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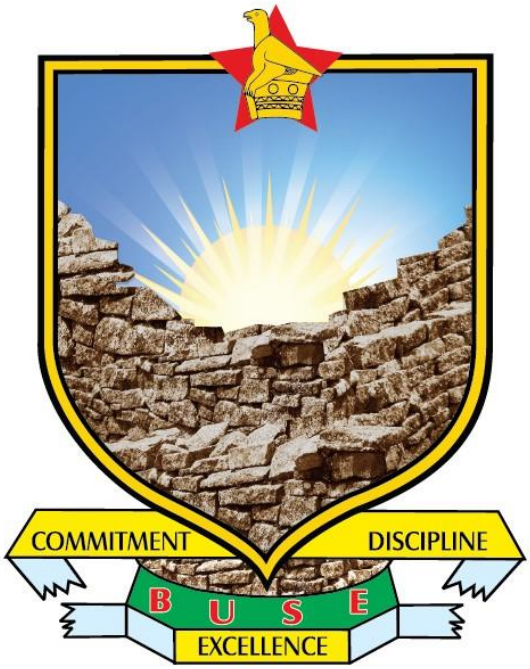

Effect of temperature and duration of heating during *Ziziphus mauritiana* (masau) juice making on the quantity of vitamin C.

BY

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A Research Project Submitted in Partial Fulfilment of the Requirements for the Bachelor of Science Honours Degree in Biotechnology

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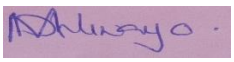
Approval form

The undersigned certify that they have read the dissertation 'Effect of temperature and duration of heating during *Ziziphus mauritiana* (masau) juice making on the quantity of vitamin C' and it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering for assessment.

Signature of student:  Date: 07/06/2024

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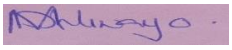
Signed:  Date: 07/06/2024

Supervisor

I, Dr. P. Jinga, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 27/09/2024

Dedication

This research project is dedicated to my mom who has been my wavering support and inspiration throughout my academic journey.

Acknowledgements

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ABSTRACT

Ziziphus mauritiana, also known as masau, is a fruit high in vitamin C that is frequently consumed for its nutritional benefits. During juice extraction, the heating temperature and duration of heating can impact the retention of vitamin C. This study aimed to determine the optimal heating temperature and duration of heating during masau juice processing to maximize the retention of vitamin C. In this study, fresh and dried masau fruits were collected and their juice was extracted using a protocol used at the BUSE Innovation Hub to produce masau juice. The extracted juice was divided into treatment groups based on temperature (70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 98 ^{0}C) and heating time (15, 30 and 45 minutes). A control group of crushed fresh masau without any heating was also included. The vitamin C content of the resulting juice samples was then analysed using UV Visible Spectrophotometer. The findings demonstrated that the heating temperature and heating duration had a substantial impact on the vitamin C content of masau juice. Higher heating temperatures and longer heating duration reduced vitamin C levels. The optimal temperature and duration of heating for maintaining vitamin C in masau juice were found to be 70 ^{0}C and 30 minutes, respectively. These findings show that heat processing can degrade vitamin C in masau juice underlining the necessity of optimizing processing conditions to preserve nutritional value of Masau juice. This study provides valuable insights for masau juice producers to optimize their processing methods and preserve the nutritional quality of this important fruit. Further research is needed to identify the specific mechanisms and kinetics of vitamin C degradation during heating, as well as to create effective heat treatment techniques to reduce vitamin C loss in masau juice production. The data can be used to optimize the processing parameters for commercial masau juice production.

Contents

CHAPTER 1

INTRODUCTION

1.1 Background of study

Ziziphus mauritiana, (Masau in Shona)- is a wild fruit growing abundantly within the Zambezi Valley in Zimbabwe. Masau fruit is nutritious and scrumptious in taste, having a higher content of vitamin C than apples or citrus fruits. They are very rich in vitamin C amounting from 15 mg to 43.8 mg per 100g (Nyanga *et al*., 2013).

Vitamin C or ascorbic acid is a water soluble vitamin which plays a function in controlling infections and recovery of wounds and is a powerful antioxidant which could neutralize harmful loose radicals. It is also needed to make collagen, a fibrous protein coactive tissue that is weaved through numerous systems within the body, immune system, bone, cartilage, blood and others (Chan, 2020).

Vitamin C supplements and foods rich in vitamin C can improve the absorption of poorly absorbed iron, lower blood pressure in both healthy adults and those with high blood pressure, lower the risk factors for heart disease and have been linked to reduced blood uric acid levels and gout (Raman and MS, 2020).

Vitamin C can be destroyed by prolonged cooking (Carr and Rowe, (2020). The longer the heating time and the longer the duration of exposure to heat, the more the loss of ascorbic acid since it is water soluble and heat liable (Lee *et al*., 2017). A study carried out on 5 green vegetables that is cabbage, ayoyo, okro, abedura and kotomie in Ghana has shown that as the heating time increases, the more vitamin C is destroyed suggesting that the boiling of vegetable should therefore be done within the shortest possible time to retain nutrients (Agbemafle *et al*., 2012).

Longer cooking time that is higher than 30 minutes led to a 95 -97 % drop in vitamin C after working on justicia galeopsis leaves (Loukou *et al*., 2020). A research carried out on local vegetables also noted a general decrease in vitamin C content with increased duration of cooking (Joshua and Suleiman, 2012). It is prudent to assume that the amount of vitamin C decreases as heating time increases.

1.2 Problem statement

Previous researches carried out on green vegetables and *Justicia galeopsis* leaves on vitamin C has shown that as heating temperature and duration of heating increases, the concentration of vitamin C also decreases. However, no research has been carried out on Masau juice since it is a new product. This therefore necessitates a research for this product so as to standardise the heating duration of masau to develop a standardized protocol for the production of masau juice with high concentration of vitamin C.

1.3 Aim

To determine the optimal masau heating temperature and duration of heating for maximum vitamin C content in masau juice.

1.4 Objectives

1. To establish the optimum heating temperature that preserves the highest vitamin C content.
2. To determine the optimum heating duration that preserves the highest vitamin C content.

1.5 Research questions

• What is the optimum heating temperature during masau juice preparation that preserves the highest amount of vitamin C?

• What is the heating duration that ensures the maximum amount of vitamin C present in masau juice?

1.6 Justification

There is no standard protocol for the production of masau juice with the highest vitamin C content. The study, the first one to be done will come up with a protocol in the production of masau juice with the highest vitamin C content. The protocol will be important since vitamin C plays an important function in the human body including preventing diseases, forming blood vessels, tendons, ligaments and proteins, mending wounds and forming scar tissue, maintaining and repairing cartilage, bone and teeth and assisting with iron absorption.

1.7 Limitations

The best method to use in this study was High Performance Liquid Chromatography, but due to the lack of a functional HPLC machine, an alternative way of quantifying vitamin C had to be used. UV Visible Spectrophotometer was used in the quantification of vitamin C.

1.8 Delimitations

The masau fruit can be harvested wet or dry. Wet masau may have been used for comparison purposes, however only dry masau was employed in this study. This would have been valuable in finding the sort of masau that provides the highest quantity of vitamin C.

CHAPTER 2

LITERATURE REVIEW

2.1 *Ziziphus mauritiana* (Masau)

Ziziphus mauritiana, a temperate-climate tree, is also known as Ber, Indian Jujube, Jujube, Desert Apple, Indian Plum, Malay Apple and Chinese Apple. It is a fruit tree from the Rhamnaceae family (Moll, 1997). This family has almost 900 species dispersed throughout 58 genera (Kaleem *et al*., 2014 and Afzal *et al*., 2017). Ziziphus is a botanical genus that includes roughly 40 species of thorny shrubs and small trees in the Rhamnaceae family. *Ziziphus mauritiana* taxonomical classification is as follows:

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Rhamnales

Family: Rhamnaceae

Genus: *Ziziphus* Mill-Jujube

Species: *Ziziphus mauritiana* (Prakash *et al*, 2021).

Figure 1: *Ziziphus mauritiana* masau fruits.

Ziziphus mauritiana is distinguished by its evergreen habit, tiny spines and many drooping branches on each plant, which are frequently ornamented with pointy stipules (Prakash *et al*., 2021). *Ziziphus mauritiana* is recognized for its quick growth, spiky appearance and exceptional lifespan reaching heights of up to 15 metres and a trunk diameter of 40 cm or more. Its distinct appearance stems from its spreading crown and relaxed twigs. *Ziziphus mauritiana* can thrive on soil with a neutral to slightly alkaline pH (7.5). However, it may swiftly adapt to shallow to deep soils such as clayey, sandy and rocky. However, sandy loam is the best soil for plant growth (Pareek, 2013).

The masau tree is a medium-sized, thorny tree with a rapidly growing taproot. The young leaves and stems are pale in colour. The fruit could be round or oval, big, medium or small with a large stone (Figure 1). Fruit from wild fruit trees can be as little as 1.8 to 2.5 cm in diameter or as large as 5 cm (plum size) in developed varieties (Nyanga *et al*., 2013). The

leaves measure 2.5-4.0 cm in length and are 1.8-3.8 cm broad. The leaves have a rounded tip and three pronounced basal veins.

Ziziphus mauritiana flowers are tiny and often yellow, white or greenish-white with up to five petals each (Figure 2 (d)). These trees are known to exhibit protandry, a process in which male reproductive organs mature before female reproductive organs (Prakash *et al*., 2021). The bark is dark grey or dull black. *Ziziphus mauritiana's* edible fruits are drupes, which are soft, meaty, crispy and sweet and have a syrupy flavour. These fruits are red, yellowish-brown or white with a globose or oblong shape and a length of 2 cm to 5 cm (0.39-2.0 inches). They have a wonderful fragrance, similar to an apple-like perfume and a nice scent (Prakash *et al.*, 2021).

As the fruit ripens, it changes colour from green to yellow to brown. Masau fruits ripen in mid-June and are available in Zimbabwe's Mashonaland Central province until late September (Nyanga *et al*., 2013). The mature fruit ranges in flavour from sweet to sour and quenches thirst. The texture and flavour of the flesh are reminiscent of apples. Yields of 80 to 130 kg/tree/year have been reported in Africa's Sahel region. In Zimbabwe, dispersed wild trees produced 4-5 tonnes per year from a 3-4- hectare area (Maposa and Chisuro, 1998). Masau is primarily sold locally, informally in urban fruit and vegetable markets. It is formally traded as jam. The local market has potential for jam, fruit slices, fruit powder, juice and as the base for alcoholic beverages. Preliminary research on market potential around masau suggests that the value of the fruit is too low to make it truly commercially interesting even at a very big scale. Figure 2 shows *Ziziphus mauritiana* tree, leaves, raw and ripen fruits and flowers.

Figure 2: *Ziziphus mauritiana* (a) tree, (b) leaves, (c) raw and ripen fruits and (d) flowers.

2.2 *Ziziphus mauritiana* distribution

Approximately 170 different species have been recorded from various geographic areas. *Ziziphus mauritiana* is primarily found in tropical Africa (Algeria, Egypt, Kenya, Libyan Arab Jamahiriya, Tunisia and Uganda), South Asia (Afghanistan, Bangladesh, India, Nepal, and Pakistan), Southeast Asia (Indonesia, Malaysia, Thailand and Vietnam), Australia and China (Mirheidari *et al*., 2022). It began in Central Asia and spread to North Africa**.** Cultivation spread south-east via Malaysia, then east into Indochina and southern China, Africa and Southern Arabia (Mishra *et al*. 2011).

Ziziphus mauritiana is a shrub or planted fruit tree that has spread spontaneously in parts of Africa. It is native to Africa and has the most widespread distribution of all Ziziphus species. It covers a substantial area known as the "Sahelian or coastal zone." *Ziziphus mauritiana*

grows in the Sahelian or coastal zone, which extends from the Atlantic (Senegal and Mauritania) to Somalia and includes locations as far away as Arabia and India. This species is well adapted to the climate conditions in this area (Yahia *et al*., 2020).

The species can be found in several regions of the Indian subcontinent, including Arunachal Pradesh, Bihar, the Himalayas, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Meghalaya, Rajasthan, Sikkim, Uttar Pradesh and the Thar Desert. It is also found worldwide in the Paleotropics, which include warm-temperate tropical and subtropical climates (Prakash *et al*., 2021).

2.3 *Ziziphus mauritiana* nutritional composition.

Ziziphus mauritiana fruits have high levels of phosphate, calcium, iron and vitamin C (Nyanga, 2013). *Ziziphus mauritiana* fruits contain sugars (sucrose, fructose, glucose and starch), proteins, organic amino acids (arginine, aspartic acid, asparagine, glutamic acid, threonine, serine and glycine), vitamins, lipids, fibre and mineral salts. *Ziziphus mauritiana* contains carbohydrates, crude protein and micronutrients like calcium, potassium, sodium, phosphorus, copper, zinc and vitamin C all of which are necessary for bone health, heart, muscle, neurological function and immune system maintenance. It has more vitamin C and iron than oranges, papaya, grapes or mango (Mahapatra *et al*., 2012).

In a study conducted by Nyanga *et al*, (2012**)** to determine the nutritional composition of fruits and thus evaluate their contribution to the diet, it was found that the masau fruits contain vitamin C (15.0-43.8 mg 100 g (-1)). The energy value is between 1516 and 1575 kJ 100 g (-1). Fruit (dry mg 100 g (-1)) potassium 1865.01 g to 2441.01 g, calcium 160.00 g to 254.00 g, sodium 185.00 g to 223.00 g, magnesium 83.00 mg to 150.00 mg. Manganese and copper contents are between 0.70 g and 1.60 g, respectively and iron and zinc contents are between 2.10 g and 4.30 g, with 0.60 g -0.90 g / 100 g (-1) dry weight. Therefore, masau fruit

is a good source of carbohydrates, proteins and micronutrients such as calcium, potassium, sodium, phosphorus, copper, iron, vitamin C and zinc.

Additionally, the dry matter composition of the sweet and sour fruits is between 21.1 g to 24.1 g 100 g (-1) of edible portion and 84.8 g to 87.2 g 100 g (-1) for the dried fruit. Crude protein ranged between 7.90 g and 8.70 g per 100g edible portion of dry weight, crude fat from 0.80 g to 1.50g, crude fiber from 4.90 g to 7.30 g, ash between 3.0 g and 4.30 g and carbohydrate between 79.5 g and 83.2 g

Table 1: Proximate composition of *Ziziphus mauritiana* fruits (Nyanga *et al.*, 2012).

Nutrient	Content (g100g)	Source of fruit	Reference
Vitamin C	96.0-500.0	Semi-arid lowlands,west Africa	(Leakey,1999)
	65.8 – 76.0	India	(Morton, 1987)
	300.0 – 500.0	Senegal	(Danthu *et al*., 2002)
	13.6	Malawi	(Saka *et a*l.,1994)
Carotene	21.0 -28.0	Semi-arid lowlands, West Africa	(Leakey,1999)
	0.02 (F.w.)	India	(Morton, 1987)
Calcium	13-Jan	Malawi	(Saka *et al*.,1994
	499	Nigeria	(Lockett *et al*., 2000)
	712.5	Nigeria	(Eromosele *et al*., 1991)
	25.6	India	(Morton, 1987)
	4	Rajasthan, India	(Rathore, 2009)
Copper	0.2	Nigeria	(Lockett *et al*., 2000)
	0.6	Nigeria	(Eromosele *et al*., 1991)
Iron	6.3	Nigeria	(Eromosele *et al*., 1991)
	17.9	Nigeria	(Lockett *et al*., 2000)
	0.76-1.8(F.w.)	India	(Morton, 1987)
	1.8(F.w.)	Rajasthan, India	(Rathore, 2009)
Magnesium	49.9	Nigeria	(Lockett *et al*., 2000)
	227	Nigeria	(Eromosele *et al*., 1991)
	0.5	Malawi	(Saka *et al*., 1994)
Manganese	3.5	Nigeria	(Eromosele *et al*., 1991)
	1.14	Nigeria	(Lockett *et al*., 2000)

Phosphorus	13	Nigeria	(Eromosele *et al*., 1991)
	2.2	Malawi	(Saka *et al*., 1994)
	144	Nigeria	(Lockett *et al*., 2000)
	9(F.w.)	Rajasthan, India	(Rathore, 2009)
Potassium	17.3	Malawi	(Saka et al., 1994)

2.4 Ethanomedicinal use of *Ziziphus mauritiana*

Ziziphus mauritiana has a long history in traditional medicine where it has been used to treat a wide variety of illnesses and health concerns. The plant has been shown to have therapeutic characteristics that can aid with fever, ulcers, asthma, depression, anxiety and inflammation (Afzal *et al*., 2017). The *Z. mauritiana* plant's leaves have been used to treat abscesses, diarrhoea, gonorrhoea, swelling, wounds, asthma, fever and liver disease (Owolarafe *et al*., 2020).

Furthermore, the fruit has long been used in traditional medicine as an analgesic, powerful wound healer, sedative and tonic. It has been applied to ulcers and cuts, as well as to treat asthma. Also, the fruit has demonstrated promising antitumor effects in various tests (Yahia *et al*., 2020).

2.5 Traditional use of *Ziziphus mauritiana*

It serves as a sedative (Afzal *et al*., 2017). It also has noteworthy wound healing effects and has been demonstrated to be effective against asthma (Ashraf *et al*., 2015). This plant's fruits, seeds and leaves have been shown to have antioxidant properties (Gupta, 2018). In contrast, the plant's bark and pulp have been shown to have cytotoxic effects on a variety of cancer cell

lines (Mishra *et al*, 2011). Plants from the genus Ziziphus have traditionally been used as medicinal remedies to treat a variety of diseases and physical abnormalities. These include chest and respiratory issues, mouth and gum irritation, acne and scabies. Furthermore, findings suggest that the leaves of Ziziphus species are useful for skin whitening of the neck and face as well as for treating hair growth difficulties (Yahia *et al*., 2020).

The roots of Ziziphus species are known for their characteristic bitter taste and have a long history of usage in treating chronic fever, old wounds and ulcers as well as headaches and cephalalgia. In contrast, the leaves have a bitter taste and cooling effects. They have been used as anthelmintic and antipyretics and have been shown to be effective in the treatment of stomatitis, asthma and typhoid fever (Prakash *et al*., 2021).

Traditional practices have used several sections of the Ziziphus plant for their healing effects. The roots are reported to be beneficial in addressing imbalances associated with cephalalgia, fever, ulcers and sores. The bark is used to treat boils, diarrhoea, dysentery, gingivitis and ulcers. The seeds have been shown to help cure asthma, burning sensations, coughing, diarrhoea, insomnia and vomiting. The leaves are useful for treating asthma, diarrhoea, leucorrhoea, obesity, stomatitis, syphilitic ulcers, typhoid fever and wounds. The fruits are thought to help with vomiting, constipation, nausea, leprosy, thirst, anorexia, exhaustion, wounds and ulcers (Dhileepan, 2017).

2.6 Phytochemistry profile of *Ziziphus mauritiana*

Extensive phytochemical research has established that the genus Ziziphus, a member of the Rhamnaceae family, is a valuable reservoir of various chemicals such as cyclopeptide alkaloids, lupane and ceanothane triterpenes (Panseeta *et al*. 2011). A preliminary phytochemical investigation of *Ziziphus mauritiana* revealed the presence of various bioactive components, including alkaloids, flavonoids, tannins, saponins and

cardioglycosides (Afzal *et al*., 2017). Furthermore, *Z. mauritiana* fruits are known for their high levels of carotene, important fatty acids and vitamins A and C (Orwa *et al*., 2009). The plant's antibacterial capabilities are mostly attributable to its many bioactive chemicals, which include alkaloids, flavonoids, phenolic compounds, tannins and terpenoids (Yahia *et al*., 2020).

2.7 Pharmacological activities/profile of *Ziziphus mauritiana*

Ziziphus mauritiana exhibits a wide range of beneficial qualities, including anticancer, antidiabetic, antibacterial, antioxidant and antitumor activity (Afzal *et al*., 2017).The fruits of *Z. mauritiana* have been shown to treat a variety of diseases, including allergies, constipation, depression, insomnia, sedative characteristics and vitamin A and C deficiencies. They are also recognized for their nutritional and anti-allergenic qualities (Lamien-Meda *et al*., 2008). Figure 3 illustrates the pharmacological actions of *Z. mauritiana* (Jha *et al*., 2023).

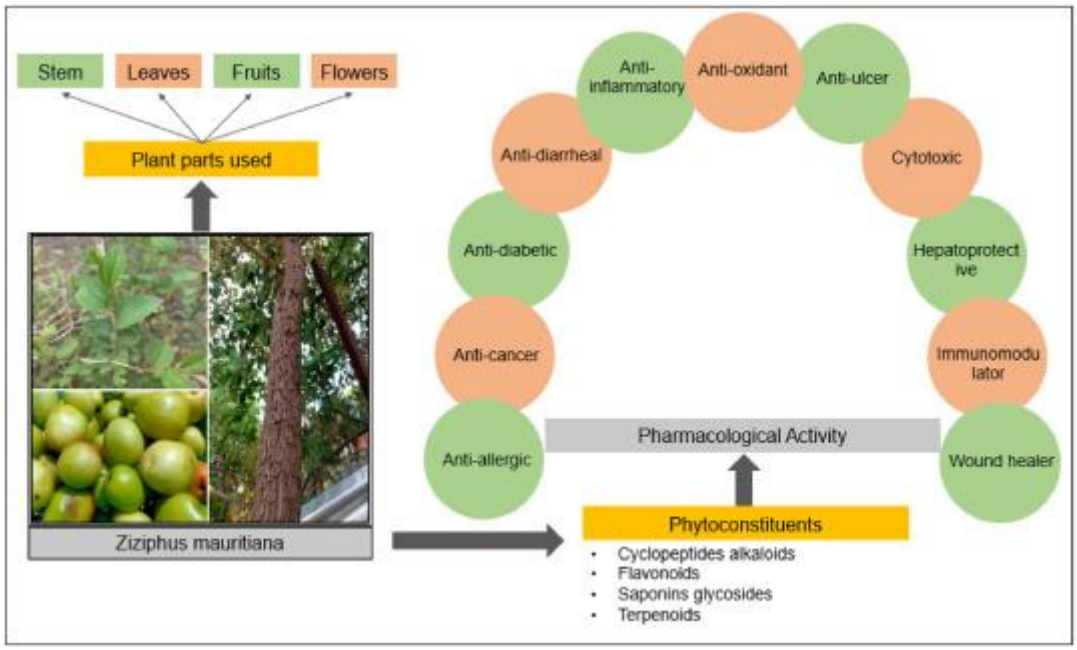

Figure 3: Pharmacological activities of *Ziziphus mauritiana*.

2.8 Non-fermented fruit products

Ziziphus mauritiana is a multi-purpose tree cultivated mostly for its fruits. It begins giving fruit 6-8 years after planting and rises in productivity until the tree reaches 15-20 years old. *Z. mauritiana* fruit can be eaten fresh, dried, candied or pickled, in juice or as masau butter. It can be made into flour, paste, juice, syrup or alcoholic beverages. The young leaves are tasty and cooked as a vegetable in Indonesia. Ripe fruits are typically eaten raw in India, however they are also cooked occasionally. Slightly under-ripe fruits are candied using a pickling procedure in which they are immersed in a salt solution with the salinity gradually increasing from 2 to 8%.

In Zimbabwe's Zambezi valley, dried fruit powder is used for baking, jam and a traditional loaf. Tulimara - Speciality Foods for Africa (SFA), Private Limited Zimbabwe manufactures jam and sun dried fruit slices (masau snacks) from the pulp of ripe masau fruits (Kalikiti, 1998). Western Sudan and Zambia produce gingerbread-like cakes prepared from dried and fermented pulp (Figure 4). Fruits and bark are used to manufacture dyes and medicinal formulations (Ecocrop, 2013 and Orwa et al., 2009). Indian jujube wood is reddish, finely grained, robust and long-lasting. It can be utilized for rural house construction, posts and tool production. It produces great firewood.

The Indian jujube tree is home to lac insects and also serves as food for the tasar silkworm, which produces highly valued silk in India (Orwa *et al*. 2009). It provides a minor source of pollen for bees (Orwa *et al.*, 2009). This thorny tree has the potential to be used as an agroforestry plant, providing windbreaks and living fences. It is consumed by livestock and its leaves provide excellent fodder for sheep and goats (Ecocrop, 2013 and Orwa *et al*., 2009).

Figure 4: Traditional masau (*Ziziphus mauritiana*) cake made by rural communities in Muzarabani, Zimbabwe (Nyanga *et al*., 2012).

2.9 Masau fermented products

Masau wine is prepared by soaking *Ziziphus mauritiana* fruits for several hours and letting them ferment at room temperature (Chivero *et al*., 2001). Kachasu, a common name for traditionally fermented and distilled robust alcoholic spirits, is prepared from masau fruits fermented for 4-7 days (Figure 5). In Malawi, dried *Ziziphus mauritiana* fruits are fermented and distilled to produce a strong alcoholic beverage and a wine known as mlunguzi is made from a combination of Uapaca kirkiana and *Ziziphus mauritiana ((*Kaaria, 1998 and Jumanne *et al*., 1992). Table 2 shows other wild fruits which are fermented into alcoholic beverages in Zimbabwe (Gadaga *et a*l., 1999).

Figure 5: Mass of fermented masau (*Ziziphus mauritiana*) fruits from rural community in Muzarabani, Zimbabwe (Nyanga *et al*., 2012).

Table 1: Other wild fruits which are fermented into alcoholic beverages in Zimbabwe (Gadaga *et al*., 1999)

Botanical name	Family name	English name	Shona/Ndebele
Balanites aegyptica	Balanitaceae	Torchwood	nyahoko
Bequeritiodendron magalismontanum	Sapotaceae	milk plum	muhorongwa/umhlautshwa
Berchemia discolour	Rhamnaceae	bird plum	munyii/umcaga
Cassia petersiana	Fabaceae	monkey pod	muremberembe
Diospyros mespiliformis	Ebenaceae	jackal berry	mushenje/umdlawu
Garcinia huillensis	Clusiaceae	granite garcinia	mutunduru
Grewia monticola	Malvaceae	donkey berries	mutongoro/umtewa
Pappea capensis	Sapindaceae	indaba tree	chitinunu/uzagogwane
Popowia obovata	Annonaceae	monkey fingers	munyani/umkozombo
Rhus tenuinervis	Anacardiaceae	nana berry	mudzambuya/umkungu
Syzygium cordatum	Myrtaceae	water berry	mukute/umdori

2.10 Environmental impact of *Ziziphus mauritiana*

Plants in the Ziziphus genus can endure severe stress induced by drought, salt and in rare circumstances, waterlogging. Cultivated jujubes are thus good for planting in places unsuitable for other crops, such as marginal or degraded areas, as long as the appropriate genotypes for alkali or sodic soils are used (Hebbara *et al*., 2002 and Dagar *et al*., 2001). Indian jujube can be used to fix sand in coastal dunes (Orwa *et al*., 2009 and Ali *et al*., 2006). Indian jujube grafted on *Ziziphus nummularia* can thrive in salty soils (Ecocrop, 2013).

2.11 History of vitamin C

Vitamin C knowledge can be classified into three groups. The scurvy era, which lasted from the fourteenth to the nineteenth century, was marked by the recognition of the scurvy

syndrome as well as the search for a cause and a remedy. The second stage, around 1900 to 1980, comprises the chemical isolation and characterization of the antiscorbutic factor, ascorbic acid, demonstrations of the substance's nutritional necessity and metabolic research that confirmed the vitamin's human metabolism and need to prevent scurvy. The most recent period, from approximately 1970 to the present, is defined by greater awareness of the vitamin's extra-scorbutic effects, such as antioxidant protection and immunological competence.

Vitamin C, also known as ascorbic acid, is a small molecule discovered in the 1920s by Albert Van Szent Gyorgyi, who discovered that it might prevent and treat scurvy (El-Ishaq and Obirinakem, 2015). Scurvy is a potentially lethal disease induced by a prolonged absence of fruit and vegetable consumption. The term 'scurvy' refers to the illness induced by a prolonged shortage of vitamin C. It comes from 'scorbutus' (Latin),'scorbut' (French), and 'Skorbut' (German). Scurvy was a prevalent disease in the world's fleets, infecting an estimated 2 million sailors.

Scurvy also occurred on land, as many cases did during Ireland's 'great potato famine' of 1845. Axel Holst and Theodor Frölich accidentally created scurvy in guinea pigs, which, like humans, require vitamin C in their diets. In 1928, Albert Szent-Györgyi isolated a chemical known as 'hexuronic acid' from adrenal glands. Four years later, Charles Glen King discovered vitamin C in his laboratory and determined that it was identical to 'hexuronic acid'. In 1933, Norman Haworth identified the chemical structure of vitamin C (Carpenter, 2012).

Vitamins are organic chemicals that must be obtained from the diet because an organism lacks the enzymes required to create them or cannot produce adequate quantities (Combs and McClung, 2008). Only non-human species, including monkeys, guinea pigs, fish and birds produce vitamin C (Klesczewska, 2000). Although most animals can produce their vitamin C

requirements, humans have mutations in the DNA coding of gulonolactone oxidase, the primary enzyme responsible for ascorbic acid synthesis. Due to this mutation, an external vitamin C supplement is necessary.

Humans derive the majority of their vitamin C from fruits and vegetables. Citrus fruits and other types, such as cantaloupe, watermelon, berries, pineapple, strawberries, cherries, kiwi fruits, mangoes and tomatoes are especially high in vitamin C. Furthermore, vegetables such as cabbage, broccoli, Brussels sprouts, bean sprouts, cauliflower, mustard greens, peppers, peas and potatoes are considered the principal source of vitamin C due to their high concentration and year-round availability.

2.12 Chemical structure of vitamin C

Vitamin C is the generic term for l-ascorbic acid, however it is also known as ascorbate and antiscorbutic vitamin. The molecule of l-ascorbic acid is made up of six asymmetrical carbon atoms ($C_6H_8O_6$) and is structurally linked to glucose, as shown in Figure 6 (Velisek and Cejpek, 2007). It has a molecular weight of 176, a melting point of 190–192 °C and a density of approximately 1.65 g/cm3. Ascorbic acid is soluble in water (300 g/L at 20 °C), alcohol (20 g/L at 20 °C), but insoluble in chloroform, ether and benzene. It dissolves to produce a clear, colourless to slightly yellow solution. It has two distinct pKa values, 4.2 and 11.6. A 5% (w/v) solution in water has a pH range of 2.2 to 2.5.

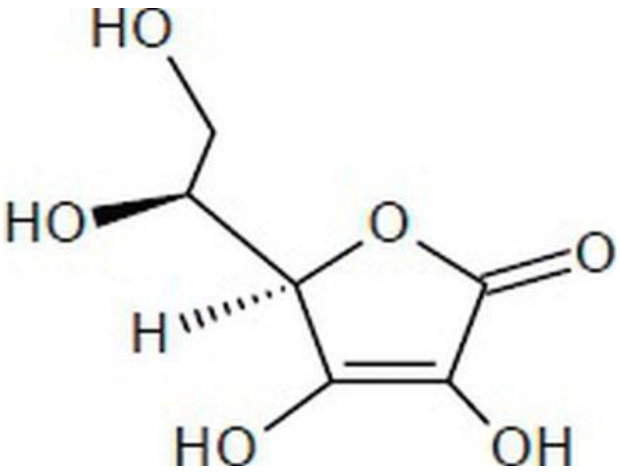

Figure 6: Chemical structure of vitamin C.

The molecular structure of ascorbic acid determines both its physical and chemical properties. It is a weak, water-soluble, unstable organic acid that readily oxidizes or degrades in light, aerobic conditions (oxygen), high temperatures, alkalis, humidity, copper and heavy metals. Ascorbic acid is typically found as a white or slightly yellowish crystalline powder. In dryness, its crystalline state is chemically stable. L-ascorbic acid is highly soluble in water, but insoluble in alcohol, chloroform, ether and benzene. In water, it produces a clear, colourless, slightly yellow solution that oxidizes (Velisek and Cejpek, 2007 and Calder, *et al.*, 2007).

Ascorbic acid has many derivatives, such as sodium l-ascorbate (sodium ascorbate), calcium l-ascorbate (calcium ascorbate), zinc-ascorbate, 6-palmityl-l-ascorbic acid (ascorbyl palmitate) and ascorbyl monophosphate calcium sodium salt. Ascorbic acid is produced from sodium ascorbate via cation exchange. Sodium ascorbate is synthesized by reacting methyl-d-sorbosonate (or ketogulonic acid methyl ester) with sodium carbonate. Ascorbic acid combines with calcium carbonate in water and ethanol, producing calcium ascorbate, which is then separated and dried.

Ascorbyl palmitate is produced by reacting ascorbic acid with sulfuric acid and then esterifying it with palmitic acid. The reaction of ascorbic acid (alone or in combination with sodium ascorbate) with calcium hydroxide and sodium trimetaphosphate yielded sodium

calcium ascorbyl phosphate. The early ascorbic acid compounds outperformed ascorbic acid in terms of light resistance and skin irritation.

2.13 Properties of vitamin C

Ascorbic acid is a colourless, odourless, crystalline molecule with a somewhat sour flavour and visual properties. It has a pleasant, crisp and acidic flavour. Antiscorbutic properties are only present in the L-Isomer. At 73 degrees Fahrenheit, vitamin C has a solubility greater than or equal to 100 mg/mL. It is not soluble in ether, chloroform, benzene, petroleum ether, oils, fats or fat solvents. It has a melting point of roughly 1900 ^{0}C (374 ^{0}F) (with decomposition), a boiling point of approximately 553 ^{0}C (10270° F), a density of 1.694 g/cm3, a molar mass of 176.12 g/mole, a PH 3 (5% sol), a vapour density auto ignition of 6600 ^{0}C, reactivity O and is stable under normal conditions.

It oxidizes readily, particularly in the presence of copper and iron, but not in the presence of aluminium. As a result, foods prepared with copper utensils lose ascorbic acid rapidly. Alkalis quickly destroy this vitamin, but it remains quite stable in weak acid solutions. As a result, baking soda is toxic, but steam heating eliminates relatively little ascorbic acid. However, cooling has no deleterious effects on this vitamin. Citrus fruits and tomato juice can be canned with minimal ascorbic acid loss.

2.14 Roles of vitamin C

Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that is found naturally in some foods, added to others and sold as a dietary supplement. Because humans, unlike other animals, cannot synthesis vitamin C on their own, it is an essential nutritional component (Li and Schellhorn, 2007). Vitamin C is essential for the growth, development and repair of all bodily tissues. It plays a role in numerous bodily functions, including

collagen production, iron absorption, immune system function, wound healing and cartilage, bone and tooth maintenance (Ratini, 2024).

Vitamin C is essential for the manufacture of collagen, L-carnitine, and some neurotransmitters; it also plays a role in protein metabolism (Li and Schellhorn, 2007). Collagen is a key component of connective tissue that aids in wound healing. Vitamin C is an important physiological antioxidant that has been demonstrated to replenish other antioxidants in the body, such as alpha-tocopherol (vitamin E) (Jacob and Sotoudeh, 2002**).** Antioxidants can guard against the damage caused by damaging molecules, radicals, poisonous compounds and pollutants such as cigarette smoke. Free radicals can accumulate and contribute to the development of health disorders such as cancer, cardiovascular disease and arthritis.

In addition to its biosynthetic and antioxidant actions, vitamin C promotes immunological function and enhances the absorption of nonheme iron, which is found in plant-based meals. Scurvy is caused by insufficient vitamin C intake and symptoms include weariness or lassitude, extensive connective tissue weakness and capillary fragility (Li and Schellhorn, 2007).

2.15 Sources of vitamin C

Fruits and vegetables are the best sources of vitamin C (USDA, 2019). Citrus fruits, tomatoes, tomato juice and potatoes are key sources of vitamin C in the American diet. Red and green peppers, kiwifruit, broccoli, strawberries, Brussels sprouts and cantaloupe are all good providers of the nutrient (USDA, 2019).

2.16 Dietary supplements of vitamin C

Supplements often contain vitamin C in the form of ascorbic acid, which has the same bioavailability as naturally occurring ascorbic acid in foods like orange juice and broccoli. Other types of vitamin C supplements include sodium ascorbate, calcium ascorbate, other mineral ascorbates, ascorbic acid with bioflavonoids and combination products, such as Ester-C, which combines calcium ascorbate, dehydroascorbate, calcium threonate, xylonate and lyxonate.

2.17 Vitamin C Deficiency

Acute vitamin C deficiency causes scurvy (Wang and Still, 2007). The onset of scurvy varies depending on vitamin C body storage, however symptoms might arise within a month of low or no vitamin C intake (less than 10 mg/day) (Wang and Still, 2007).Initial symptoms may include weariness (most likely due to decreased carnitine biosynthesis) and gum irritation (Francescone and Levitt, 2005). As vitamin C deficiency progresses, collagen production is reduced and connective tissues are weakened, causing joint discomfort, poor wound healing, hyperkeratosis and corkscrew hairs (Li and HE, 2007).

Scurvy symptoms may also include sadness, bleeding gums and tooth loosening or loss due to tissue and capillary fragility (Stephen and Utecht, 2001). Iron deficiency, anaemia can also arise as a result of insufficient vitamin C intake, which causes increased bleeding and decreased nonheme iron absorption (Francescone and Levitt, 2005). Bone disease can exist in youngsters (Weinstein *et al*., 2001). Left untreated, scurvy is lethal.

2.18 Effect of temperature and duration of heating on the quantity of vitamin C.

Because vitamin C is a water-soluble and temperature-sensitive, it is rapidly destroyed during cooking and high temperatures and long cooking durations have been linked to particularly severe vitamin C losses (Lee *et al*., 2017). Temperature changes impact vitamin C, which

implies that cooking can destroy some of the vitamin naturally found in food. It is highly unstable, oxidizing quickly when exposed to heat and the type of cooking method utilized impacts vitamin C in diverse ways. Vitamin C is impacted by temperature variations, breaking down the most when exposed to high temperatures over extended periods of time. Temperature and heating time both results in a considerable loss of vitamin C (Farah *et al*., 2020). It was discovered that at 90 ^{0}C for one hour, vitamin C lost around 36.6 % ($p< 0.003$) of its contents. In contrast, incubating vitamin C at 4 Cá' for 240 hours resulted in an 11.4% loss of its contents (p 0.013). It was discovered that incubating vitamin C at -18 Cá' caused no substantial change in its contents (Farah *et al*., 2020).

These results were obtained after soaking ground pepper in 500 millimetres of distilled water for 24 hours with constant shaking and then filtering the solution. The filtrate is then redox titrated to determine the quantity of vitamin C. At each incubation period, equal quantities of the filtrate (20 ml) were used to evaluate the influence of the following on the levels of vitamin C pH ranges from 3-8, temperature of -18, 14, 37, 40, 60, 90 Cau and metal salts (ZnCl2, MgCl2, FeCl2) of the same concentration (0.1 M). After each incubation period, the amount of vitamin C in the treated filtrate was calculated.

A study conducted by Essoddom *et al*., (2020) on the effect of temperature on the degradation of vitamin C (ascorbic acid) contained in infant supplementary flours during the preparation of porridges found that vitamin C is destroyed at temperatures ranging from 85 ^{0}C to 95 ^{0}C, particularly after 10 minutes of cooking time. The initial amount of 66.67 mg for 10N by 10 enhanced flour declines to 2.64 mg after 2 minutes of cooking time and approaches zero after 20 minutes (0.50 mg). The initial vitamin C value of the enriched 10Ne10 flour decreased from 67.74 milligrams to 3.04 milligrams after 2 minutes of cooking time and subsequently to 0.40 milligrams after 20 minutes (Essoddom *et al*., 2020). Table 3 depicts the effect of heat treatments at various cooking times that is 2, 3, 5, 7, 10, 12, 15 and

20 minutes, at temperatures ranging from 85 to 95 ^{0}C, of porridges made with flours enhanced with baobab and parkia pulp (10Nx10 and 10Ne10) (Essoddom *et al .*, 2020).

Table 3: Heat degradation of vitamin C in porridges.

	Vit C (10N x 10)I = 66.67 + 2.01mg		Vit C (10Ne10)I = 67.74 + 1.00mg	
Cooking time	Vit C in porridges 10N x 10 (mg)	Deterioration rate (%)	Vit C in porridges 10Ne10(mg)	Deterioration rate (%)
2min	2.64+ 0.63	96.04	3.04+ 0.59	95.51
3min	2.64 ± 0.45	96.04	2.84 ± 0.36	95.81
5min	1.75 + 0.21	97.37	1.80 ± 0.30	97.34
7min	1.75 + 0.10	97.37	1.75 ± 0.16	97.42
10min	1.31 + 0.31	98.03	1.40 ± 0.22	97.93
12min	1.20+ 0.27	98.2	1.31 ± 0.32	98.07
15min	0.88 ± 0.61	98.68	0.95 ± 1.51	98.6
20min	0.50 ± 0.52	99.25	0.40 ± 0.26	99.41

Temperature and boiling duration have an effect on vitamin C concentration, indicating a decrease in vitamin C content in all four citrus juices (Bofars, 2022). These findings were from a study on the Effect of Heated Temperature Environment on vitamin C in four citrus juices: citrus lemon, citrus reticulate, citrus paradise and citrus sinensis. The rate of loss of vitamin C during boiling is determined by temperature and duration. This is due to the fact that the high heat used to raise the temperature of fruit juices kills the enzyme ascorbic acid oxidize, which is prevalent in fruits and vegetables, before much vitamin C is oxidised (Bofars, 2022).

The four citrus fruit samples were obtained from a Tripoli market. Different temperatures, namely room temperature and heated temperatures of 30 ^{0}C, 40 ^{0}C, 50 ^{0}C, 60 ^{0}C and 70 ^{0}C, were investigated at 10 minutes, 15 minutes and 20 minutes. The titration method was used to determine the vitamin C content in citrus fruits. Cooking by either boiling or steaming resulted in 95-99 % loss of vitamin C (Kinyi *et al.*, 2022).

Agbemafle *et al*., (2012) investigated the effects of boiling on the nutritional composition of selected vegetables consumed in Ghana. Five green vegetables were chosen: cabbage (*Brassica oleracea*), ayoyo (*Corchorus olitorius*), okro (*Abelmoschus esculentus*), adeduru *(Solanum torvum*), and nkontomine (*xanthosama sagitifolium*). Equal weights of veggies were boiled for varying amounts of time (5, 10, 15 and 20 minutes). The study's findings clearly reveal that heating duration dramatically reduced the concentration of both nutrients that is beta-carotene and vitamin C. Increasing or extending the boiling time resulted in a constant decline in nutrient concentration, with vitamin C having the greatest loss.
Different studies have been conducted on pepper, porridges, citrus juices and vegetables so as to investigate the effect of heating temperature and heating duration on the quantity of vitamin C but however there is a knowledge gap because such studies have not been done on masau. There is no standard protocol for the production of masau juice with the highest vitamin C content. The study's findings will lead to the manufacturing of masau juice with the greatest vitamin C content. The protocol will be important since vitamin C plays a crucial role in the functioning of the human body thus it should be consumed in the maximum amount feasible.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

The study was carried out in 2023 at Bindura University of Science Education in Mashonaland Central Province in Zimbabwe. Preparation of samples was done at the university's Innovation Hub at FSE campus and quantification of vitamin C was done at Astra Campus.

3.1.1 Instruments and apparatus

Genesys 10S UV Visible Spectrophotometer (Figure 7) with a resolution of 1.8nm dual beam optics and wavelength range of 190-1100 nm was used for the quantification of vitamin C in the masau juice samples.

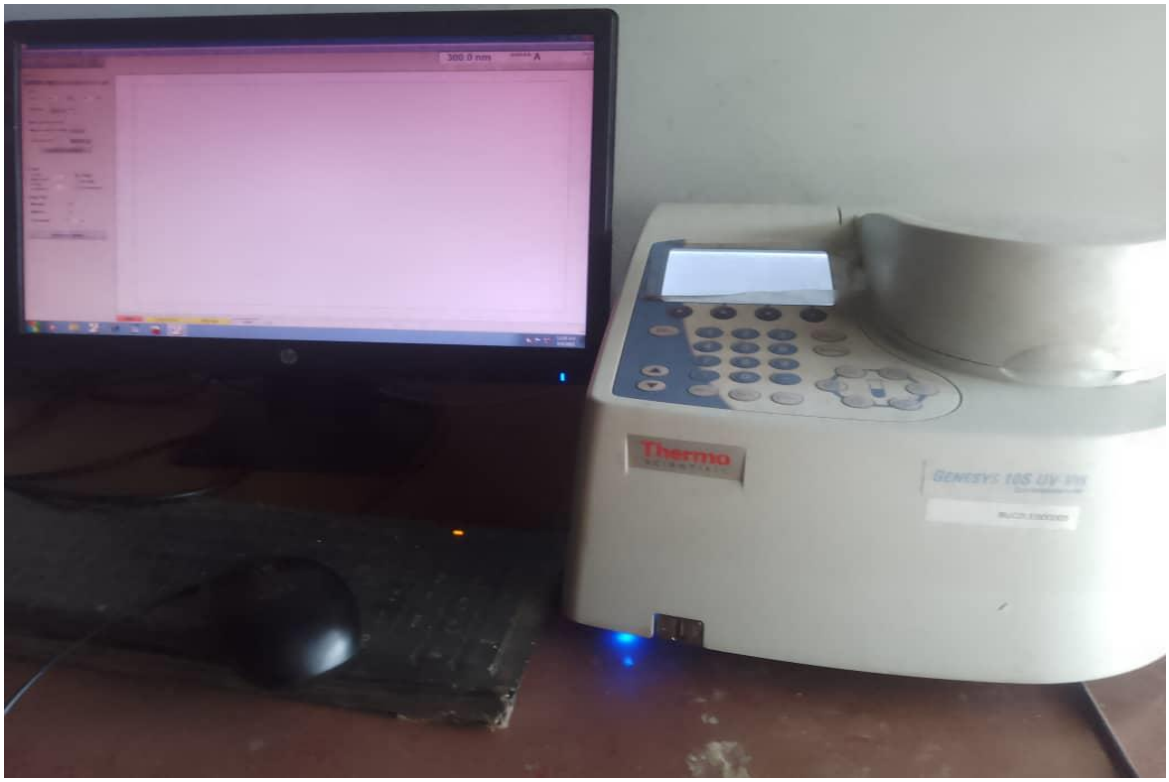

Figure 7: Genesys 10S UV Visible Spectrophotometer.

3.1.2 Reagents and chemicals

All the chemicals and reagents used for the experiment were analytical grade.

3.1.3 Raw materials

Fully ripened dried as well as fresh *Ziziphus mauritiana* fruits were collected from the local market of Bindura. The fruits were transported to the university's food processing workshop at the Innovation Hub.

3.2 Fruit preparation

The fruits were manually hand graded to remove damaged and diseased fruit. They were washed thoroughly using sodium hypochlorite solution to remove dirt and microbes and then rinsed with distilled water. The fruits were then dried in a shaded place to remove excess moisture.

3.3 Juice extraction

The dried *Z. mauritiana* fruits were used. A measured quantity of distilled water was boiled. A proportion of the masau fruits was then added to the boiling water for a measured period of time (15, 30 and 45 minutes). The fruits were crushed to separate the seeds and the pulp (Figure 8).

Figure 8: Freshly squeezed masau.

Different sizes of sieves (58 nm, 45 nm, 38 nm and 25 nm) aperture were used to separate solid material from juice pulp. The juice was left to cool to a temperature of 25^{o} C as well as allowing it to settle the pulp sediments that could not be removed by the sieves then packed in in 1% v/v hypochlorite sterilized bottles (Figure 9).

Figure 9: Bottles of prepared samples of masau juice.

3.4 Experimental design

Different heating temperatures were used at different heating durations during preparation of masau juice (Table 4). The control was prepared by crushing fresh masau then extracting the juice without boiling.

Table 2: Experimental design

Heating temp (^{O}C)	Heating duration	Number of replicates
70	15	3
	30	3
	45	3
80	15	3
	30	3
	45	3
90	15	3
	30	3
	45	3
98	15	3
	30	3
	45	3

3.5 Quantification of vitamin C

UV Visible Spectrophotometer was used to quantify the Vitamin C. UV Visible Spectrophotometer was carried out as described by Desai and Desai, (2019) which involves preparation of calibration curve from 5 different concentrations prepared from the stock solution, sample extract preparation and finally quantification of vitamin C.

3.5.1 Preparation of standard ascorbic acid solution

3.5.1.1 Preparation of stock solution

A series of vitamin C standard solutions with known concentrations was prepared. One gram of ascorbic acid (purity 99 %) was weighed using a precision balance and placed into a 100 ml volumetric flask to make a stock solution of 10 g/L (Figure 10). The volume was made up to the mark using distilled water. A magnetic stirrer was used to help the ascorbic acid dissolve.

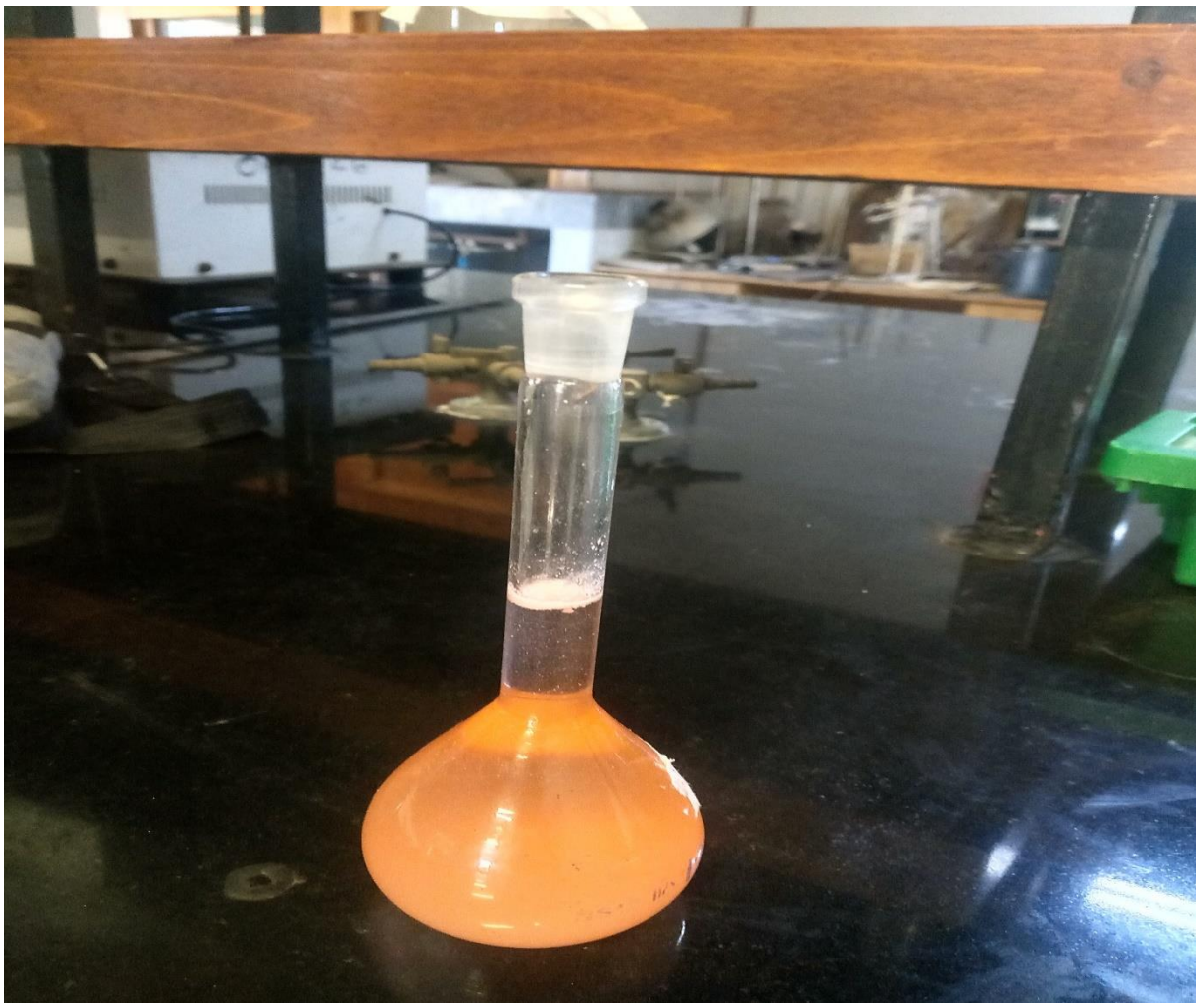

Figure 10: Ascorbic Acid stock solution.

3.5.1.2 Preparation of standard solutions

From this stock solution, serial dilutions were made to obtain 5 different solutions of ascorbic acid with concentrations of 0.625 mg/L, 1.25 mg/L, 2.5 mg/L, 5 mg/L, as well as 10 mg/L (Figure 11), where 1 is 10 mg/L 2 is 5 mg/L, 3 is 2.5 mg/L, 4 is 1.25 mg/L and 5 is 0.625 mg/L. Multiple replicates of each standard concentration were prepared so as to account for variability and ensure accuracy.

Dilution of the stock solution was carried out using a dilution which is $\mathbf{V_1M_1 = V_2M_2}$ where V1 is the initial volume, M1 is the initial concentration, M2 is the concentration after mixing and V2 is the total final volume.

Preparation of first dilution

Step 1: Determining the volume of stock solution required.

The total volume of the diluted solution made was 1 litre (1000 mL), the initial concentration (m1) was 10g/L, while the ultimate concentration (m2) was 5 g/L. The final volume (v2) was 1000 ml.

To find the value of v1, the equation was rearranged: m1v1 = m2v2.

v1 = (m2v2) / m1

Substituting the values: v1 = (5 g/L) (1000 mL) / 10g/L v1 = 500 mL.

So 500 mL of the 10 g/L stock solution was measured.

Step 2: Calculating the volume of diluent needed.

In-order to dilute the 500 mL of stock solution to a total volume of 1000 mL, a diluent was added, in this case distilled water was added. To calculate the volume of the diluent, the volume of the stock solution was subtracted from the final volume.

Volume of diluent equals final volume - The volume of stock solution

Volume of diluent = 1000 mL minus 500 mL

Volume of diluent: 500 mL

To make a total volume of 1000 mL, 500 mL of diluent, distilled water was combined with 500 mL of stock solution.

Step 3: Mixing the stock solution and diluent.

500 mL of the stock solution was poured into a conical flask, followed by 500 mL of the diluent in this case distilled water. The conical flask was gently swirled to ensure complete mixing.

For the remaining concentrations, steps 1 to 3 were repeated.

Figure 11: Different concentrations of ascorbic acid.

3.6 Preparation of calibration curve

UV Visible Spectrophotometer was turned on and allowed to sit for at least 15 minutes before running any samples thus allowing it to warm up for accurate readings. The

wavelength of light to analyse the samples with was then set on the UV Visible Spectrophotometer. The cuvettes were properly cleaned before use by way of rinsing thoroughly with distilled water.1 cuvette was filled with distilled water then wiped on the outside with lint-free paper to remove any fingerprints and the outside was checked first for any dirt. It was ensured that the cuvette is aligned properly with any grooved sides out of the beam path and that it is also standing upright.

The other cuvettes were filled with the 5 different concentrations of ascorbic acid and also wiped with lint-free paper on the outside to remove any fingerprints. The cuvettes were inserted into the spectrophotometer in the correct orientation. The lid was secured to prevent ambient light from entering the system. The absorbance of each standard solution was measured at the selected wavelength of 258 nm. The calibration curve was constructed using Microsoft Excel. The calibration curve was constructed by plotting the concentration of vitamin C on the x-axis and the corresponding absorbance on the y-axis. The absorbance of the standard solutions were read at 258 nm against blank.

3.7 Sample extract preparation

The prepared masau juice samples were diluted using dilution factor 5 using distilled water.

3.8 Determination of vitamin C

The absorbance of the extract was measured using a spectrophotometer at a wavelength of 258 nm. A cuvette containing the prepared blank solution was used to calibrate the spectrophotometer to point zero. Samples of the extract were placed into the cuvette (Figure 12) and the lid was secured. Readings were taken when the figure in the display window became steady. The spectrophotometer was blanked each time with the prepared blank solution before the readings were taken. The prepared solutions were read at 258 nm against

blank by spectrophotometer for the analysis. The operation was repeated three times for each sample and the average readings were recorded. The absorbance obtained was extrapolated on a vitamin C standard curve.

Figure 12: Loading of cuvettes into the UV Visible Spectrophotometer.

3.9 Data analysis

The data was analysed using R and R Studio, a statistical software. One-way ANOVA was used to determine the levels of difference of heating temperature and duration of heating on the final vitamin C content of masau juice.

CHAPTER 4

RESULTS

4.1 Results for the quantification of vitamin C

Table 5: Results for the quantification of vitamin C.

Sample	Dilution factor	Ordinate 1	Ordinate 2	Ordinate 3	Concentration 1 (mg/L)	Concentration 2 (mg/L)	Concentration 3 (mg/L)
Control	5	0.282	0.325	0.309	12.31	15.7	14.45
A	5	0.404	0.429	0.416	22.03	24.04	23.06
B	5	0.498	0.534	0.511	29.59	32.51	30.61
C	5	0.421	0.451	0.427	23.44	25.8	23.94
D	5	0.415	0.456	0.43	22.92	26.23	24.19
E	5	0.36	0.35	0.027	18.53	17.73	17.4
F	5	0.465	0.455	0.141	26.92	26.15	26.76
G	5	0.385	0.339	0.066	20.55	16.89	20.56
H	5	0.353	0.37	0.04	17.98	19.37	18.12
I	5	0.318	0.298	0.004	15.15	13.54	15.08
J	5	0.319	0.071	0.306	15.25	14.22	13.3
K	5	0.345	0.085	0.336	17.32	16.62	16.76
L	5	0.303	0.003	0.294	13.94	13.22	15.24

Table 6: Calculated actual quantity of vitamin C

Sample	Average Concentration	Average x Dilution factor 5
Control	14.15	70.77
A(70^{o},15min)	23.04	115.22
B(70^{0},30min)	30.9	154.52
C(70^{o},45min)	24.39	121.97
D(80^{0},15min)	24.45	122.23
E(80^{o},30min)	17.88	89.43
F(80^{o},45min)	26.61	133.05
G(90^{o},15min)	19.33	96.67
H(90^{o},30min)	18.49	92.45
I(90^{o},45min)	15.56	72.8
J(98^{o},15min)	14.26	71.28
K(98^{o},30min)	16.9	84.5
L(98^{o},45min)	14.1	70.67

The study shows variations in vitamin C concentrations among masau juice samples subjected to different temperature and heating durations. Temperature variations can

influence chemical reactions, potentially impacting absorbance readings and calculated concentrations. Accurate temperature control is crucial for reliable analytical results. These results (Table 8) provide valuable data for understanding the vitamin C content in masau juice and the effects of processing parameters on its nutritional quality. The observations derived from the analysis reveal notable trends across the samples subjected to varying conditions. The analysis performed for samples of masau juice at 70 ^{0}C at 15 minutes, 30 minutes and 45 minutes the f-value was 0.17 and the p-value 0.693. This concludes that there is no significant difference on the quantity of vitamin C as boiling duration increases.

The analysis performed for samples of masau juice at 80 ^{0}C at 15 minutes, 30 minutes and 45 minutes the f-value was 0.398 and the p-value 0.548. This concludes that there is no significant difference on the quantity of vitamin C as boiling duration increases.

The analysis performed for samples of masau juice at 90 ^{0}C at 15 minutes, 30minutes and 45minutes the f-value was 14.36 and the p-value 0.0681. This concludes that there is a significant difference on the quantity of vitamin C as boiling duration increases.

The analysis performed for samples of masau juice at 98 ^{0}C at 15 minutes, 30 minutes and 45 minutes the f-value was 0.008 and the p-value 0.929. This concludes that there is no significant difference on the quantity of vitamin C as boiling duration increases.

The analysis performed for samples of masau juice at 70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 98 ^{0}C at 15 minutes the f-value was 26.35 and the p-value 0.000442. This concludes that there is a significant difference on the quantity of vitamin C as heating temperature increases.

The analysis performed for samples of masau juice at 70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 98 ^{0}C at 30 minutes the f-value was 19.93 and the p-value 0.000173. This concludes that there is a significant difference on the quantity of vitamin C as heating temperature increases.

The analysis performed for samples of masau juice at 70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 98 ^{0}C at 45 minutes the f-value was 23.85 and the p-value 0.000638. This concludes that there is a significant difference on the quantity of vitamin C as heating temperature increases.

CHAPTER 5

DISCUSSION, SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

5.1 Discussion

The fruit preparation process comprising of hand grading, thorough washing and moisture removal plays a pivotal role in ensuring the selection of high-quality fruits and minimizing the risk of microbial contamination in the juice. By meticulously hand-selecting and grading the fruits, any damaged or low-quality ones can be identified and discarded, ensuring that only the best fruits are used in the juice extraction process (Sishu *et al*., 2023). Additionally, thorough washing of the fruits helps to remove any dirt, pesticides or contaminants present on the surface further enhancing the safety and cleanliness of the final product. Moreover, the careful removal of excess moisture from the fruits prior to extraction helps to concentrate their flavor and nutritional content resulting in a more flavorful and nutrient-rich juice. This step also helps to prevent dilution of the juice and ensures a more efficient extraction process.

The methodical approach to juice extraction involving boiling and crushing of the fruits, is essential for facilitating the efficient separation of seeds and pulp from the juice. Boiling the fruits softens them, making it easier to extract the juice, while crushing helps to break down the fruits' cellular structure releasing more juice and flavor. This process ultimately leads to the extraction of pure masau juice, free from seeds and pulp and preserves the natural taste and nutritional value of the fruits. The careful fruit preparation and extraction techniques employed in this process are vital for maintaining the quality, safety and nutritional value of the final product. By ensuring the selection of high-quality fruits and employing efficient extraction

methods, producers can deliver a superior-quality juice that meets consumer expectations for taste, freshness and nutritional benefits (Sishu *et al*., 2023).

5.1.1 Preparation of calibration curve

The preparation of standard ascorbic acid solutions for the quantification of vitamin C content in masau juice samples involved calculations and precise laboratory techniques. Through serial dilution of a stock solution, standard solutions ranging from 0.625 mg/L to 10 mg/L were accurately prepared, serving as crucial references for spectrophotometric analysis. This calibration process allowed for the creation of a calibration curve, correlating absorbance values at a specific wavelength with known concentrations of vitamin C (Maruza *et al*., 2017). The reliability of the calibration curve was ensured through rigorous evaluation of linearity, precision and accuracy. Despite efforts to minimize experimental errors, the validity of results depended on the accuracy of standard solution preparation and the consistency of spectrophotometric measurements.

The preparation of a calibration curve is a critical step in spectrophotometric analysis, aiding in the quantification of analytes within a sample. In the provided data, the preparation of the calibration curve involved the use of standard solutions with known concentrations of the analyte (in this case, vitamin C) and measuring their corresponding absorbance values. The standards, with concentrations ranging from 1.25 mg/L to 10.00 mg/L, were subjected to spectrophotometric analysis, resulting in absorbance values for each standard solution. These data points were then used to construct the calibration curve, plotting absorbance against concentration.

The observed variations in sample concentrations highlight the importance of precise experimental conditions and data analysis in spectrophotometric analysis. Factors such as dilution, temperature and measurement techniques can significantly influence the accuracy and

reliability of the results obtained. Understanding these nuances is essential for drawing meaningful conclusions from experimental data and advancing scientific knowledge in analytical methodologies.

5.1.2 Results from the quantification of vitamin C

The findings demonstrated a pattern of decreased vitamin C concentration as temperature and heating duration increased. This is consistent with prior research into the thermal stability of vitamin C, which has been shown to decline at high temperatures. Vitamin C's heat sensitivity comes from its susceptibility to oxidation and enzymatic breakdown. The observed decrease in vitamin C content with increasing temperature and heating duration can be explained by a variety of factors, including the acceleration of vitamin C oxidation, which leads to its degradation and loss of activity, as well as the promotion of vitamin C enzymatic degradation as a result of enzyme activation. These factors contribute to the overall reduction in vitamin C content as the processing conditions become more severe.

The optimal heating temperature and duration of heating for maintaining vitamin C in masau juice were found to be 70^0 C and 30 minutes respectively. This shows that a moderate temperature and shorter heating duration are optimal for reducing vitamin C loss throughout the juice-making process. The study's findings have practical significance for the commercial production and preservation of masau juice.

It is also crucial to note that factors other than temperature and heating duration can alter the amount of vitamin C in masau juice. These considerations include storage conditions, light exposure and the inclusion of additional components such as antioxidants or enzymes. Future research could look into the combined effects of these aspects to create comprehensive guidelines for maintaining vitamin C during masau juice production and storage, contributing to further advancements in food industry practices. The findings of this study hold broader implications beyond masau juice, offering valuable insights for optimizing processing

techniques for other fruit juices rich in vitamin C (Maruza *et al*, 2017). Through continued investigation and application of findings, the project aims to contribute to the improvement of nutritional quality in fruit juice products, promoting consumer health and well-being.

5.2 Summary

The study investigated the impact of temperature and duration of heating during the juice making process of *Ziziphus mauritiana* (locally known as masau) on the quantity of vitamin C present in the final fruit juice product. *Ziziphus mauritiana* is a fruit that is rich in vitamin C, a crucial antioxidant nutrient. However, the vitamin C content can be affected by the processing conditions used to extract the juice. Juice samples were prepared by subjecting the fruit to different heating temperatures (70 ^{0}C, 80 ^{0}C, 90 ^{0}C and 98 ^{0}C and heating durations (15 minutes, 30 minutes and 45 minutes). A UV Visible Spectrophotometer was then used to quantify the vitamin C in the juice samples and the results were compared across the different processing conditions. The results showed that both temperature and duration of heating had a significant effect on the vitamin C content of the masau juice. Higher temperatures and longer heating durations resulted in greater losses of vitamin C. The juice heated at 98° C for 45 minutes had the lowest vitamin C content, while the juice heated at 70° C for 30 minutes had the highest Vitamin C content. This study provides valuable insights into the optimal juice extraction conditions to preserve the nutritional quality of masau fruit specifically in terms of retaining the beneficial vitamin C content. The findings can guide juice producers and processors on the appropriate temperature and duration of heating to maximise the retention of vitamin C during masau juice production. This is important for ensuring the final juice product maintains its nutritional value and health-promoting properties.

5.3 Recommendations

Based on the findings and conclusions of the study, the following recommendations are proposed:

- Develop standardized protocols for masau juice production, incorporating optimal heating times and temperatures to maximize vitamin C retention. These protocols should be rigorously tested and implemented in commercial production facilities to ensure consistent quality.
- Invest in High Performance Liquid Chromatography (HPLC) or other advanced analytical techniques for precise quantification of vitamin C in masau juice. This will enable more accurate monitoring of vitamin C levels and facilitate quality control measures.
- Conduct further research to investigate the impact of harvesting methods (wet vs dry masau fruit) on vitamin C content. Understanding the influence of harvesting practices will inform agricultural practices and optimize fruit quality.
- Educate consumers about the nutritional benefits of masau juice and the importance of proper storage and consumption to preserve vitamin C content. Public awareness campaigns can promote the consumption of Masau juice as a nutritious dietary supplement.
- Foster collaboration between researchers, agricultural experts and food industry stakeholders to continue exploring innovations in masau juice production. Collaborative efforts can lead to the development of novel processing techniques and value-added products to enhance market competitiveness.

5.4 Conclusions

The research conducted on the preparation of Masau juice and its vitamin C content has yielded valuable insights into optimizing the production process to maximize nutritional benefits. It

was observed that variations in heating duration and heating temperature significantly impact the vitamin C content of Masau juice. The optimal temperature and duration of heating for maintaining vitamin C in masau juice were found to be 70 ^{0}C and 30 minutes, respectively. The findings highlight the importance of careful control over processing parameters to preserve the nutritional integrity of Masau juice.

There is need for standardized protocols in Masau juice production to ensure consistent and optimal levels of vitamin C. The study also corroborated previous research indicating the susceptibility of vitamin C to degradation with prolonged heating, emphasizing the importance of efficient heat management during processing.

Despite limitations such as the absence of High Performance Liquid Chromatography (HPLC) for precise quantification and the focus on dry masau fruit, the study provides a foundational understanding of factors influencing vitamin C retention in Masau juice. These insights pave the way for further research and development aimed at refining production protocols and enhancing the nutritional quality of masau juice.

REFERENCES

Ashraf, A., Sarfraz, R.A., Anwar, F., Shahid, S.A. and Alkharfy, K.M. (2015). Chemical composition and biological activities of leaves of Ziziphus mauritiana L. native to Pakistan. Pakistan Journal of Botany, 47 (1), 367-376.

Afzal, S., Batool, M., Ch, B. A., Ahmad, A., Uzair, M. and Afzal, K. (2017). Immunomodulatory, cytotoxicity and antioxidant activities of roots of Ziziphus mauritiana. Pharmacognosy Magazine, 13 (2), 262-265.

Agbemafle, R., Obodai, E. A., Adukpo, G. E. and Amprako, D. N. (2012). Effects of boiling time on the concentrations of vitamin c and beta-carotene in five selected green vegetables consumed in Ghana. Pelagia Research library, 3(5), 2815-2820.

Ali, S. A., Bonkoungou, E., Bowe, C., DeKock, C., Godra, A. and Williams, J. T. (2006). Ber and other jujubes. Fruits for the future. Southampton Centre for Underutilized Crops, Southampton, 119-129.

Bofars. M. S. (2022). Effect of Heated Temperature Environment on Vitamin C in Four Fruit Citrus Juice. South Asian Research Journal of Nursing and Healthcare, 4(6), 71-83.

Calder, P. C., Albers, R., Antoine, J. M. Blum, S., Bourdet-Sicard, R., Ferns, G. A. and Zhao, J. (2009). Inflammatory disease processes and interactions with nutrition. The British Journal of Nutrition, 101(1), 1-45.

Carpenter, K. J. (2012). The discovery of vitamin C. Annals of nutrition and metabolism, 61(3), 259–264.

Carr, A. C. and Rowe, S. (2020). Factors Affecting Vitamin C Status and Prevalence of Deficiency: A Global Health Perspective. Nutrients, 12(7), 1963-1964.

Combs, G. F. and McClung, J. P. (2008). The vitamins: fundamental aspects in nutrition and health. Academic press, (6).

Chan, T. H. (2020). The Nutrition Source, Harvard College, School of Public Health. https://nutritionsource.hsph.harvard.edu/.

Chivero, E.T., Mutukumira, A.N. and Zvauya, R. (2001). Partial purification and characterisation of a xylanase enzyme produced by a micro-organism isolated from selected indigenous fruits of Zimbabwe. Food Chemistry 72(2), 179-185.

Dagar, J. C.; Singh, G. and Singh, N. T. (2001). Evaluating forest and fruit trees for rehabilitation of semiarid alkali-sodic soils in India. Arid Land Research and Management, 15 (2), 115-133.

Jha, D., Hangargekar, P., Akbar, M., Ashish, Parihar, A.S., Kashyap, S., Joshi, A. and Rahman, M. A. (2023). Ziziphus mauritiana: An in-depth review of its medicinal attributes and pharmacological activities. Intelligent Pharmacy, 2 (2), 274-283.

Desai, P. A. and Desai, S. (2019). Uv Spectroscopic Method for determination of Vitamin C (Ascorbic Acid) content in different fruits in South Gujarat Region. International Journal of Environmental Science and Natural Resources, 21(3), 41-44.

Dhileepan, K. (2017). .Biological control of Ziziphus mauritiana (Rhamnaceae): feasibility, prospective agents and research gaps. Annals of Applied Biology, 170 (3), 287-300.

Ecocrop. (2013). Ecocrop database. Feedipedia, Animal feed resources information system. FAO, Rome, Italy. https://www.feedipedia.org/node/16485.

El-Ishaq, A. and Obirinakem, S. (2015). Effect of Temperature and Storage on Vitamin C Content in Fruits Juice. International Journal of Chemical and Biomolecular Science, 1(2), 17-21.

Essodolom, P., Chantal, B. E., Mamatchi, M. and Kous'anta, A. (2020). Effect of temperature on the degradation of ascorbic acid (vitamin c) contained in infant supplement flours during the preparation of porridges. International Journal of Advanced Research. 8(3), 116-121.

Farah, H.S., Alhmoud, J.F., Al-Othman, A., Alqaisi, K.M., Atoom, A.M., Shadid, K., Shakya, A. and AlQaisi, T. (2020). Effect of pH, Temperature and Metal Salts in Different

Storage Conditions on the Stability of Vitamin C Content of Yellow Bell Pepper Extracted in Aqueous Media. SRP, 11(9), 661-667

Francescone, M.A. and Levitt, J. (2005). Scurvy masquerading as leukocytoclastic vasculitis: a case report and review of the literature. Cutis, 76(4), 261-266.

Gadaga, T.H., Mutukumira, A.N., Narvhus, J.A. and Feresu, S.B. (1999). A review of traditional fermented foods and beverages of Zimbabwe. International Journal of Food Microbiology, 53 (1), 1-11.

Gupta, N. (2018). Morphological and physico-chemical characterization of ber (Ziziphus mauritiana Lamk.) genotypes in semi-arid zone of Punjab. International Journal of Chemical Study, 6 (5), 2353-2356.

Hebbara, M.; Manjunatha, M. V., Patil, S. G. and Patil, D. R. (2002). Performance of fruit species in saline-waterlogged soils. Karnataka Journal of Agricultural Science, 15 (1), 94-98.

Jacob, R.A. and Sotoudeh, G. (2002). Vitamin C function and status in chronic disease. Nutrition in clinical care, 5(2), 66-74.

Joshua, Z. P. and Suleiman, M. M. (2012). The effect of cooking time on the vitamin C, dietary fiber and mineral compositions of some local vegetables. Science World Journal, 7 (1), 29-30.

Jumanne, A., Maghembe, J. and Seyani, J. (1992). Multipurpose trees used by small-scale farmers in Malawi. Results of an ethnobotanical survey. Int. Centre for Research in Agroforestry.

Kaaria, S. (1998). Ziziphus mauritiana - A valuable tree for arid and semi-arid lands. Forest, Farm and Community Tree Network (FACT Net), Winrock International. https://winrock.org/ziziphus-mauritiana-a-valuable-tree-for-arid-and-semi-arid-lands/.

Kaleem, W. A., Muhammad, N., Khan, H. and Rauf. A. (2014). Pharmacological and phytochemical studies of genus Zizyphus. Middle East Journal of Scientific Research, 21 (8), 1243-1263.

Li, Y. and Schellhorn, H. E. (2007). New developments and novel therapeutic perspectives for vitamin C. The Journal of nutrition, 137(10), 2171-2184.

Loukou, A. L., Assi, R. S., Atchibri-Anin, L. and Brou, K. (2020). Impact of cooking time on nutritive characteristics of justicia galeopsis leaves consumed in Cote Divoire, International Journal of Innovation and Scientific Research, Volume 48 (2), 146-153.

Kalikiti, F. (1998). Ziziphus mauritiana in Siavonga district, Zambia. International Workshop on Ziziphus mauritiana, 13-16.

Kinyi, H. W., Tirwomwe, M., Ninsiima, H. I. and Miruka, C. O. (2022). Effect of Cooking Method on Vitamin C Loses and Antioxidant Activity of Indigenous Green Leafy Vegetables Consumed in Western Uganda. International Journal of Food Science.

Kleszczewska, E. (2000). L-Ascorbic acid - clinical use, toxicity, properties, methods of determination and application in chemical analysis. Die Pharmazie, 55 (9), 640-644.

Lamien-Meda A., Lamien. C.E., Compaoré, M. M., Meda, R. N., Kiendrebeogo, M., Zeba, B. and Nacoulma, O. G. (2008). Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules, 13 (3), 581-594.

Lee, S., Choi, Y., Jeong, H. S., Lee, J. and Sung, J. (2017). Effect of different cooking methods on the content of vitamins and true retention in selected vegetables. Food science and biotechnology, 27(2), 333–342.

Li, Y. and Schellhorn, H.E. (2007). New developments and novel therapeutic perspectives for vitamin C. Journal of Nutrition, 137 (10), 2171-2184.

Mahapatra, A. K., Mishra, S., Basak, U. C. and Panda, P. C. (2012). Nutrient analysis of some selected wild edible fruits of deciduous forests of India: an explorative study towards non-conventional bio-nutrition. Advance Journal of Food Science and Technology 4 (1), 15-21.

Maposa, M. and Chisuro, D. (1998). Importance of Ziziphus mauritiana (masau) in the Mukumbura area of Zimbabwe- From a farmer's and extensionist's point of view. In: International Workshop on Ziziphus mauritiana, Harare, Zimbabwe, 13-16.

Maruza, I. M., Musemwa, L., Mapurazi, S., Matsika, P., Munyati, V. T. and Ndhleve, S. (2017). Future prospects of Ziziphus mauritiana in alleviating household food insecurity and illnesses in arid and semi-arid areas: A review. World Development Perspectives, 5, 1-6.

Mirheidari. F., Khadivi, A., Saeidifar, A. and Moradi, Y. (2022). Selection of superior genotypes of Indian jujube (Ziziphus mauritiana Lamk.) as revealed by fruit-related traits. Food Science and Nutrition, 10 (3), 903-913.

Mishra, T., Paice, A.G. and Bhatia, A. (2011). Use of seeds of Malay apple (Ziziphus mauritiana) and related species in health and disease. In Nuts and Seeds in Health and Disease Prevention, 733-739.

Moll, E.J. (1997). Trees of Southern Africa. C. Struik Publishers, Cape Town, Johannesburg.

Nyanga L.K., Gadaga T.H., Nout M.J., Smid E.J., Boekhout T. and Zwietering M.H. (2013). Nutritive value of masau (Ziziphus mauritiana) fruits from Zambezi Valley in Zimbabwe. Food Chemistry, 138 (1), 168–172.

Nyanga, L. K., Nout, M. J. R., Smid, E. J., Boekhout, T. and Zwietering, M. H. (2012). Genotypic and phenotypic characteristics of yeasts isolated from masau (Ziziphus mauritiana) fruits and their fermented products in Zimbabwe. The 23rdInternational ICFMH Symposium on Global Issues in Food Microbiology, Abstract book, 128.

Orwa, C. (2009). Agroforestree Database: a tree reference and selection guide version 4.0. http://www. Worldagroforestry. Org/sites/treedbs/treedatabases. asp.

Owolarafe, T.A., Salawu, K., Ihegbor, G.O., Ononamadu, C.J., Alhassan. and Wudil, A.M. (2020).Investigation of cytotoxicity potential of different extracts of Ziziphus mauritiana (Lam) leaf Allium cepa model. Toxicol Reports, 7, 816-821.

Panseeta, P., Lomchoey, K., Prabpai, S., Kongsaeree, P., Suksamrarn, A., Ruchirawat, S. and Suksamrarn, S. (2011). Antiplasmodial and antimycobacterial cyclopeptide alkaloids from the root of Ziziphus mauritiana. Phytochemistry, 72 (9), 909-915.

Pareek, S. (2013). Nutritional composition of jujube fruit. Emirates Journal of Food and Agriculture, 25(6), 463–470.

Prakash, O., Usmani, S., Singh, R., Singh, N., Gupta, A. and Ved. A. (2021). A panoramic view on phytochemical, nutritional, and therapeutic attributes of Ziziphus mauritiana Lam.: a comprehensive review. Phytotherapy Research, 35 (1), pp. 63-7.

Raman, R. and MS, R. (2020). (7) Impressive Ways Vitamin C Benefits Your Body. Healthline.

Ratini M. (2024). The Benefits of Vitamin C. What can vitamin C do for your health? https://www.healthline.com/nutrition/vitamin-c-benefits

Stephen, R. and Utecht, T. (2001). Scurvy identified in the emergency department: a case report. The Journal of emergency medicine, 21(3), 235-237.

Sishu, N.K., Das, U. and Selvaraj, C.I. (2023). Indian Jujube a potential fruit trees to improve the livelihood. Saudi Journal of Biological Sciences, 30(9), 103769.

U.S. Department of Agriculture. (2019). Agricultural Research Service. FoodData Central. https://fdc.nal.usda.gov/.

Velisek, J. and Cejpek, K. (2007). Biosynthesis of food constituents: Vitamins. 2. Water-soluble vitamins: part 1-a review. Czech Journal of Food Science, *25*(2), 49-64.

Wang, A.H. and Still, C. (2007). Old world meets modern: a case report of scurvy. Nutr Clin Pract, 22(4), 445-448.

Weinstein, M., Babyn, P. and Zlotkin, S. (2001). An orange a day keeps the doctor away: scurvy in the year 2000. Pediatrics, 108(3), 55.

Yahia. Y., Benabderrahim, M.A, Tlili. N., Bagues, M. and Nagaz, K. (2020). Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two Ziziphus Mill. Species. PLoS One, 15 (5).

APPENDICES

Appendix 1: Summary for the analysis of data at 70 ^{0}C

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
temp	1	0.142	0.1420	0.17	0.693
Residuals	7	5.858	0.8369		

Appendix 2: Summary for the analysis of data at 80 ^{0}C:

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
temp	1	7.02	7.02	0.398	0.548
Residuals	7	123.37	17.62		

Appendix 3: Summary for the analysis of data at 90 ^{0}C

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
temp	1	33.75	33.75	14.36	0.00681 **
Residuals	7	16.45	2.35		

Appendix 4: Summary for the analysis of data at 98 ^{0}C:

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
temp	1	0.023	0.0228	0.008	0.929
Residuals	7	18.908	2.7011		

Appendix 5: Summary for the analysis of data at 15 minutes:

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
temp	1	148.59	148.59	26.35	0.000442 ***
Residuals	10	56.39	5.64		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix 6. Summary for the analysis of data at 30 minutes*:*

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
temp	1	253.1	253.05	17.93	0.00173 **
Residuals	10	141.1	14.11		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix 7. Summary for the analysis of data at 45 minutes:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
v1	1	276.5	276.49	23.85	0.000638 ***
Residuals	10	115.9	11.59		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1