncles and secondary branches reaches 10–20 mm in length (PROTA 2020. Corolla is beaked, elongate, with divaricate tails at the apex in bud. It can be also whitish or yellow-green. Grey tomentose is up to 25 mm long. The tomentose is shallow-tubular with markedly recurved lobes. It has cream-colour with calyx cup-like and densely downy.

The fruit of *V. lanciflora* is 2.5-4 cm long and yellow when ripe (Figure 5). The yellow-brown, globose fruit is up to 30 mm in diameter. They are the sweetest when somewhat overripe, so they are often left in the sun for a few days before consumption. The light brown fruit is considered one of the best wild fruits in its native range. Ripe fruits are collected from October to December (PROTA 2020).

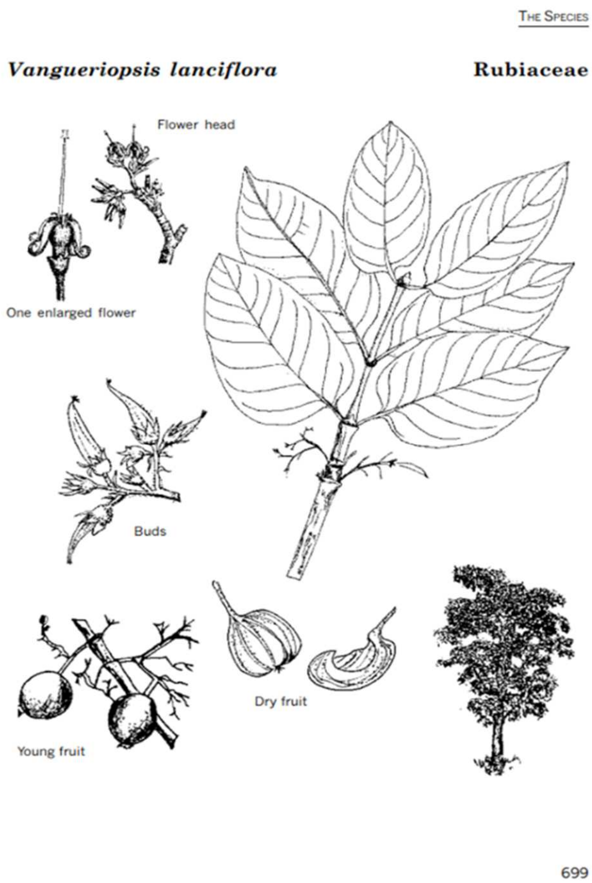

Figure 4. Botanical description of of *V. lanciflora* (ICRAF 2008)

Figure 5. The fruit and fruit pulp of *V.lanciflora* (Source: A. Maňourová's archive 2019)

2.5.2. Propagation and treatment

Vegetative methods of propagation are not often developed or practised. Propagation is mainly done by seedlings or root suckers. Seed germination is poor due to the hard seed coats, and at its best, the germination rate reached 60 % after 40 days. For better results, the seed is soaked in cold water for 24 hours. In general seedlings give better results compared to other methods of plant propagation. Although regeneration from coppicing and root suckers is good, the growth often succumbs to drought and fire. The species is light demanding and should be planted after partial clearance of vegetation. The leaves and fruit are attacked by insects (ICRAF 2008).

2.5.3. Habitat, ecology and distribution

Vangueriopsis lanciflora is common in medium to higher altitudes, in deciduous woodlands, specifically in Miombo woodlands. It occurs often in open grassland with scattered trees, on Kalahari desert, or sometimes even on rocky ground, at elevations of 900 - 1,740 metres, where it is retained in old cultivations and fields for their edible fruit (Tropical Plants Database 2021).

The tree is widely distributed in parts of Central Africa and in tropical South Africa specifically in: Western and Southern Tanzania, Malawi, Mozambique, Zambia, Zimbabwe, Botswana, Namibia, Angola and South Africa (Figure 6).

Places where *V. lanciflora* is located are classified as Aw by Köppen (wet tropical savanna). The mean annual rainfall is approximately 1200 mm, with a dry period of about four months, when each month receives 40 mm of rain on average but there can be months of no rain at all (Climate data 2020). Soils vary according to bedrock and edaphic conditions. Laterization is the dominant soil-forming process and low fertility oxisols can be found there (Woodward 2019). The main pollinators are bees (Tropical Plants Database 2017).

Covering much of Southern Africa, the Miombo woodlands are a vast region of tropical grasslands, savannas and shrublands. Named for the oak-like "miombo" trees (*Brachystegia* spp.) that dominate the area. Miombo woodlands cover about 352 million hectares (47 % of the total land area in Zambia). Soils of the Miombo woodlands are generally nutrient-poor (Campbell 1996).

Figure 6. Distribution of *V. lanciflora* (Source: MapChart, Author 2021)

2.5.4. Utilization

The fruits are valued as a food in its native area and are predominantly harvested from the wild, consumed by local communities and occasionally also sold in markets. The fruit can be eaten fresh or dried (Figure 7).

The tree is sacred to the local people who use the hard wood for making spoons and drinking cups for infants to bring them strength (PROTA 2020). The wood can be used for poles, constructions, turnery, tool handles and carving. It is also a popular source of firewood and charcoal. Charcoal preparation is a crucial activity in Miombo woodlands and is increasingly becoming a lucrative, however, unsustainable business. A traditional kiln can take an average volume of 13.96 m^3 of billets of various tree species to produce 20 to 30 charcoal bags each weighing 40 to 55 kg depending on species used (Abdallah & Monela 2007).

The plant is also of medicinal value, it is used as oral ingestion: a decoction of the root is administered to barren women to induce pregnancy. Bark is used to treat mumps (PROTA 2020). As mentioned above, living plant *in situ* has cultural importance being part of many traditions, such as rituals and magic: “When a person suffers from nightmares and headaches, he is likely to be advised by his African doctor (traditional doctor) to plant a cutting of the plant” (Gilges 1955).

Figure 7. Fresh and dried fruit of *V. lanciflora* (Source: J. Staš’s archive)

2.6. Related species and taxonomy

The genus *Vangueriopsis* is found in Central and South Africa (from D.R. Congo to Tanzania and South Africa). It was originally described by Walter Robyns in 1928 and contained 18 species in two subgenera *Brachyanthus* and *Rostranthus*. Since then most of the species have been transferred to *Vangueriella* and currently only four species names remain valid. Species: *Vangueriopsis lanciflora, Vangueriopsis longiflora, Vangueriopsis rubiginosa* and *Vangueriopsis shimbaensis* (Verdcourt 1987).

Vangueriopsis longiflora is a tree growing about 13 metres in height. The edible fruit is gathered from the wild for local use. The tree also supplies timber and fuel for local use and is sometimes planted to provide shade and bee forage. The trees predominantly grows in lowland forests and thickets. The wood is white and perishable, it is used for firewood and domestic utensils (Tropical Plants Database 2021).

Vangueriopsis shimbaensis looks very similar to *V. longiflora*. However, the tree occurs mainly in Tanzania and is easily distinguished from this species due to its longer stipules, 2.6 - 3.4 cm long (Davis 2010).

2.7. Lyophilization (freeze-drying)

The classic sun or air drying is a very well-known process even in low-income countries but it has its limitations. One of the major disadvantages is that during the processing, the nutritional value decreases. It has been verified that for heat-sensitive materials, such as fruits and vegetables, the effect of the drying process on product quality, particularly in the retention of nutrients, colour, and texture, is a critical factor (Extrifit 2017). The dehydration techniques commonly employed to preserve fruits and vegetables are solar drying, heated air drying, microwave drying, osmotic dehydration, foam-mat, spray-drying, freeze-drying, and fluidized-bed drying. Freeze drying is the best method for obtaining a high-quality dried product (for heat sensitive materials). Proper freeze drying can reduce drying times by 30 % (Marques et al. 2006).

The first documented signs of preservation by means of frost and low pressure appeared during the time of the Inca Empire. Sub-zero night temperatures together with low pressure (high in the mountains) caused the products to dry out slowly, conserving the food for much longer time and making it easier to transport. The method of lyophilization (freeze-drying) as we know it today was discovered by the French scientist Dr. Jacques Arsène d'Arsonval and his assistant Frédéric Bordas in the laboratories of the Collège de France (Chalupa 2020).

During lyophilization, a triple point occurs and there is water in all three states. Food is frozen down to -20 °C and most of the water evaporates or sublimes under the pressure. It is then dried in a special chamber in which the temperature reaches - 60 °C, at the same time, the pressure is reduced to the state of vacuum. This ends the process, the pressure drops and the lyophilizate is formed, which must be stored well so that the food does not come into contact with the air. Dehydrated fruits have received special attention because they retain the characteristics of natural products, especially valuable minerals and vitamins, reduce the transportation costs, and have an appropriate moisture level to prevent the growth of the moulds, which cause deterioration in fresh fruits. The freezing process also suspends the enzymatic activity, which is responsible for the rot and mould of food and slows down the multiplication of microbes. In addition, there are no oxidative changes (Extrifit 2017; George & Ratta 2002). Freeze-drying appears, therefore, as a promising technique for dehydration of thermal-sensitive materials, such as *V. lanciflora* fruits (Marques et al. 2006). It is typically used to preserve perishable materials, with the goal of extending their shelf life and preparing them for transport.

One of the disadvantages is that this method is relatively expensive. It costs about five times more than the conventional drying. The costs are also variable depending on the product, the packaging material and processing capacity (Ratti 2001).

2.7.1. Storage of freeze-dried products

Lyophilized products are extremely hydroscopic, they must be sealed in air-tight containers to prevent rehydration from atmospheric exposure. Freeze dryers can be configured with a "stoppering" capability to seal the product while it is still under partial vacuum inside the unit. Typically, stoppering is done on vials with partially inserted stoppers. The shelves are collapsed so that each shelf pushes down the vials/stoppers located on the adjacent shelf. It is also common to backfill with an inert gas such as dry nitrogen before stoppering the product (Barley 2020).

One of the most important questions which follows from the text above is how to store freeze-dried product once the package has been opened. These freeze-dried fruits are fragile and highly sensitive to light, heat, oxygen and moisture. Keeping freeze-dried foods protected from these elements is the key to getting the maximum shelf-life.

3. Objectives of the Thesis

There are two main objectives of the thesis.

1) To summarize the scientific knowledge about the species *Vangueriopsis lanciflora* and to make an overview of its current and potential uses.

2) To evaluate the nutritional content of the freeze-dried fruit pulp for ash, crude fat, crude fibre, protein, saccharides (nitrogen free extract - NFE), B-group vitamins and vitamin C.

4. Materials and methods

The methodology consisted of a theoretical part (literature review) and a practical part (laboratory analyses) carried out in laboratories of the Department of Food Science and the Department of Microbiology, Nutrition, and Dietetics at the Faculty of Agrobiology, Food and Natural Resources CZU, in May 2020.

The first, literature review part, was done by a comprehensive information search through scientific databases such as Web of Science, Google Scholar, Science Direct, Researchgate, JSTOR and plant database PROTA followed by reviewing several scientific books, as well as bachelor's and master's theses, appended by FAO and World Bank statistic information. All these sources are listed in 'References'.

In the second part, the content of macronutrients (ash, moisture, crude fat, fibre, protein and saccharides- NFE) along with micronutrients such as B-group vitamins: thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinic acid (vitamin B_3), pathothenic acid (vitamin B_5), pyridoxine (vitamin B6), pyridoxamine (vitamin B6), pyridoxal (vitamin B_6), folic acid (vitamin B_9) and ascorbic acid (vitamin C) was analysed according to standardized AOAC methods. Crude fibre was determined using Filter Bag Technology with ANKOM Fiber Analyzer, the protein content was analysed by the Kjeldahl method using Kjeltec apparatus, crude fat determination was done according to the Soxhlet method and vitamins were analysed by HPLC. Results were evaluated in Excel spreadsheet. The plant samples were tested in triplicate for each method and in three independent experiments. Results were expressed as mean values with standard deviation (mean ± SD).

4.1. Data collection and evaluation, study site

The data collection was conducted by co-supervisors of this thesis in November 2019 in Mongu, Zambia, and surrounding areas. The fruits were obtained from the local farmers as well as from the fruit market directly in Mongu. A voucher specimen of *V. lanciflora* was taken and stored in Herbarium of FTA, CZU.

Mongu lies in 1,035 metres above sea level and it is the capital city of Western Province in Zambia (Figure 8). Mongu's climate is classified as tropical. Average daily temperature in Mongu is 23.1 °C with average annual rainfall of 1,070 mm. Whereas in June, the level of precipitations is the lowest (0-1 mm), January is the rainiest month (269 mm on average). October is the hottest month of the year (19.2 °C on average), while July is considered to be the coldest month of the year (10.5 °C on average). The variation in annual temperature is around 8.8 °C (Climate Data 2020).

Mongu belongs to the agroecological region II - and receives between 800 mm and 1,000 mm of annual rainfall. Mongu is also part of Zambezian flooded grassland specifically Barotse floodplain. Zambian rivers and low-lying areas are prone to flooding, and there are extensive permanent swamps. Around 10 % of the country is in this biome and it is a sole ecoregion. Plants, animals and people have evolved to this cycle which in Zambia has been fairly reliable, and it brings a number of ecological advantages (Ministry of Agriculture and Ministry of Fisheries and Livestock 2016).

Figure 8. Western province of Zambia, with its capital city Mongu (Source: Dignity 2018)

4.2. Chemical analysis

All laboratory analyses were performed at least in triplicate based on AOAC methodology. The final result is then arithmetic average of those measurements in complying with the standard deviation.

List of laboratory devices used for the evaluations:

Dry matter and ash - Muffle furnace – LH 15/13 (Nabertherm, USA)

Crude fibre analysis – ANKOM 200 Fibre Analyzer (ANKOM Technology, USA) Crude

Protein analysis – Kjeltec 2400, Digestion unit – MB 442 (FOSS, Denmark)

Crude fat analysis – SER 148 (VELP Scientifica, Italy)

Drying oven –UN55 Single DISPLAY (Memmert, Germany)

Laboratory scales – AE200 (Mettler Toledo, Czech Republic)

Centrifuge – 4600 RPM

4.2.1. Sample preparation

The samples were stored in a freezer at -60 °C. They were then defrosted and separated into pulp, seeds and skin. The pulp was crushed together with liquid nitrogen in a mortar with pestle to obtain homogeneous mixture. They were wrapped in aluminium foil and lyophilized. Dried samples were finely homogenized with the use of laboratory hammermill and blender to obtain the final product: yellow powder with a pleasant fruit aroma. This was the initial sample for our analyses.

4.2.2. Lyophilization

Freeze drying occurs in three phases: Freezing, Primary Drying (Sublimation) and Secondary Drying (Adsorption) (Millrock technology 2020).

The steps we have taken to lyophilize the fruit pulp can be summarized as follows:

- Pre-treatment
- Freezing (Thermal Treatment) at atmospheric pressure
- Primary Drying (Sublimation) under vacuum
- Secondary Drying (Desorption) under vacuum
- Removal of Dried Product from Freeze Dryer

4.2.3. Dry matter and ash determination

The crucibles were placed in a desiccator freshly charged with a drying agent. At first, 2 g of sample were put into clean porcelain crucibles of known weight which have been dried for 24 hours and removed from the oven with wire tongs.

The crucibles with samples were returned to the oven set at 105 °C for 3 hours and weighed again to obtain dry matter weight. The weight was recorded, and the dry sample was determined as the difference between the gross dry weight and crucible weight. The percent dry matter was expressed as the ratio of the dry sample weight to the wet sample weight times 100.

Results were counted by formula:

$$DM = \frac{\mathrm{w2} - \mathrm{w0}}{\mathrm{w3}} \times 100$$

w0 – weight of empty crucible (g)

w2 – weight of crucible with sample after drying (g)

w3 – sample weight (g)

The crucible and the dry sample remaining after the dry matter determination may be used for the ash determination. Before proceeding, the crucible and dry sample weights must be known. The crucibles were transferred to a cool muffle furnace and the furnace was pre-set at 600 °C. The samples were removed and placed in a desiccator with tongs, cooled and weighed following the procedure outlined above. The percent of ash was expressed as the ratio of the sample ash weight to the dry sample weight times 100.

Results were counted by formula:

$$A = \frac{\mathrm{w3} - \mathrm{w0}}{\mathrm{w2}} \times 100$$

w0 – weight of empty crucible (g)

w2 – sample weight (g)

w3 – weight of crucible after burning (g)

4.2.4. Crude fibre analysis

Crude fibre determination was performed using Filter Bag Technology on ANKOM 200 Fibre Analyzer. Filter bags were pre-dried at 103.5 °C, after noting the bag weight, 1 g of sample was added; the bag was sealed and placed into a carrier (three samples in one partition). For each analysis, one bag was left blank and added into the carrier as a control. Carrier was put into the vessel, loaded with additional weight and poured by 1.45 litres of distilled water and 12ml of 98 % H_2SO_4. The same procedure was repeated with 25 g NaOH poured by 2 litres of distilled water. The filter bags were dried with filter paper and placed in 250 ml of acetone. After three minutes, bags were dried in the oven for four hours (103.5 °C). The weight of each bag was recorded and the samples were put in porcelain crucibles of known weight.

The weight of burned content was noted and the crude fibre calculated using the formula: $CF = \frac{\mathrm{w3}+\mathrm{w4}-\mathrm{w5}-(\mathrm{w1}\times\frac{\mathrm{w3}}{\mathrm{w1}})}{\mathrm{w2}} \times 100$

w1 – weight of empty filter bag (g)

w2 – sample weight (g)

w3 – weight of filter bag after drying (g)

w4 – weight of empty crucible (g)

w5 – weight of crucible after annealing (g) c – correction factor of empty filter bags, $c = \frac{w3}{w1}$

4.2.5. Protein analysis

Protein content was determined by Kjeldahl method on Kjeltec 2400 apparatus. 0.5 g of sample was put into glass digestion tube together with one mineralisation tablet Kjeltabs CK (3.5 g K_2SO_4, 0.4 g $CuSO_4.5H_2O$), 10 ml of H_2SO_4 (96 %) and two times 5 ml of H_2O_2. The nitrogenous substances were converted into ammonium ions, from which ammonia was liberated after basification of the mineralizate, which was then determined by titration after steam distillation with 0.1 M HCl. The results were provided in % N × 6.25.

4.2.6. Crude fat analysis

Crude fat determination was done by Soxhlet method performed on SER 148 apparatus. 2-3 grams of the ground sample was weighed into the extraction cartridge. The cartridge was attached to the extraction unit of the Soxhlet system with a magnetic ring. 50 ml of extraction solvent (petroleum ether) was measured into the crucible. First analysis was done with 70 ml of extraction solvent. The extraction cartridge was let into a boiling solvent and let the first phase of extraction take place (25 minutes). Then the extraction cartridge was lifted over the boiling solvent and the second extraction phase was allowed to proceed (20 minutes). After drying, the extraction crucibles cooled down in a desiccator for about 20 minutes and their weight was noted. Using the obtained data, we calculated the fat content in the analysed sample.

Results were counted by formula: $F = \frac{w3-w1}{w2} \times 100$

w1 – weight of empty extraction glass (g)

w2 – sample weight (g)

w3 – weight of extraction glass after the analysis (g)

4.2.5. Nitrogen-free extract (NFE)

Nitrogen-free extract (NFE) consists of carbohydrates, sugars and starches. When crude lipid, crude protein, ash and crude fat are added and the sum is subtracted from the dry matter, the difference is NFE (Tamilnadu 2010).

$$NFE = DM - (CL + CP + ash + CF)$$

NFE – nitrogen free extract (%)

DM – dry matter (%)

CL – crude lipid (%)

CP – crude protein (%)

CF – crude fat (%)

4.2.6. Vitamins

4.2.6.1. Chemicals and reagents

Standards of thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinic acid (vitamin B_3), pathothenic acid (vitamin B_5), pyridoxine, pyridoxamine, pyridoxal (vitamin B6), folic acid (vitamin B_9), ascorbic acid (vitamin C), and monopotassium phosphate (KH_2PO_4) were purchased from Sigma Aldrich (Prague, Czech Republic). Analytical grade methanol (MeOH) was purchased from VWR Chemicals (Radnor, USA). Phosphoric (H_3PO_4) and hydrochloric (HCl) acids were purchased from Lachner chemicals (Neratovice, Czech Republic) and PENTA Chemicals (Prague, Czech Republic), respectively.

4.2.6.2. Sample preparation

To prepare samples for the vitamin content determination, 50 mg of plant material were extracted in aqueous acid solution (0.1 M HCl). The solution was kept either in the oven at 121 °C for 30 min (in the case of analysis of the compounds of the vitamin B complex) or at room temperature for 30 min in an orbital shaker (in the case of vitamin C analysis). Afterwards, the samples were thoroughly vortexed (for 1 min), soaked for 5 min in an ultrasonic bath (room temperature), and centrifuged at 9,000 rpm for 15 minutes. Resulting solution was transferred to HPLC vials and submitted to the HPLC-UV analysis (Figure 9).

4.2.6.3. High performance liquid chromatography (HPLC) – UV/Vis analysis

HPLC analysis was carried out on UltiMate 3000 HPLC system that was hyphenated with an UV/Vis detector (ThermoFisher, Waltham, USA). Separation of particular vitamins was performed on an ACE C8 column (250 × 4.6 mm, 5 µm, 100 A; Aberdeen, Scotland). Compounds were separated using a gradient elution employing mobile phase A (water with 50 mM KH_2PO_4 and 50 mM H_3PO_4) and B (MeOH) as follows: 0 min, 95:5 (% A:B); 3 min, 95:5; 16.5 min, 55:45; 19.5 min, 20:80; 22.5 min, 20:80; 24.5, 95:5 (total run: 25 min). Flow was set at 1 mL/min, the injection volume was 10 µL, and column temperature was maintained at 40 °C. UV absorption was monitored at wavelengths between 190 and 400 nm. Compounds were quantified under 205 nm. Evaluation of acquired data was performed using Chromeleon 7.2 Software (ThermoFisher). Peak areas of standards of each analyte obtained at six concentration levels (100, 50, 20, 10, 5, and 2 μg/mL) were plotted against the corresponding response using weighed linear regression to generate calibration curves. Each sample was extracted in triplicate. Results were expressed as mean values with standard deviation (mean ± SD) in mg/g dry weight (DW).

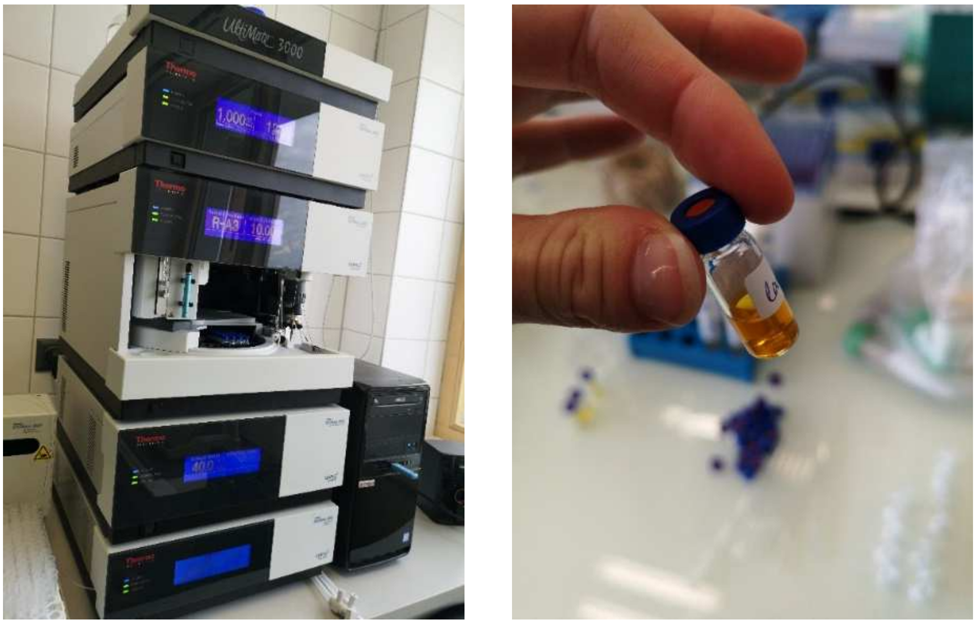

Figure 9. HPLC and sample in a vial (Source: Author)

5. Results and Discussion

The results were determined using the methods described above. Only one study on the nutritional content of *V. lanciflora* fruits have been published so far, though no study determining the dietetic values from lyophilizate was found elsewhere. Documentation of the exact nutritional properties of *V. lanciflora* is crucial, forming the basis for increased consumption and utilization of the species not only in Zambia.

5.1. Nutritional composition of *Vangueriopsis lanciflora* fruits

The results of the determinations of dry matter content and proximate composition (protein, crude fat, crude fibre, ash and NFE) along with vitamins of the fruit pulp are summarised in the Table 2.

As can be seen from the table, the dry matter value after lyophilization is 90.76 %. The content of ash was 2.55 %, most often it is in the range of 2-5 % of ashes in dry matter and expresses essentially the total content of inorganic substances (Škarpa 2010). Ashes are also used to calculate the content of nitrogen-free extractives. Nitrogen-free extract - NFE, consisting of carbohydrates, sugars, starches, and other saccharides, was 76.65 %. Protein analysis showed value of 4.8 %, while crude fat and crude fibre content were 1.74 % and 5.02 %, respectively.

As mentioned above, vitamin B_6 comes in 3 closely related derivatives, which were detected as follows: pyridoxal (trace amount), pyridoxine (769.94 mg/100 g) and pyridoxamine (30.47 mg/100 g). The value of thiamine was 909.24 mg/100 g, however the highest value was reached by vitamin C, 1148.26 mg/100 g. Nicotinic acid was found in *V. lanciflora* only in trace amount and the lowest value displayed folic acid (27.5 mg/100 g). Panthothenic acid and Riboflavin were detected in values 805.07 mg/100 g and 473.72 mg/100 g, respectively.

Table 2. Contents of individual analysed components after lyophilization in *V. lanciflora* fruit pulp

Micronutrients	mg/100g	SD
Thiamine (B_1)	909.24	199.82
Riboflavin (B_2)	473.72	118.47
Nicotinic acid (B_3)	trace amonut	-
Panthothenic acid (B_5)	805.07	98.03
Pyridoxal (B_6)	trace amonut	-
Pyridoxine (B_6)	769.94	113.00
Pyridoxamine (B_6)	30.47	8.04
Folic acid	27.50	7.47
Vitamin C	1148.26	303.28
Macronutrients	**g/100g**	**SD**
Dry matter	90.76	0.021
Ash	2.55	0.350
Crude fibre	5.02	0.373
Protein	4.8	0.252
Crude fat	1.74	0.059
NFE	76.65	

SD – standard deviation

5.2. Macronutrients comparison

The information on the nutritional content of *V. lanciflora* is limited, though a study of Malaisse (1997) was used for comparison with the results of this study. However, the research from 1997 worked with fresh fruits, which might be the reason why the values differ from our results, counted from lyophilizate. Therefore, we also decided to compare the data with other known fruit tree species (apple, baobab and strawberries) as well as with another Zambian NUS – *Strychnos cocculoides* and *Strychnos pungens* (Přinosilová 2021).

In the above mentioned study from Mallaise (1997), the dry matter of fresh fruit of *V. lanciflora* was 22 %, the remain was water (78 %). The dry matter content of lyophilized *V. lanciflora* pulp was much higher 90.76 % and the moisture was 9.24 %. Crude fibre was higher in lyophilizate (5.02 %), whereas ash was higher in fresh *V. lanciflora* (3.7 % compared to 2.55 % in lyophilizate). In case of crude fat, there was not a big difference: 2.3 % in fresh form and 1.74 % in lyophilizate (Table 3). The protein content of fresh *V. lanciflora* was 2 %, which corresponds to the fact that the protein in most fruits comprises less than 5 % (Saka et al. 1994). However, in the lyophilizate, *V. lanciflora* has considerably higher protein content of 4.8 %. The Recommended Dietary Allowance (RDA) for protein is a 0.8 grams per kilogram of body weight. Protein helps our body to maintain a proper fluid balance, builds and repairs tissues, transports nutrients, and provides other essential functions (USDA National Nutrient Database 2015). Even though *V. lanciflora* cannot be generally considered as rich in proteins, compared to other fruits such as apple, the content is still much higher.

Table 3. Macronutrient comparison of different tropical fruits, both in fresh and lyophilized form

g/100 g	Analysed	Condition	Dry matter	Ash	Crude fibre	Protein	Crude fat
Vangueriopsis lanciflora	Malaisse (1997)	fresh	22.0	3.7	2.2	2.0	2.3
Vangueriopsis lanciflora	Author (2020)	lyophilized	**90.76**	**2.55**	**5.02**	**4.8**	**1.74**
Strychnos cocculoides	Sake et al. (2008)	fresh	27.8	3.7	17.9	11.5	6.0
Strychnos cocculoides	Přinosilová (2021)	lyophilized	89.65	8.97	12.97	3.1	10.47
Apple (*Malus domestica*)	Jaiswal (2020)	fresh	14	0.3	2.4	0.3	0.2
Apple (*Malus domestica*)	Abdualrahman (2015)	lyophilized	83.5	0.27	1.83	0.25	0.35
Strawberry (*Fragaria × ananassa)*	Kowalska et al. (2018)	fresh	7.53	ND	2.0	0.7	0.3
Strawberry (*Fragaria × ananassa)*	El-Beltagy et al. (2005)	lyophilized	88.69	3.08	17.62	5.6	6.38
Baobab (*Adansonia digitata*)	Oyeleke et al. (2012)	fresh	88.8	4.5	6.1	3.5	0.4
Baobab (*Adansonia digitata*)	Leatherhead Food Research (2009)	lyophilized	87.6	ND	50.57	2.68	0.52

ND- not determined

The pulp of wild fruits is typically low in fat and protein (Kalenga 1994), while the kernels are good sources of fat and protein (Saxon 2005). However, in case of *Strychnos cocculoides* there is very high fat content of 10.47 %. The RDA for fat in adults is 20 % to 35 % of total calories from fat. That is about 44 grams to 77 grams of fat per day (National Institutes of Health 2005). Though, fat content in *V. lanciflora* fruits was quite low, only 1.74 % in lyophilizate and 2.3 % in fresh form.

The average ash content ranged from 0.27 % to 8.97 %. The high ash content indicates that the fruits and potentially kernels may be good sources of minerals. The highest ash content had *Strychnos cocculoides* 8.97 %, while *V. lanciflora* was found to have significantly lower ash content, as mentioned above.

Fiber is known to help to prevent many diseases prevalent such as maintaining digestive and cardiovascular health (Gordon 2002). It also helps to regulate blood sugar levels (Garg et al. 2000). Baobab had the highest crude fiber content of 50.57 %, while the lowest value appeared in lyophilized apple (1.83 %). Fresh *V. lanciflora* contained 2.2 % of fibre, whereas lyophilized was found to have more fibre (5.02 %). It was similar in baobab and strawberries, where there was significantly higher fibre (from 6.1 % to 50.57 %) and (from 2.0 % to 17.62 %). Baobab had also very high dry matter content, almost the same as in fresh form (88,8 % fresh and 87.6 % lyophilized). Hence that, moisture content was low (11.2 %).

Energy value is almost ten times higher in the freeze-dried fruits, reflecting the fact that much higher amount of fruit is need to produce the lyophilizate. For instance, *V. lanciflora* contains 90.76 % of dry matter and only 9.24 % of water. For this reason, lyophilized fruits has a very low weight - to produce 1 kg of freeze-dried fruit, 7-10 kg of fresh fruit must be processed. There is usually a big difference in fibre. Lyophilized fruits contain almost more than 10 % fibre as well as NFE. This comparison reveals that freeze-drying can be explored as a viable method for processing of tropical fruits while retaining the maximum amount of their nutrients (Chalupa 2020).

5.3. Comparison of vitamins B and C

Vitamin C is relatively unstable to heat, oxygen and light. The retention of this nutrient can be used as a quality index of dried products because if vitamin C content is well retained, the other nutrients are also generally preserved. Based on this context, it has been confirmed that for heat-sensitive materials, such as fruits, the effect of the drying process on product quality, particularly in the retention of nutrients, colour, and texture, is a critical factor in the selection of a drying method. Freeze-drying is considered as the best method for obtaining a high-quality dried product (Marques et al. 2006).

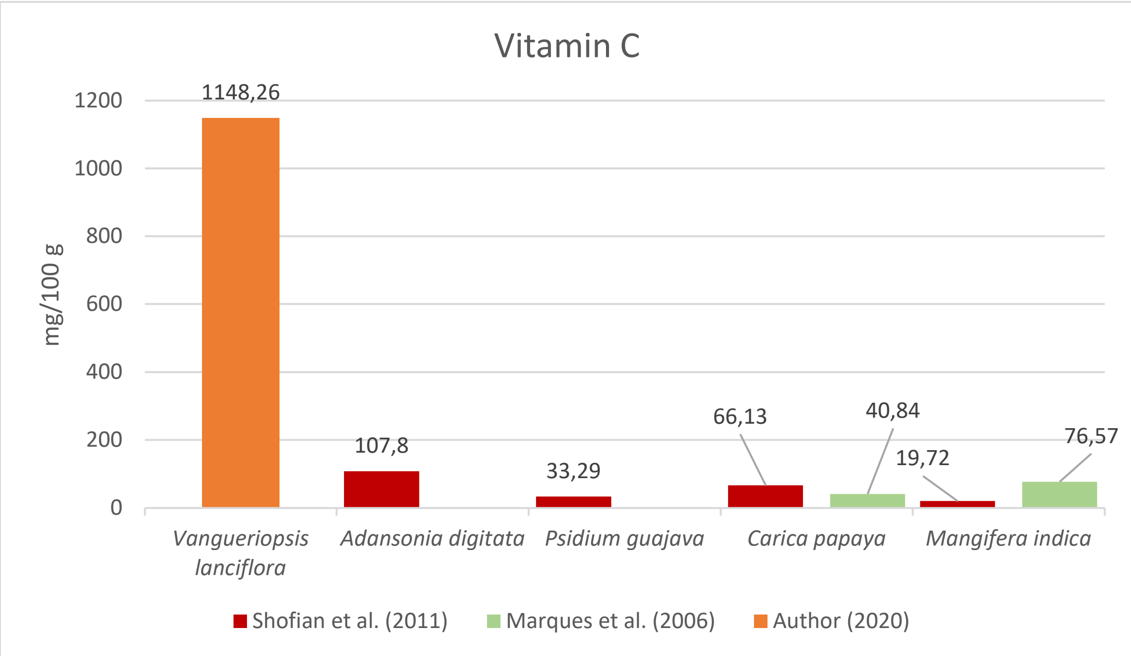

Figure 10. Comparison of vitamin C content in lyophilizates

Figure 10 demonstrates the comparison of vitamin C content of *V. lanciflora* with other selected tropical fruits: baobab, guava, papaya and mango preserved by freeze-drying method. Analyses showed that the content of vitamin C was the highest in *V. lanciflora*. Values from two different studies on the same fruits (papaya and mango) from Margues et al. (2006) and Shofian et al. (2011) differed, especially in the case of mango (19.72 or 76.57 mg/100 g detected). Baobab fruit pulp can be also considered as valuable source of vitamin C (107.8 mg/100 g), but the content in guava was lower (33.29 mg/100 g).

To cover the daily dose of vitamin C, only 6.53 grams of lyophilized *V. lanciflora* is needed. The main importance is the intake of a small amount (only few grams), as it is obvious from Table 4.

Table 4. Daily intake of lyophilizate for vitamin C (g)

	RDA of lyophilizate (g)*
V. lanciflora	**6.53**
Strychnos cocculoides	10.67
Strychnos pungens	4.23

*Adults 25-30 years (mg/day)

These vitamin C rich crops may replace food supplements in developed countries (for example in Czech Republic), very effectively. With the increasing trend of food supplements, natural supplements from fruit lyophilizate can be seen as an attractive health-promoting offer.

5.4. *V. lanciflora* as potential superfruit

To date, the term “superfruit” does not have an official definition, but fruits marketed as superfruits are generally high in a variety of nutrients and consequently associated with health benefits. Commonly known superfruits include açai berry, goji berry, chia and mangosteen. Together with the study of Přinosilová (2021) our results show the potential of Zambian NUS to become a superfruits due their high vitamin B and C content (Table 5). *V. lanciflora* was found to have the highest contents of thiamine 909.24 mg/100g, panthothenic acid 805.07 mg/ 100g and pyridoxamine 30.47 mg/100 g. Pyridoxal was found in all fruits only in trace amounts. Although all those fruits are nutritious, some stand out because of their high levels of nutrients.

Table 5. Vitamins B and C comparison among various fruits from lyophilizate

mg/100 g	***Vangueriopsis lanciflora***	***Strychnos cocculoides***	***Strychnos pungens***	***Mangifera indica***	***Ficus verrulosa***	***Psidium guajava***	***Adansonia digitata***
Analysed	Author (2020)	Přinosilová (2021)	Přinosilová (2021)	FoodData central (2019)	Tauchen (2020)	FoodData central (2020)	FoodData central (2019)
Thiamine (B1)	**909.24**	206.15	778.02	0.062	901.48	0.067	0.078
Panthothenic acid (B5)	**805.07**	238.52	804.40	ND	734.26	ND	ND
Folic acid	27.50	21.9	**99.03**	0	84.87	0	ND
Pyridoxal (B6)	tr	tr	tr	ND	tr	ND	ND
Pyridoxamine (B6)	**30.47**	6.74	7.58	0.334	11.15	0.11	2.414
Vitamin C	1148.26	703.04	**1772.76**	76.57[1]	1469.49	33.29[2]	107.8[2]

tr– trace amount, ND – not determined, 1 – Marques et al. (2006), 2 – Shofian et al. (2011)

6. Conclusion

This study provides a comprehensive overview of potential use, propagation, cultivation and benefits in human nutrition, as well as results of the nutritional content of the neglected fruit species *V. lanciflora*, native to Zambia. It is the first time, when lyophilization (freeze-drying) was used to prepare the samples for dietetic determination. Also, our study brings the first results in terms of vitamin C and B examination in the fruit. Based on our findings it can be concluded that *V. lanciflora* is a rich source of nutrients, especially – vitamin C (1148.26 mg/100 g) and vitamin B_1 (909.24 mg/100 g). Only 5.23 grams of lyophilizate is needed to cover the recommended daily allowance for vitamin C, therefore freeze-dried *V. lanciflora* fruits may possibly serve as natural energy rich snacks. Due to the short durability of fresh fruits, it is reasonable to process *V. lanciflora* to be available throughout the year. Apart from common drying methods, freeze-drying might be a good solution, helping Zambia to also extend its commodities for export.

V. lanciflora as well as other Zambian neglected species mentioned in the study, have great potential to become superfoods thanks to their high levels of vitamins B and C. Yet there are many research gaps concerning the species which need to be fulfilled, e.g. detailed micronutrient analysis, determination of secondary metabolites content as well as the examination of antinutritional substances and digestibility of the fruits.

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Appendices

List of the Appendices:

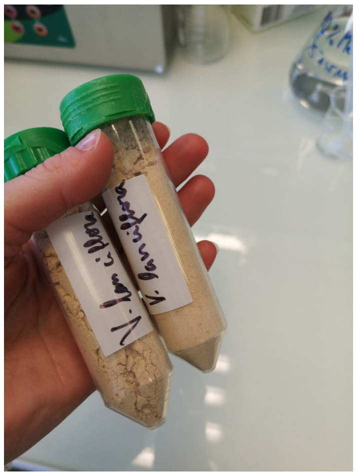
Appendix 1: **Analysis**

(Author's personal photo documentation)

Fruit without seed

Grounding fruit pulp together with nitrogen in a mortar with pestle

In action



Initial sample for our analyses

SER 148 apparatus – crude fat analysis

Kjeltec 2400 apparatus – protein analysis, Digestion unit – MB 442 (FOSS, Denmark)

Appendix 2: **V. lanciflora habitus**

Straight cylindrical trunk (Hyde 2016)

Bark with brownish-pink underbark (Baumann 2009)

Opposite leaves (Baumann 2009)

Leave (Baumann 2009)

Tree in blossom (Stevens 2004)

Tree in blossom (Stevens 2004)

Unriped fruits (Burrett 2011)

Mature tree (Stevens 2004)