Optimizing pasteurization of *Ziziphus mauritiana* (masau) non-alcoholic juice.

By

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Approval form

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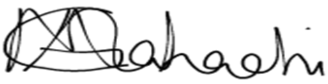
Signature of student: 
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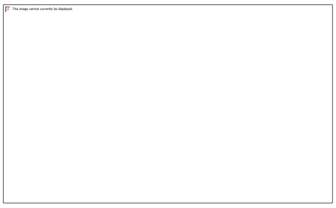
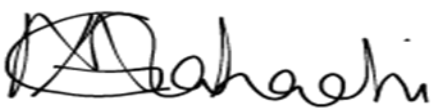
Signature of Supervisor:  Date: 27/09/24

Signature of Chairperson of Department:  Date: 27/09/24

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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.

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Dedication

I dedicate this project to my lovely mother Nellie Dhliwayo, whose moral and financial support has remained unrelenting.

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List of abbreviations

ANOVA- Analysis of Variance

Cfu - Colony forming unit

Cm- Centimetre

HTST- High-Temperature Short Time

Kg- Kilogram

l- Litre

LTLT- Low-Temperature Long Time

M - Metre

ml - Millilitre

% - Percentage

PET- Polyethylene Terephthalate

pH - Hydrogen Ion Concentration

Spp- species

UV- Ultraviolet

UHT- Ultrahigh Temperatures

˚C- Degree Celsius

˚F- Degree Fahrenheit

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Abstract

Native to several regions of Asia and Africa, *Ziziphus mauritiana* is also known as masau in Zimbabwe. It is a tropical fruit used for juice production because of its unique flavour and nutritious composition. Pasteurization is an essential step in the manufacturing chain that ensures safety and extends the juice's shelf life. To maintain the exceptional properties of masau fruit juice and ensure microbial safety, the pasteurization process was optimised. This study set out to investigate the effects of temperature and duration on pasteurization on the physicochemical properties, nutritional value, and sensory features of masau juice. Masau fruits from Muzarabani were graded, washed with tap water, and dried to get rid of extra moisture. Following that, the fruits were juiced using a system developed at Bindura University of Science Education. Subsequently, the juice was pasteurised in a water bath at different temperatures (55, 65, 72, 84, and 95 ˚C) and for different amounts of time (15, 20, and 30 minutes). Additionally, a control group of unpasteurized masau juice was made. Both the experimental and control groups were maintained in consistent storage conditions and monitored for changes in pH, colour, appearance, and sensory attributes over time in order to evaluate the impact of the various pasteurization techniques. The study examined the effects of pasteurization on the nutritional value and quality of masau juice. Pasteurization at 72 ˚C for 15 minutes emerged as a promising compromise and by selecting this temperature-time combination as the optimal pasteurization conditions masau juice, producers can strike a balance between microbial safety and product quality. Extended pasteurization periods led to reduced taste intensity, darker colouring, and possibly even the disintegration of heat-sensitive ingredients, particularly at elevated temperatures. Higher temperatures changed the juice's sensory characteristics significantly, even if they increased microbiological safety. The findings demonstrated how challenging it is to optimise pasteurization while maintaining microbiological safety and sensory preservation. Future research could focus on looking into alternative pasteurization procedures or technologies, which would increase the general acceptability of masau juice and lessen the influence on sensory qualities while preserving microbiological safety. The correct pasteurization parameters, such as temperature and duration, can be carefully chosen to ensure microbial inactivation while preserving desired sensory attributes. This comprehensive approach can raise the overall quality and acceptance of the pasteurised masau juice product among consumers.

Contents

Chapter 1

Introduction

1.1 Background of the study

Masau trees are found in dry and semi-arid environments in the world. The medium-sized masau (*Ziziphus mauritiana*) tree is thorny and has a taproot that is fast growing, which is a crucial adaptation to drought conditions (Padhy, 2021). The species has naturalized in several southern African countries, including Zimbabwe, where it is considered to be indigenous. It is commonly grown in Mozambique, where it spreads up the Zambezi valley. Researchers from Israel, Malawi, Senegal, and Zimbabwe have participated in an extensive study on the chemical and physiological qualities that were initially started in India (Gichora, Kojwang and Bosu, 2017).

The size and shape of masau fruit vary; it can have a somewhat large stone and be round, oval, or oblong, as well as huge, medium, or small. Fruit from wild trees may have a diameter of just 1.8 to 2.5 centimetres, but fruit from developed cultivars may have a diameter of up to 5 cm (plum-sized). The fruit starts out green, then ripens to yellow, and then brown. In the Zambezi valley of Zimbabwe's Mashonaland central region, masau fruits begin to ripen in mid-June and are available until the end of September. In Zimbabwe, naturally growing, distributed wild trees produce 4 to 5 tons per year of masau fruits from a 3 to 4 hectare area (Haavisto-Meier, 2018). The fruit is thirst-quenching and has a sour taste intermingled with sweetness. In the Sahel region of Africa, yields of 80 to 130 kilograms/tree/year have been reported (Von Maydell, 1986).

The improvement of rural communities' socioeconomic conditions through job creation, increased family income, and the provision of a year-round supply of high-quality, safe beverages are all possible benefits of optimizing and controlling the processing of locally grown fruit products, such as traditionally fermented foods. In Zimbabwe, the fruit is naturally fermented before being distilled to make the potent alcoholic beverage known as Kachasu (Tredgold, 1986; Gadaga et al., 1999). The highly nutritious fruits (masau) are planted in many areas because they are an excellent source of energy, vitamins, and money when sold at the local market. Masau trees are utilized as a hedge because their spines make an effective living fence. In the Zambezi valley of Zimbabwe, dried fruit powder is made into jam, a traditional loaf of bread, and baked goods (Kadzere, 1998). Ripe fruits are typically eaten raw in India, but stewing is also sometimes done. Several companies produce jam and sun-dried fruit slices (masau snacks) from the pulp of mature masau fruit for the market for example Tulimara - Speciality Foods for Africa (SFA). In Zambia and Western Sudan, cakes composed of dried and fermented pulp resemble gingerbread (Kalikiti, 1998). The majority of rural residents in arid regions obtain biomass-based products from the masau tree, including fruits, fuel (firewood), fodder, fertilizer (organic manure, forest litter), building materials (poles), and medicinal plants (Morton, 1987).

The masau fruit can also be taken as juice, candied, pickled, or dried fruits (Fact, 1998). Generally speaking, the juice is described as the fluid that can be extracted from cells or tissues (Merriam-Webster, 1991). Research has demonstrated that the *Z. mauritiana* plant contains antioxidant chemicals (Dahiru and Obidoa 2007; Bhatia and Mishra 2009; Gupta 2018). Masau fruit juice is better for the body's efforts to fight illness and infection because of its health advantages and higher antioxidant content than chemically made food supplements, which can have negative side effects including high blood pressure. The antioxidants may make effective antibacterial agents than conventional antibiotics, which may have unpleasant side effects. Particularly in rural areas without convenient access to medical services, the use of fruit juice as an antibacterial agent is encouraged. Moreover, extracts from the trees' fruits, seeds, leaves, roots, and bark are used in traditional medicine to treat fever, inflammatory illnesses, skin ailments, and insomnia (Morton, 1987).

Technologists around the world have worked to develop methods of processing tropical fruits and vegetables through pre-processing storage techniques, preparative procedures, extraction, and preservations using chemical and natural preservatives, packaging, and pasteurization (Ihekoronye and Ngoddy, 1985). Pasteurization is the act of heating a product to a specific temperature and holding it there until nearly all potentially harmful microbes are eliminated (This was developed by Louis Pasteur, 1960). Thermal processing is the most widely used technique for pasteurizing fruit juices and drinks to extend their shelf life. The most popular methods for pasteurizing juice are low-temperature long time (LTLT) and high-temperature short time (HTST). Pasteurization of juice is based on a 5-log reduction of the most significant public health-relevant resistant bacteria (USFDA, 2001).

Several time-temperature combinations could be used to carry out the operation. Fruit juice has traditionally been pasteurized by batch heating for an extended time at 63 to 65 degrees Celsius (D'Amico et al. 2006). This technique causes unfavourable quality changes, and high-temperature short-time treatment has taken its place. HTST takes far less time than batch heating, it can minimize any negative quality changes. Orange juice is processed by HTST at 90 to 95 ˚C for 15 to 30 seconds, making it the most widely used method for heating fruit juice at the moment (Braddock, 1999). Fruit juices can be pasteurized at 65 ˚C to 85 ˚C for 15 to 45 minutes. Apple juice is subjected to HTST treatment for 25 to 30 seconds at 77 to 88 ˚C (Moyer & Aitken, 1980). Other time and temperature combinations can also be utilized for fruit juices. The juice is then cooled to room temperature following this heat treatment. Pasteurization can also be carried out at high temperatures (90 ˚C) for a brief period. For apple juice, for instance, you may do this at 95 ˚C to 98 ˚C for 15 to 30 seconds.

Temperatures above 85 ˚C are produced by thermal inertia when containers under 1litre are hot-filled and quickly closed. Juices can be heated to ultrahigh temperatures (UHT), which is frequently done in larger plants.

1.2 Problem statement

Masau juice has been commonly packaged without pasteurization. This has led to spoilage of the beverage due to fermentation. There is need to develop the best pasteurization conditions to prevent spoilage and lengthen the shelf life of the juice.

1.3 Aim

This research aims to examine the effects of different temperatures and time intervals on the pasteurization of masau juice produced with additives to extend its shelf life.

1.4 Objectives

(i) To evaluate the effects of various pasteurization temperatures on the microbial safety and quality attributes of masau juice.

(ii) To determine the impacts of different pasteurization durations on the stability and sensory qualities of masau juice with preservatives.

(iii) To identify the optimal temperature and duration for pasteurizing masau juice to maximize safety and quality.

1.5 Research questions

(i) What is the optimum temperature for pasteurization of masau juice?

(ii) What is the optimum time for the pasteurization of masau juice?

(iii) What is the optimum temperature and time for maintaining the quality attributes (colour, flavour, nutrients) of pasteurized masau juice?

1.6 Justification of the study

There are currently no pasteurization conditions for masau juice, and this has resulted in losses due to early fermentation of the juice. Pasteurization helps in eliminating harmful microorganisms that can cause foodborne illness such as *Salmonella* and *Escherichia coli* (*E. coli*). The study will fill this critical gap by identifying pasteurization conditions that will extend the shelf-life of masau juice the most.

1.7 Limitations

One of the primary limitations in pasteurizing masau juice is its sensitivity to heat. Masau juice contains delicate flavour compounds and nutrients that can be compromised at high temperatures. Therefore, optimizing pasteurization involves finding the right balance between achieving microbial safety and preserving the sensory and nutritional qualities of the juice. The colour and flavour of masau juice are key attributes that contribute to its consumer appeal. High-temperature processing during pasteurization can lead to undesirable changes in colour and flavour, impacting the overall sensory experience of the juice. Certain microorganisms may exhibit resistance to conventional temperatures and time posing a limitation to the efficacy of the process. This resistance can compromise the microbiological safety of the juice.

1.8 Delimitations

The study is delimited to only masau juice with preservatives. The maximum temperature that can be tested is 95 ˚C because temperatures above 95 ˚C can start to degrade important nutrients and alter the sensory properties of the juice.

1.9 Hypotheses

H_1: There is a significant difference between the pasteurized and non-pasteurized juice in terms of quality, taste and color.

H_0: There is no significant difference between pasteurized and non-pasteurized juice in terms of quality, taste and color.

1.10 Definition of terms

Pasteurization is a heat-treatment process that destroys pathogenic microorganisms in certain foods and beverages (Ramesh, 2020).

Masau (*Ziziphus mauritiana*), also known as Indian jujube, Indian plum, Chinese date, Chinese apple and dunks is a tropical fruit tree species belonging to the family Rhamnaceae.

Family: Rhamnaceae

Genus: *Ziziphus*

Kingdom: Plantae

Species: *Z. mauritiana* (Alhassan, Abubakar and Ibrahim, 2020)

Masau juice is a delightful non-alcoholic beverage made from the masau fruit, which grows in certain regions of Zimbabwe.

Chapter 2

Literature review

2.1 *Ziziphus mauritiana*

Native to Asia and Africa, *Ziziphus mauritiana*, popularly called the "masau" tree, is a tropical fruit. Its well-liked juice is prone to infection and spoiling. Although pasteurization is a popular technique for extending the shelf life of juices, it is unknown what exactly the ideal pasteurization settings are for masau juice.

2.2 Description, classification and distribution of masau

The Rhamnaceae family includes the tropical fruit tree species *Ziziphus mauritiana* known as masau (Figure 1). One hundred species of trees and shrubs in the genus *Ziziphus* can be found growing in temperate, tropical, and subtropical climates worldwide (Williams, 2006). Since its discovery in the Torres Strait of Britain in 1893, the fruit has spread (Amin et al., 2018). Indian jujube, Indian plum, Chinese date, Chinese apple, beri, and dunks are some other names for it. With a trunk diameter of 40 cm or more, a spreading crown, stipular spines, and a profusion of drooping branches, masau is a prickly, evergreen shrub or small tree (Kaudo et al., 2022). This species frequently produces other kinds without spines. The bark has large vertical fissures that are reddish and fibrous within, and it might be dark grey, dull black, or reddish (Dubey et al., 2011). The ends of the branches droop as they expand out. When the branches are young, they are often gently tomentose (Ghazanfar et al., 2011). From 2 to 6 cm in length and 1.5 to 5.9 cm at their broadest point, the leaves are either solitary or in pairs. Insects pollinate the tiny, hermaphrodite, greenish-white or yellow flowers, which have an unpleasant odour (Kennard and Winters 1960). The flowers are in sessile or short stalk axillary cymes.

The fruit is shaped and sized differently (Dahlia et al., 2019). Its shape might be oval, obovate, oblong, or round, and its diameter can vary from 1 to 2.5 cm, depending on the variety. The flesh is described as crisp and white. When slightly under ripe, this fruit has a slight sweetness and aroma. The fruit has smooth, tight, and glossy skin. It is indigenous to Malaysia, Afghanistan, South East Asia, North and Eastern Africa, Australia, North India, and South East China (Naghmouchi and Alsubeie, 2021). In Southern Africa, the fruit is widely grown in Mozambique, Malawi and Zimbabwe (Coates Palgrave, 1997). Its cultivation is spreading and it is gaining importance (Sharif et al., 2019). The fruit dominates in the Mashonaland province for example the Muzarabani district and the lower Zambezi valley (Musemwa et al 2018).

Masau is a species that can be found in both wild and naturalised forms in the arid and semi-arid regions of Pakistan and India (Uddin et al., 2022). Its range stretches from the Himalayan foothills, where it can reach elevations of over 1500 metres, to the southern desert region (Azam Ali et al., 2006). The masau tree has become widely introduced worldwide, surpassing the small list of nations that have been officially recorded to date. Brazil, for example, has embraced the cultivation of masau and included it into its varied fruit-bearing environment (Silva et al., 2022). Analogously, masau has been successfully introduced in India, where local residents are becoming more and more accustomed to its use and cultivation (Rao et al., 2021). Although it grows all over Zimbabwe, only the lower lying regions in the Dande valley see fruiting (Gonese, 2002). The survival of rural communities in Zimbabwe depends on masau fruit. The fruit is consumed fresh as well as sold at local markets. Extra fruit can be preserved by sun drying.

Figure 1: Masau tree, credit: Caroline Alice Mahachi, 04 May 2024.

2.3 Uses of Masau

Masau fruits

Masau fruit has a particular flavour and is used in many culinary recipes (Figure 2). It is frequently used in Zimbabwe to create sauces, jellies, and jams, which give food a sweet and tangy flavour (CCS HAU, 2019). The fruit adds a delicious tropical flavour to delicacies including pies, cakes, and ice cream (Shi and Moy, 2005). A tart and mildly alcoholic beverage is produced by fermenting masau fruit pulp with water and sugar (Nyanga, 2012). The fruit can also occasionally be used to create fruit smoothies and liquids, which is a tasty and nutritious alternative (Adilah et al., 2023). Traditional herbal medicines use the masau fruit, which has long been known to have medicinal qualities. It is thought to have anti-inflammatory and antioxidant qualities, among other health benefits (Rao et al., 2021). To treat conditions including respiratory problems, fever, and digestive disorders, the fruit is frequently eaten fresh or in the form of extracts, decoctions, or infusions. The masau fruit is advantageous for human consumption as well as a valuable source of feed for animals. It is a good addition to animal diets due to its high nutritional value, especially for raising poultry and cattle (Amin et al., 2019).

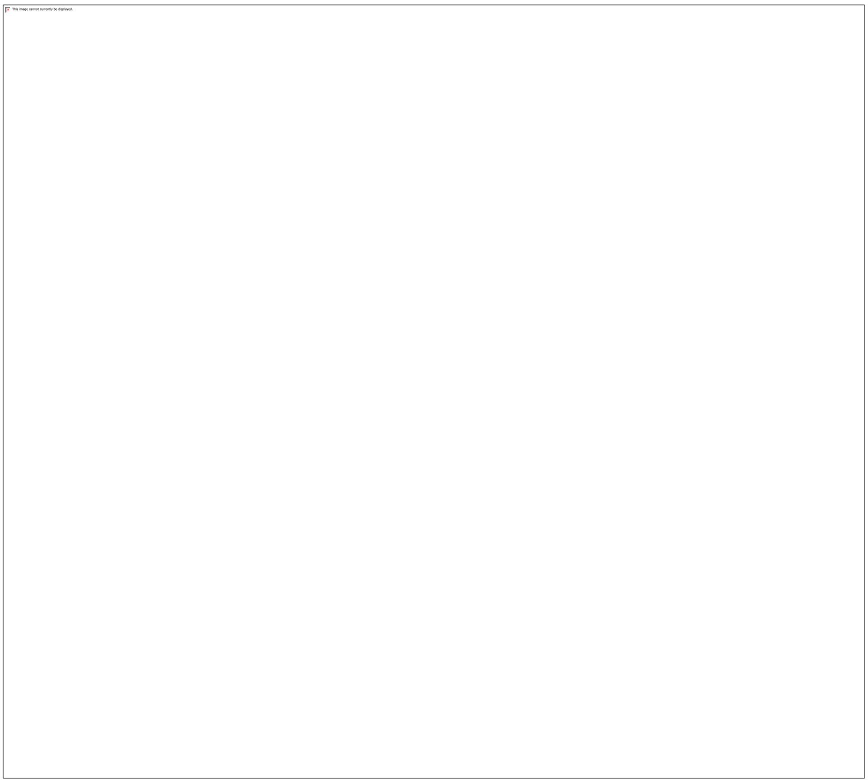

Figure 2: Masau fruits, credit: Caroline Alice Mahachi, 02 May 2024.

Masau leaves

The possible therapeutic characteristics of masau leaves have led to their lengthy history of usage in traditional medicine. They are frequently used in herbal treatments to address a range of conditions. The leaves may be helpful in treating wounds, skin infections, and gastrointestinal issues because they are thought to have anti-inflammatory and antibacterial qualities (El Maaiden et al., 2020). To maximise its therapeutic potential, masau leaf infusions or extracts are made, applied topically, or ingested as herbal teas. Masau leaves are occasionally utilised in food preparations, especially in several ethnic cuisines. Young, tender Masau leaves are utilised in some areas as a leafy green vegetable in cooking. They can be added to stir-fries, stews, and soups to provide the food a special taste and nutritional boost (Sen and Bhattacharyya, 2023). The leaves' well-known somewhat bitter flavour goes well with a variety of savoury meals. Masau leaves have been used as a valuable source of feed for animals (Figure 3). They are nutrient-rich and can be fed to cattle, sheep, and goats. To improve the nutritional value and support animal health, the leaves are frequently dried and used to animal feed formulations (Khan et al., 2009). They are a sustainable feed alternative for raising animals because of their accessibility and possible advantages. Applications for masau leaves exist in the environmental field as well. The leaves' fibrous quality makes them suitable for composting and mulching. By releasing nutrients gradually as the leaves break down, mulching with masau leaves can help conserve soil moisture, inhibit weed growth, and improve soil fertility (Sidibe, 2010). Moreover, composting masau leaves yields nutrient-rich organic matter that may be used to plants as a natural fertiliser.

Figure 3: Masau fruits and leaves, credit: Caroline Alice Mahachi, 06 June 2024.

Masau roots

The roots of the Masau tree have been used traditionally in medicine because of their possible therapeutic benefits. They are frequently used in herbal treatments to treat a variety of illnesses. The roots are effective in treating conditions including toothaches, digestive issues, and skin infections since they are thought to have antibacterial, anti-inflammatory, and analgesic effects (Rao et al., 2021). The roots of the masau tree are used to make preparations like decoctions and infusions, which can be used topically or taken internally for possible therapeutic benefits. Masau tree roots can be used to make crafts and artwork. The roots can be carved, sculpted, and used to construct beautiful products because of their complex and distinctive shapes (Seth, 2003). These creative works might be used as decorative accents or as cultural relics. The large root system of the Masau tree is important for controlling erosion and stabilising the soil. The spreading, deep roots aid in stabilising the soil, reducing erosion, and enhancing soil structure. Planting Masau trees and allowing their root systems to spread can help stabilise slopes and conserve soil in places that are vulnerable to landslides and erosion (Fischer, 2012). Because of their capacity to bind and hold the soil together, roots serve as a buffer against environmental deterioration and the loss of fertile topsoil.

Masau seeds

The seeds of masau may find use in industry. Rich in fatty acids, the oil collected from the seeds is used to make soap, cosmetics, and biodiesel (Pandey et al., 2023). The oil content of masau seeds is one of its main applications. There is a lot of oil in the seeds, which can be extracted and utilised in cooking. Because of its high content of unsaturated fatty acids, the oil derived from seeds is a healthy substitute for conventional cooking oils. Moreover, masau seeds may be ground into a paste or powder and added to skincare items. A common component of cosmetics and beauty products, the seed extract is prized for its nourishing and moisturising qualities. Masau seed oil was investigated as a potential source of biodiesel (Veeranan and Ganapathy, 2023).

2.3 Juice

The most general definition of juice is the extractable fluid contents of cells or tissues (Rajauria and Tiwari, 2018). It is also defined as fermentable but unfermented juice, intended for direct consumption, obtained mechanically from sound, ripe fruits, preserved exclusively by physical means (Poonia, 2018). The juice can be clear or turbid, and it can have been concentrated and then reconstituted with water suitable for the purpose of maintaining the juice's essential composition and quality factors. Adding sugars or acids can be allowed, but they must be approved in the relevant standard (Bates et al., 2001). While it is evident that expressing the liquid from a whole or chopped fruit yields fruit juices in many cases, this is not always the case. Squeezing the flesh of a peeled mango, for instance, does not produce much juice until the flesh is comminuted. Even so, the finished product is a thick purée.

Comminuted apples, on the other hand, produce a juice that is easily extracted. Mango puree would become less thick if water was added, but it is still not regarded as juice. Although the liquid extracted from lemons, limes, and other highly acidic fruits is undoubtedly juice, it is too sour to drink straight without first diluting it with sugar and water to make lemonade or limeade. Fruits belong to the diverse and intricate substance group category of foods. The substance classes that are relevant include carbohydrates, acids, minerals, water-soluble vitamins, polyphenols (tannins), including the colourful anthocyanins, amino acids, aroma compounds, carotenoids, fibres, and other bioactive chemicals. In essence, they are moved into the purée or the squeezed juice during processing (Bates et al., 2001).

2.4 Health benefits of fruit juices

Fruit consumption contributes to the prevention of numerous degenerative illnesses, including cancer and heart issues. Research spanning decades has demonstrated the importance of fruits and vegetables as dietary components that can lower the risk of a number of chronic diseases, many of which have been linked to chronic inflammation. Fruit juices are low in sodium and high in potassium, which support normal blood pressure. Their fat-free content is also good for the cardiovascular system. Vitamin C, phosphate, and carbs can all be found in good amounts in masau juice. By warding off disease, vitamin C boosts immunity, combats free radicals, and helps skin look more vibrant. Fruit juices have been linked to a significant reduction in the progression of Alzheimer's disease and the onset of cancer, according to numerous investigations (Delichatsios and Welty, 2005; Matthews, 2006; Rico et al., 2007; Dai et al., 2006; Cutler et al., 2008; Kyle et al., 2009; Holt et al., 2009). The fruit contains 20 to 30 % sugar, 2.5 % protein, and 12.8 % carbohydrate. The fruits' sweet-sour flavour is caused by several acids, including glucose, fructose, oxalic, succinic, and malic. Because it contains a lot of antioxidants, it helps to repair liver and kidney malfunction and revitalise bodily cells. Given its high antioxidant content, the fruit can be used to treat a variety of skin conditions. Various digestive diseases are naturally treated with masau. The fruit promotes the regular function of several digestive organs by aiding in the release of enzymes. In dry form, it also relieves constipation. Free radicals in the blood circulation system, which can lead to heart obstruction and numerous other cardiovascular issues, are naturally captured by flavonoids. A significant amount of antioxidant, anti-inflammatory, and anti-cancerous properties are present in several other flavonoids. These aid in the prevention of cancer cells from forming, treat a variety of digestive issues, lessen allergies, and ultimately boost immunity.

2.5 Spoilage

A change in a food's flavour, aroma, or appearance that renders it unpalatable to the consumer is referred to as food spoiling (Aneja et al., 2008). Fruit juices, especially masau juice, can spoil for a number of reasons, including enzymatic reactions, physical deterioration, and microbiological infection. Fruit and vegetable juice spoilage is mostly caused by the growth of their innately osmophillic and acid-tolerant microflora. Fruit juices, especially masau juice, can deteriorate due to microbial infection. In the juice, moulds, bacteria, and yeast can grow when the right circumstances are present, such as high moisture content and room temperature. These microbes have the potential to cause fermentation, bad flavours, colour and texture changes, and other effects that make the juice dangerous to drink (Brennan et al., 2015).

Fruit juices have high sugar content, ideal water activity for microbial growth, and a low pH that makes them more prone to fungal and yeast spoiling because most bacteria prefer to grow at neutral pH, which eliminates a significant portion of bacterial contamination (Patil et al., 2011; Bevilacqua et al., 2011). In the fermented masau juice, fructose was discovered to be the most prevalent remaining sugar. Nonetheless, fructose is assimilated by yeast more slowly, which leads to its increased residual sugar content in the finished fermented product

(Mocke, 2013). Fruit juice deterioration is also caused by enzymatic processes. Masau juice can be made with naturally occurring enzymes that are still active after extraction. By producing browning, flavour alterations, and nutritional loss, these enzymes have the ability to catalyse reactions that lower the juice's quality. To stop enzymatic spoiling, use appropriate processing methods such heat treatment or enzyme inactivation (Galanakis, 2020). With time, pectinases and amylases, among other enzymes, can induce cloudiness, odd tastes, and textural changes in juice.

Masau juice can deteriorate physically as a result of exposure to light and oxygen, for example. Exposure to oxygen may trigger oxidative reactions that reduce the juice's flavour, nutritional value, and browning. Improper sealing or defective packing might allow oxygen to get into the juice. Furthermore, photochemical reactions that result in off-flavors and nutritional degradation can be accelerated by exposure to light. Nutrient loss and flavour changes result from the breakdown of vitamins and other components by UV radiation. These types of spoiling can be lessened by packaging masau juice in materials that are both light- and oxygen-barrier (Barba et al., 2017). Good hygiene during processing and packing is essential to keep masau juice and other fruit juices from going bad. Microbial contamination can be reduced by employing clean equipment and keeping processing facilities sanitary. Additionally, the use of suitable storage conditions, including pasteurization or refrigeration, might increase the shelf life of masau juice by preventing enzymatic activity and microbiological growth (Kalia and Gupta, 2012).

2.6 Sources of contamination

A number of things can taint fruit liquids, including masau juice. Fruit juices may get contaminated throughout many phases of manufacturing, processing, and distribution. Masau juice contamination can come from three different kinds of sources: chemical, biological, and physical. Bacteria, viruses, and fungi are examples of biological pollutants. During the harvesting and processing phases, these bacteria may get into the fruit juice. For instance, if the fruits used to make masau juice are not well cleaned or sanitised beforehand, bacteria from the soil, airborne spores, irrigation water, or other environmental sources may be present in them. Harmful germs can also be introduced into the juice by using inadequate hygiene standards when handling and preparing the fruit.

As fruits and vegetables are grown, harvested, and transported to facilities for fruit sorting and juice extraction, they come into contact with a wide range of potentially spoiling bacteria. The majority of bacteria that are first seen on entire fruit surfaces are found in the soil. Yeasts and moulds are frequently present in fruit and fruit juices due to insect damage. Potential sources of microbial contamination include flavourings, water, filling lines, processing equipment, and other chemicals (Wareing and Davenport, 2004; Lawlor et al., 2009). Fresh fruit juices are particularly prone to deterioration because, when being handled, their liquid contents come into contact with air and environmental bacteria. Indicators of raw material quality include lactic acid bacteria, heat-sensitive moulds, and yeasts. For the pasteurization of fruit juice, spore-forming bacteria and other heat-resistant fungi like *Bacillus coagulans* and *Clostridium pasteurianum* are employed as targets (Lima Tribst et al., 2009).

Fruit crops that are subjected to agricultural methods like pesticide and fertiliser application might result in chemical pollutants found in fruit juices. These chemicals' residues could cling to the fruits and taint the juice as it is being extracted. Moreover, lubricants or cleaning agents may contaminate machinery used in processing and juicing if it is not cleaned properly. Foreign materials that could unintentionally get into fruit juice are referred to as physical pollutants. This can contain any extraneous element that might unintentionally mix with the juice during manufacture or packaging, such as pieces of wrapping material or metal fragments from machinery. Good agricultural methods in fruit growth, appropriate sanitation and hygiene procedures during processing, frequent testing for pollutants, and adherence to food safety rules are all necessary to guarantee the safety and purity of masau juice.

2.7 Factors affecting shelf life of juices

Fruit juices have a limited shelf life due to various factors that can affect their quality and safety, such as the type of fruit, storage conditions, processing methods, and the presence of microorganisms. Shelf life is defined as the time to reach a microbial population of 6 log cfu/ml which is determined experimentally (Andres et al. 2001). It can also be defined as the time period within which the food is safe to consume and/or has an acceptable quality to consumers (Man, 2015). The existence of microorganisms, such as bacteria, yeasts, and moulds, can significantly reduce the shelf life of fruit juices. These microorganisms can cause spoilage, which can lead to off-flavours, changes in colour, and even potential health risks. Intrinsic factors include pH, oxidation–reduction potential, water activity, availability of nutrients, and the presence of antimicrobial compounds.

Juice production and storage can be made less contaminated and have a longer shelf life with the use of good sanitation and hygiene practices. Extrinsic factors include storage temperatures and times, relative humidity conditions during storage, and the characteristics of the packaging material. Fruit juice shelf life can be significantly impacted by storage conditions, which include temperature, humidity, and light exposure. Masau juice, like other fruit juices, should be stored in a cool, dark place to guard against bacterial, yeast, and mould deterioration. Extrinsic factors like temperature also affect how long juices last on the shelf. Factors like temperature, time, and heat distribution within the juice must all be taken into account when optimising pasteurization processes in order to achieve microbial safety while maintaining the juice's sensory qualities (Calugar et al., 2021). Proper refrigeration can also help slow down chemical reactions that can degrade the juice's nutritional content and flavour.

In order to ensure that all microorganisms are effectively inactivated, the juice must also have uniform heat distribution (Brown et al., 2019). Freshly squeezed, unpasteurized orange juice has a shelf life of less than 20 days at 1 ˚C. Low temperature storage is necessary during the manufacturing process because it increases the shelf life by slowing down degradatory reactions and limiting microbial growth. Therefore, the combination of reduction in chemical, biochemical, and microbial kinetics can extend the shelf life of both fresh and processed foods (Hartel and Heldman, 1997; Bates et al., 2001; Sandhu and Minhas, 2006; Raccach and Mellatdoust, 2007). The shelf life of Masau juice can be increased by adding preservatives and additives like potassium sorbate and ascorbic acid (vitamin C), which can help inhibit the growth of microorganisms, prevent oxidation, and preserve the juice's freshness and flavour. To guarantee the juice's safety and quality, though, these additives must be used in the right amounts and in accordance with food safety regulations.

2.8 Preservation of fruit juices

Food preservation is the process of preventing the growth of harmful organisms and deterioration (Aneja et al., 2008). Fruit juice preservation is dependent on several factors, including pasteurization, low pH, refrigeration, and preservative addition. Numerous efficient preservation techniques have developed to counteract spoiling, despite the multiplicity of pathways leading to deterioration. Preserving the nutritional value and quality of food while avoiding spoiling is a fundamental tenet of food preservation. Freshly made, unprocessed juice is commonly the standard of excellence because fresher juices are generally of superior quality (Sizer and Balasubramanian, 1999). As mentioned, even in the best of situations, this substance has a very short shelf life of a few hours or days. Pasteurization is one of the main techniques used to preserve masau juice. In order to eradicate hazardous bacteria from juice without drastically altering its flavour or nutritional value, pasteurization entails heating the juice to a particular temperature for a predetermined amount of time (Ağçam et al, 2018). This method aids in preserving the quality of masau juice while prolonging its shelf life.

Preservative addition is another way to keep masau juice fresh. Preservatives like citric acid and ascorbic acid are frequently added to fruit juices to help stop enzymatic browning and slow the growth of bacteria that cause spoiling. But to make sure the juice is safe, preservatives must be used within legal bounds. Masau juice preservation also heavily depends on packaging. The juice should be shielded from light and oxygen exposure, which can cause degradation, by using carefully chosen packaging materials to avoid contamination. In order to keep the juice fresh and avoid microbiological contamination, containers must be sealed properly. Refrigeration is an additional technique for preserving masau juice in addition to these ways. Maintaining the juice at low temperatures prolongs its shelf life by delaying enzymatic reactions and microbiological growth. It is crucial to remember that refrigeration might not be adequate for long-term preservation, particularly in the case of commercially marketed masau juice.

2.8.1 Pasteurization

The process of short-term low-temperature heating of liquids, including wine and beer, to kill unwanted microbes and extend their shelf life was discovered by Louis Pasteur, a French scientist who is credited with coining the term "pasteurization". Thermal pasteurization is a mild form of heat treatment that deactivates relatively heat-sensitive microorganisms, such as moulds, yeasts, and vegetative bacteria that cause food poisoning or food deterioration (Lewis and Heppell, 2000). Pasteurization is a food preservation technique that involves gentle heat treatment, usually at a temperature below 100 ˚C (212 ˚F), to kill germs and extend the shelf life of both packaged and unpackaged goods (such milk and fruit juices). Additionally, spoiling enzymes become dormant. In order to avoid any recontamination, fruit juice is normally pasteurised by heating it to a temperature of between 55 and 95 ˚C for 15 to 30 minutes, then quickly chilling it.

The juice is packaged in a sterile container to preserve its quality and safety until it is consumed, after it has been pasteurised and cooled. This heat treatment prolongs the juice's shelf life and guarantees its safety for ingestion by eliminating dangerous bacteria, yeast, and moulds. Pasteurization also aids in reducing microbial deterioration and enzymatic activity, preserving the juice's sensory attributes. Pasteurization is currently a standard procedure in the dairy industry and other food processing sectors to preserve food and guarantee food safety. Finding the best temperature and time combination to minimise the microbial load and maintain the juice's qualitative characteristics is the key to optimising the pasteurization process for masau juice. Usually, the optimisation procedure entails testing various temperature-time combinations and assessing quality alterations following pasteurization. The efficiency of pasteurization can also be influenced by variables like pH and the inclusion of preservatives, in addition to combinations of temperature and time. A study examined how pasteurization affected the microbial reduction and sensory characteristics of juice in glass and polyethylene terephthalate (PET) bottles (da Costa Ribeiro et al, 2019). Both glass and PET bottles' microbial load significantly decreased after pasteurization, according to the study's findings. On the other hand, compared to PET bottles, glass bottles showed a somewhat greater degree of microbiological decrease. It is possible that some microbes were able to survive the pasteurization procedure due to PET's gas permeability, which accounts for this discrepancy. Regarding the sensory qualities of juice preserved in glass versus PET bottles after pasteurization, the study discovered no statistically significant differences. The sensory qualities of the juice were successfully preserved in both forms of packaging.

2.8.2 Chemical preservatives

Chemical preservatives are additives used to food and drink to keep them fresher longer and stop spoiling. Chemical preservatives are frequently employed in fruit juices, such as masau juice, in order to stop microbial development, stop fermentation, and maintain the juice's original flavour and colour. Preservatives are added to the juice to help ensure that it is safe to consume for an extended amount of time. Fruit-based goods may contain benzoic acids, potassium sorbate, sorbic acids, sulphur dioxide, and p-hydroxyl benzoic acids as preservatives (Bates et al., 2001; ICMSF, 2018). Sorbic acid and benzoic acid are two examples of preservatives that can be used separately or in combination. A prominent natural antioxidant used as a preservative in fruit juices is ascorbic acid, sometimes known as vitamin C. By preventing the enzymes responsible for these reactions from acting, it helps to keep the juice from oxidising and browning. Another naturally occurring substance present in citrus fruits, citric acid, is also employed as a preservative in fruit juices because of its acidic qualities, which aid to decrease the juice's pH and prevent microbial growth. Synthetic preservatives like potassium sorbate and sodium benzoate are frequently used in fruit juices to stop bacteria, mould, and yeast from growing. These preservatives prolong the juice's shelf life by interfering with bacteria' metabolic processes.

Fruit juices are also preserved with sulphites, such as sulphur dioxide, to prevent microbial development and enzymatic browning. Considering the higher solubility of the salts, potassium sorbate and sodium benzoate are the recommended forms. Foods can contain up to 0.1% of potassium sorbate and sodium benzoate (Chipley 2020). Below pH 4.0, both works best. Benzoates are especially effective against mould and yeast, and federal restrictions in the United States limit their use to 0.1 percent. Up to pH of 6.5, sorbic acid works over a wider pH range. Low temperatures and benzoates and sorbates are frequently combined to increase the shelf life of juice drinks with little to no processing (Somogyi, et al., 1996).

Despite the fact that sorbates and benzoates are found in nature, consumers' misconceptions about their safety and the trend towards choosing "all natural" products have made these so-called "chemical preservatives" unpopular. It is misleading to assume that better quality is implied by the phrase "no preservatives added." It is difficult to convince consumers that using these preservatives properly may improve quality and safety while reducing waste. Certain processors are still misusing preservatives. It is overuse of benzoates in particular. The high benzoate content in some pasteurised juices causes a harsh aftertaste. While excess in sufficiently pasteurised juice is not hazardous, it is ironically totally unneeded. In food processing, the adage "if a little is good, more is better" is completely inappropriate. Sanitary practices should always come first, regardless of the preservative used. Fruit juices can be preserved and have their shelf life extended using a variety of methods, chemical preservatives being just one of them (Table 1).

Table 1: Common juice preservatives and additives.

Ingredient	**Use**
Sulphur Dioxide	Retards microbial and enzymatic activity
Benzoates	Antimicrobial at pH <4.5
Sorbates	Antimicrobial at pH <6.5
Carbon Dioxide	pH reduction, anaerobic atmosphere
Ascorbic Acid	Retards enzymatic browning
Dimethylpyrocarbonate	Antimicrobial
Glucono Delta-Lacton	Slowly converts to gluconic acid, reducing pH and acting as a preservative
Niacin	Vitamin B3, provides nutritional value

2.8.3 Refrigeration

Fruit juices, especially masau juice, can be preserved by refrigeration, which is a generally recognised technique. In order to prolong the juice's shelf life, enzymatic processes and microbiological growth are slowed down by storing it at low temperatures. For the storage of masau juice, appropriate packaging is essential in addition to refrigeration. To further maintain the juice's quality, airtight bottles or containers can aid in preventing oxidation and contamination. Making sure the packing material is appropriate for long-term refrigeration and doesn't react with the juice's acidic composition is crucial. In addition, extending the shelf life of masau juice necessitates maintaining constant refrigeration temperatures. Temperature variations have the potential to hasten the juice's degradation and impair its sensory qualities. In order to maintain the quality and safety of masau juice, it is advised to store it at a consistent and suitable refrigeration temperature.

Chapter 3

Materials and methods

3.1 Raw material

Fully ripened fresh masau fruits were collected from Muzarabani. The fruits were transported to the university Innovation hub, where they were manually hand graded to remove damaged and diseased fruits. They were washed thoroughly to remove dirt and rinsed with distilled water. Fresh masau fruits are slimy hence they were sun dried to remove excess moisture.

3.2 Cleaning, sorting and inspection

Begin by thoroughly washing your hands and making sure that all utensils and equipment are clean and properly sanitized. Prior to juicing, the fruits were washed, thoroughly inspected and sized (fruit-dependent). Inspection and removal of defective or spoiled fruits was crucial, during the production process of masau juice. In solid packs one bad piece of fruit can cause a defect in one container, but after juicing that same piece of defective fruit can end up contaminating an entire lot of juice. Inspection was done manually, observing and removing spoiled fruits (Figure 4).

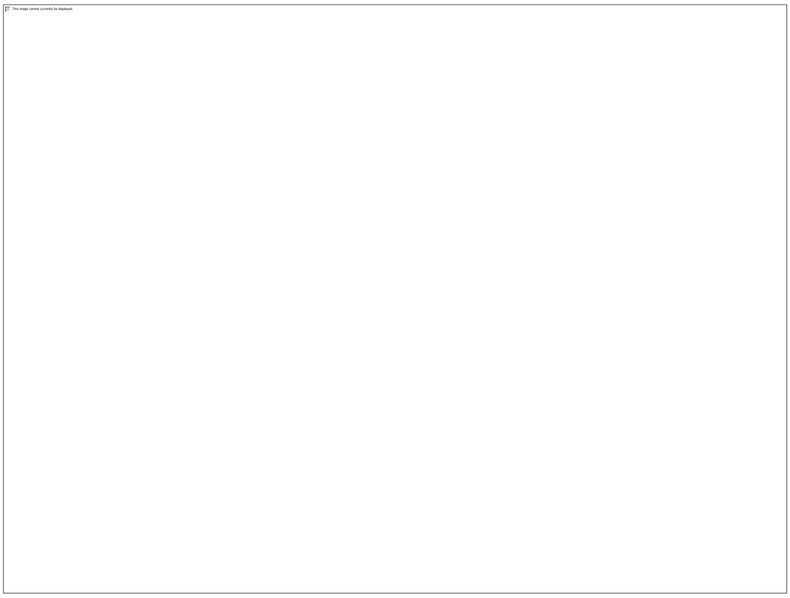

Figure 4: Grading of masau fruits.

3.2.1 Preparing masau juice

The juice was made using a protocol developed at Bindura University of Science Education. The protocol involved transforming the fruits into a liquid form and blending it with some additives as well as preservatives. A thermometer was inserted into the juice to monitor its temperature throughout the process (Figure 5).

Figure 5: Monitoring masau juice temperature.

3.2.2 Pasteurization

The objective of pasteurization was to ensure the safety of the juice by destroying harmful, yeasts and molds while preserving its quality attributes. The experiment involved a batch of masau juice bottles with consistent physical attributes (size and shape) and juice content. A water bath method was used to pasteurize the juice. The batch was split into two groups, experimental and control groups ensuring that both groups are identical in all aspects. This was to ensure that any differences observed between the groups could be attributed to the pasteurization process. The filled and sealed bottles from the experimental group were gently placed into the pot. The water was heated gradually until it reached the required pasteurization temperature (55,65,72,84 and 95 ^oC). Once the desired temperature was reached, it was maintained for a specific time period (15, 20 and 30 minutes) using a thermometer to monitor. Three replicas were used for each condition to ensure reliable results. During the pasteurization of masau juice, the control group involved not subjecting any bottles to the heating process. The control bottles were handled in the same way as the experimental bottles, except that they were not exposed to heat.

3.2.2.1 Pasteurization at 55, 65, 72, 84 and 95 ^oC

A water bath temperature was raised and maintained at 55, 65, 72, 84 and 95 ^oC with varying durations 15, 20 and 30 minutes. Four containers were taken out of the water bath after the allotted fifteen minutes had passed and the temperature had been sustained by regulating the gas stove flame. The containers were let to cool at room temperature and tagged with masking tape with the bottle number, the temperature, and the pasteurization period as well as the date the experiment was conducted. Following the same procedures as for the first fifteen minutes, four containers were taken out after twenty minutes. The pasteurization process was done in the same way after thirty minutes.

3.2.2.2 Control

The control group samples of masau juice involved a group of bottles that were not subjected to the heating process (pasteurization), but were handled in the same manner as the experimental group samples in terms of filling, capping and packaging. This setup allowed a direct comparison between the effects of pasteurization (heating) and those of other factors such as handling and storage. Three replicas were made for the control samples.

3.3 Storage

The control and experimental group samples were both stored under identical conditions, ensuring consistent temperature, humidity, and light exposure to minimize external variables affecting the juice. The experimental group, which consisted of the juice samples undergoing different pasteurization treatments, was stored in a cupboard under controlled conditions. Similarly, the control group, which served as a baseline for comparison with the experimental group, was also stored in a cupboard following similar guidelines to ensure consistency and accuracy in the results obtained. Exposure to light could lead to degradation of nutrients and changes in colour and flavour of the juice. Therefore, storing the samples in a dark cupboard helped in preserving the quality of the juice during storage (Figure 6).

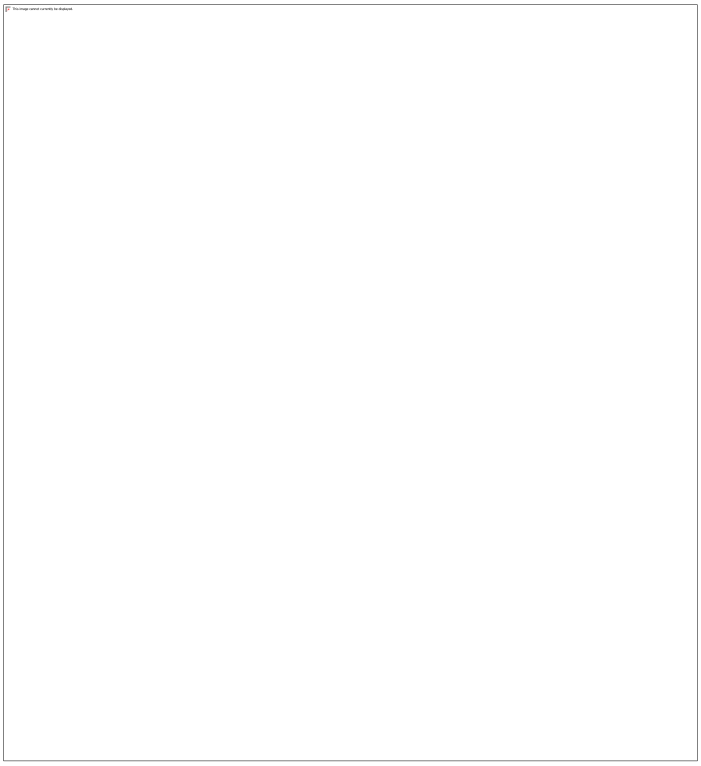

Figure 6: Masau juice in a cupboard

3.4 Monitoring

Periodic inspections were conducted at two-week intervals to track changes and ensure storage stability. This allowed the comparison of the effects of different pasteurization conditions on the juice and to identify the most effective method. During the monitoring process, several parameters were evaluated including pH level, colour and appearance, and sensory attributes through a structured sensory evaluation involving a panel of assessors. Panelists evaluated attributes using a scoring system, which allowed them to rate each characteristic on a defined scale. Data from their scores and qualitative feedback were then collected and analyzed to identify trends and significant differences among the samples, providing comprehensive insights into the sensory qualities of the masau juice.

3.5 Data analysis

To assess the impact of varying pasteurisation temperatures and durations on the physicochemical properties, nutritional value, and sensory features of masau juice, an analysis of variance (ANOVA) was performed in R Studio. Each row in the dataframe represented a juice sample, together with the measured results and accompanying pasteurisation settings. The dependent variables and independent factors of the ANOVA model were specified using the aov function.

Chapter 4

Results

4.1 Effects of temperature on pasteurization of masau juice

The experiment investigated the impact of various temperature ranges on the pasteurization of masau juice. Five different temperatures (55, 65, 72, 84, and 95 ˚C) were evaluated for their efficacy in eliminating harmful microorganisms while preserving the sensory and nutritional qualities of the juice.

4.1.1 Taste

Unpasteurized masau juice

At 2 weeks

After two weeks of storage, the taste of the juice began to change slightly (Table 3). The taste became less sweet and more acidic, as the additive may have altered the balance of natural sugars and acids in the juice.

At 4 weeks

After four weeks of storage, the taste changes became more pronounced. The sweetness continued to decrease, while the acidity became more noticeable. Additionally, the juice developed a slightly astringent or bitter taste due to the chemical reactions between the additive and the juice components.

At 6 weeks

By six weeks of storage, the taste changes became even more significant. The potassium sorbate may have effectively prevented spoilage, but the juice's flavour may have been altered considerably. The sweetness became minimal, and the acidity became quite pronounced. The astringency or bitterness also increased, which made the juice less palatable.

Pasteurized masau juice

At 55 ˚C

2 Weeks: At this temperature, the masau juice exhibited a slightly tangy and refreshing taste with subtle sweetness (Table 3).

4 Weeks: The taste became more pronounced with a balance of sweetness and acidity, providing a pleasant drinking experience.

6 Weeks: The flavour profile remained stable, maintaining its refreshing quality with a slightly enhanced sweetness.

At 65 ˚C

2 Weeks: The juice displayed a well-rounded taste with a harmonious blend of sweetness and acidity.

4 Weeks: The flavour intensified, becoming richer in sweetness while retaining its underlying tanginess.

6 Weeks: Despite some slight changes in intensity, the overall taste remained appealing and balanced.

At 72 ˚C

2 Weeks: The juice had a vibrant taste profile with a notable sweetness that was well complemented by a hint of acidity.

4 Weeks: The sweetness became more prominent, overshadowing the acidity slightly but still maintaining a pleasant flavour.

6 Weeks: There was a slight decline in freshness, but the sweetness remained dominant, albeit losing some complexity.

At 84 ˚C

2 Weeks: The juice exhibited a strong sweet flavour with minimal acidity, providing a rich and indulgent taste experience.

4 Weeks: The sweetness intensified further, almost masking the acidity entirely and resulting in a very sweet profile.

6 Weeks: The taste remained overly sweet with diminished freshness compared to earlier time points.

At 95 ˚C

2 Weeks: The juice had an overpowering sweetness that dominated the palate, lacking balance with any noticeable acidity.

4 Weeks: The intense sweetness persisted, overwhelming any other flavors present in the juice.

6 Weeks: The taste profile deteriorated significantly, becoming cloyingly sweet and losing most of its original characteristics.

Table 2: Impact of pasteurization temperature and duration on juice's sensory properties.

Juice Type	Time Period (Weeks)	Taste Description
Unpasteurized	2	Less sweet, more acidic
	4	Sweetness decreased, acidity and astringency/bitterness increased
	6	Minimal sweetness, pronounced acidity, increased astringency/bitterness
Pasteurized at 55°C	2	Slightly tangy, refreshing taste with subtle sweetness
	4	Balanced sweetness and acidity, pleasant drinking experience
	6	Refreshing quality with slightly enhanced sweetness
Pasteurized at 65°C	2	Well-rounded taste with harmonious blend of sweetness and acidity
	4	Richer sweetness, retaining underlying tanginess
	6	Slight changes in intensity, overall appealing and balanced
Pasteurized at 72°C	2	Vibrant taste with notable sweetness and hint of acidity
	4	Sweetness more prominent, overshadowing acidity slightly
	6	Slight decline in freshness, sweetness remained dominant
Pasteurized at 84°C	2	Strong sweet flavour with minimal acidity, rich and indulgent
	4	Sweetness intensified, almost masking acidity entirely
	6	Overly sweet with diminished freshness

Pasteurized at		
95°C	2	Overpowering sweetness, lacking balance with acidity
	4	Intense sweetness persisted, overwhelming other flavours
	6	Cloyingly sweet, significant deterioration of original characteristics

4.1.2 Colour

Unpasteurized masau juice

After 2 Weeks

At the end of two weeks, the color of the unpasteurized masau juice with potassium sorbate added as a preservative remained relatively stable (brown). The potassium sorbate prevents the growth of microorganisms responsible for spoilage and color degradation, thus maintaining the juice's freshness and color (Table 4).

After 4 weeks

After four weeks, some minor color changes began to occur. The natural aging process of the juice may have caused subtle color shifts. The juice became slightly darker or developed a more intense hue. However, these changes were minimal, and the overall appearance of the juice was acceptable.

After 6 Weeks

By the end of six weeks, the color changes in the unpasteurized masau juice with potassium sorbate as an additive was more noticeable. The juice appeared darker and more intense in color.

Pasteurized masau juice

Color changes in masau juice due to pasteurization (55, 65, 72, 84 and 95 ˚C) at different time intervals.

Pasteurization for 15 minutes

After 2 weeks: The juice exhibited a slightly darker appearance compared to its original color (brown), as the heat may have caused some enzymatic reactions to take place, leading to the breakdown of pigments (Table 4).

After 4 weeks: The color change became more noticeable, with the juice appearing darker and potentially more concentrated in color.

After 6 weeks: The juice experienced a significant color change, with the juice appearing significantly darker than its original color.

Pasteurization for 20 minutes

After 2 weeks: The juice exhibited a similar color change as the 15-minute pasteurization, but the darkening of the color became more pronounced (Table 5).

After 4 weeks: The color change became more apparent, with the juice appearing darker and potentially more concentrated in color.

After 6 weeks: The color change was more pronounced, with a darker and more intense hue than at the previous time durations.

Pasteurization for 30 minutes

After 2 weeks: The juice exhibited a similar color change as the 15 and 20 minute pasteurizations, but the darkening of the color became more pronounced (Table 6).

After 4 weeks: The color change continued, with the juice appearing significantly darker than its original color.

After 6 weeks: The color change was more pronounced, with the juice exhibiting a deeper and darker hue.

Table 3: Colour changes in unpasteurized and pasteurized juice (15 minutes).

Juice Type	**2 Weeks**	**4 Weeks**	**6 Weeks**
Unpasteurized with potassium sorbate	Relatively stable, brown colour	Slight darkening or more intense hue	Noticeable darkening, more intense colour
Pasteurized at 55 ˚C for 15 minutes	Slightly darker appearance	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 65 ˚C for 15 minutes	Slightly darker appearance	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 72 ˚C for 15 minutes	Slightly darker appearance	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 84 ˚C for 15 minutes	Slightly darker appearance	Darker, more concentrated colour	Significantly darker colour

Pasteurized at 95 ˚C for 15 minutes	Slightly darker appearance	Darker, more concentrated colour	Significantly darker colour

Table 4: Colour changes in pasteurized juice (20 minutes).

Juice Type	**2 Weeks**	**4 Weeks**	**6 Weeks**
Pasteurized at 55 ˚C for 20 minutes	More pronounced darkening	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 65 ˚C for 20 minutes	More pronounced darkening	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 72 ˚C for 20 minutes	More pronounced darkening	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 84 ˚C for 20 minutes	More pronounced darkening	Darker, more concentrated colour	Significantly darker colour
Pasteurized at 95 ˚C for 20 minutes	More pronounced darkening	Darker, more concentrated colour	Significantly darker colour

Table 5: Colour changes in pasteurized juice (30 minutes).

Juice Type	2 Weeks	4 Weeks	6 Weeks
Pasteurized at 55 ˚C for 30 minutes	More pronounced darkening	Significantly darker colour	Deeper, darker hue
Pasteurized at 65 ˚C for 30 minutes	More pronounced darkening	Significantly darker colour	Deeper, darker hue
Pasteurized at 72 ˚C for 30 minutes	More pronounced darkening	Significantly darker colour	Deeper, darker hue
Pasteurized at 84 ˚C for 30 minutes	More pronounced darkening	Significantly darker colour	Deeper, darker hue
Pasteurized at 95 ˚C for 30 minutes	More pronounced darkening	Significantly darker colour	Deeper, darker hue

4.1.3 pH changes

Unpasteurized masau juice

The pH of unpasteurized juice decreased slightly over time. The values indicate a gradual decrease in pH, suggesting a slight increase in acidity over the course of six weeks (Table 7).

Table 6: pH changes of unpasteurized masau juice.

Week	pH
2	3.97
4	3.88
6	3.84

At production the pH of masau juice was measured 3.95. The pH values for pasteurized juices were measured at three different time points: 15 minutes, 20 minutes, and 30 minutes. Additionally, the pH measurements were taken at three different time intervals: 2 weeks, 4 weeks, and 6 weeks after the initial treatment (Table 8).

Table 7: Displays the variations in pH of masau juice at various temperatures and times throughout its storage.

Week	Time (Minutes)	55 ˚C	65 ˚C	72 ˚C	84 ˚C	95 ˚C
2	15	3.99	3.98	3.99	3.96	3.97
	20	3.95	3.97	3.96	3.95	3.95
	30	3.98	3.96	3.94	3.94	3.93
4	15	3.95	3.94	3.94	3.96	3.97
	20	3.94	3.92	3.96	3.94	3.97
	30	3.93	3.92	3.94	3.92	3.96
6	15	3.88	3.9	3.9	3.85	3.91
	20	3.92	3.92	3.86	3.88	3.87
	30	3.9	3.84	3.65	3.88	3.86

4.2 Sensory evaluation of pasteurized and unpasteurized masau juice

The (Tables 9 to 13) shows the means of sensory attributes appearance, taste, colour, consistency and overall acceptability for pasteurized and unpasteurized masau juice. The juice pasteurized at 72 °C for 15 minutes at 2 weeks had the highest acceptance score of 6.1 on a scale ranging from 1 (extremely dislike) to 7 (extremely like) according to the hedonic score. The lowest overall acceptability score across all the conditions is 1.3, which occurs at 4 weeks and 30 minutes for the 95 °C temperature on (Table 13).

Table 8: Effect of holding time on sensory attributes of masau juice at 55 ˚C.

Weeks	**Time (minutes)**	**Appearance**	**Taste**	**Colour**	**Consistency**	**Overall Acceptability**
2	15	5.2	5.1	5.0	5.0	5.1
	20	4.3	4.2	4.1	4.2	4.2
	30	3.4	3.3	3.2	3.1	3.3
4	15	4.8	4.6	4.5	4.5	4.6
	20	3.9	3.7	3.6	3.7	3.7
	30	2.9	2.8	2.7	2.6	2.8
6	15	4.4	4.2	4.1	4.0	4.2
	20	3.5	3.3	3.2	3.2	3.3
	30	2.4	2.3	2.2	2.1	2.3

Table 9: Effect of holding time on sensory attributes of masau juice at 65 ˚C.

Weeks	Time (minutes)	Appearance	Taste	Colour	Consistency	Overall Acceptability
2	15	5.4	5.3	5.2	5.1	5.3
	20	5.3	5.2	5.1	5.0	5.2
	30	4.4	4.3	4.2	4.1	4.3
4	15	5.0	4.9	4.7	4.6	4.8
	20	4.9	4.7	4.6	4.5	4.7
	30	4.0	3.8	3.7	3.6	3.8
6	15	4.6	4.5	4.3	4.2	4.4
	20	4.5	4.3	4.2	4.0	4.3
	30	3.6	3.4	3.3	3.1	3.4

Table 10: Effect of holding time on sensory attributes of masau juice at 72 ˚C.

Weeks	Time (minutes)	Appearance	Taste	Colour	Consistency	Overall Acceptability
2	15	6.2	6.1	6.0	6.0	6.1
	20	5.3	5.2	5.1	5.0	5.2
	30	5.1	5.0	4.9	4.8	5.0
4	15	5.8	5.7	5.5	5.5	5.7
	20	4.9	4.7	4.6	4.5	4.7
	30	4.6	4.5	4.4	4.3	4.5
6	15	5.3	5.2	5.0	5.0	5.2

20	4.5	4.3	4.2	4.0	4.3
30	4.2	4.0	3.9	3.8	4.0

Table 11: Effect of holding time on sensory attributes of masau juice at 84 ˚C.

Weeks	**Time (minutes)**	**Appearance**	**Taste**	**Colour**	**Consistency**	**Overall Acceptability**
2	15	4.2	4.1	4.0	3.9	4.1
	20	3.3	3.2	3.1	3.0	3.2
	30	3.1	3.0	2.9	2.8	3.0
4	15	3.7	3.6	3.5	3.4	3.6
	20	2.8	2.7	2.6	2.5	2.7
	30	2.6	2.5	2.4	2.3	2.5
6	15	3.2	3.1	3.0	2.9	3.1
	20	2.3	2.2	2.1	2.0	2.2
	30	2.1	2.0	1.9	1.8	2.0

Table 12: Effect of holding time on sensory attributes of masau juice at 95 ˚C.

Weeks	**Time (minutes)**	**Appearance**	**Taste**	**Colour**	**Consistency**	**Overall Acceptability**
2	15	3.0	2.9	2.8	2.7	2.9
	20	2.1	2.0	1.9	1.8	2.0
	30	1.9	1.8	1.7	1.6	1.8
4	15	2.5	2.4	2.3	2.2	2.4

	20	1.6	1.5	1.4	1.3	1.5
	30	1.4	1.3	1.2	1.1	1.3
6	15	3.9	3.8	3.7	3.6	3.8
	20	3.1	3.0	2.9	2.8	3.0
	30	2.0	1.9	1.8	1.7	1.9

Table 13: Sensory evaluation of unpasteurized masau juice.

Weeks	**Appearance**	**Taste**	**Colour**	**Consistency**	**Overall Acceptability**
2	6.0	5.8	6.2	5.6	5.9
4	5.4	4.8	5.2	4.6	5.0
6	4.2	3.8	4.4	3.6	4.0

4.3 Sample with highest shelf life

Based on the results, the optimum conditions for pasteurization of masau juice involved a balance between microbial safety and sensory preservation. Pasteurization at 72 ˚C for 15 minutes emerged as a promising compromise, achieving satisfactory microbial reduction while minimizing detrimental effects on flavour and color. Determining the optimal conditions for pasteurization of masau juice is essential to balance microbial safety with sensory and nutritional quality. The study investigated various temperature-time combinations to identify conditions that achieve adequate microbial reduction while minimizing detrimental effects on juice attributes. Based on the findings, pasteurization at 72 ˚C for 15 minutes emerged as a promising compromise. This temperature-time combination demonstrated satisfactory microbial safety by effectively minimizing negative impacts on flavour, color, and nutritional content. The choice of 72 ˚C as the pasteurization temperature was informed by its ability to achieve microbial safety without causing excessive degradation of heat-sensitive compounds.

Chapter 5

Discussion, summary, recommendations and conclusions

5.1 Discussion

5.1.1 Sensory and nutritional quality

In terms of sensory and nutritional quality, prolonged pasteurization periods led to more pronounced changes in flavour and color, particularly at elevated temperatures. Juice samples subjected to longer pasteurization durations exhibited diminished flavour complexity and darker coloration, indicating potential degradation of heat-sensitive compounds.

Pasteurization at temperatures above 72 ˚C resulted in changes in color, flavour, and overall sensory attributes of the juice. Consumers may perceive differences in taste and aroma between pasteurized and non-pasteurized juice, which could influence their preferences and purchasing decisions. Additionally, changes in color intensity may impact the visual appeal of the juice, influencing consumer perception of its freshness and quality.

The findings underscore the importance of balancing microbial safety with sensory preservation during pasteurization optimization. Future research efforts could focus on exploring alternative pasteurization methods or adjunct technologies to minimize the impact on sensory attributes while ensuring microbial safety. By optimizing pasteurization conditions, it is possible to achieve a delicate balance between safety and quality, thereby enhancing the overall consumer acceptance and marketability of masau juice. However, it was observed that pasteurization at temperatures above 72 ˚C resulted in noticeable changes in sensory attributes such as color and flavour. The delicate flavour compounds of masau juice were particularly affected at higher temperatures, leading to alterations in taste profile and color intensity. This finding is in line with the limitations identified in Chapter 1, highlighting the challenge of balancing microbial safety with sensory preservation.

The exploration into the impact of different pasteurization periods on the sensory and nutritional quality of masau juice revealed important considerations regarding flavour complexity, color intensity, and nutrient retention. Prolonged pasteurization periods, especially at higher temperatures, were found to have noticeable effects on the sensory attributes of masau juice. Flavor complexity, which is a key component of consumer acceptability, was observed to diminish with increasing pasteurization duration. The delicate balance of sweet and tangy notes characteristic of masau juice was compromised, resulting in a loss of flavour depth and nuance. This decline in flavour complexity may diminish the overall sensory appeal of the juice and affect consumer preferences. Furthermore, changes in color intensity were evident following extended pasteurization periods. Masau juice subjected to prolonged heating exhibited darker hues, potentially due to the degradation of pigments and Mallard browning reactions. The visual appeal of the juice may be compromised as a result, as consumers often associate brighter, vibrant colors with freshness and quality. Thus, alterations in color intensity could influence consumer perception and purchasing behavior.

In addition to sensory attributes, the nutritional quality of masau juice may also be impacted by prolonged pasteurization. Heat-sensitive nutrients such as vitamins and antioxidants may undergo degradation or loss during extended heating, leading to reduced nutritional content in the final product. This is particularly relevant for juices marketed for their health benefits, as consumers expect them to retain their nutritional integrity. It is worth noting that while prolonged pasteurization periods may enhance microbial safety, they come at the expense of sensory and nutritional quality. Therefore, there is a delicate balance to be struck between ensuring microbiological safety and preserving the sensory and nutritional attributes of masau juice. Future research endeavours could explore alternative pasteurization methods or processing techniques aimed at minimizing the impact on sensory and nutritional quality. Techniques such as pulsed electric fields or high-pressure processing may offer viable alternatives to traditional thermal pasteurization, preserving flavour complexity and nutrient content while ensuring microbial safety.

5.1.2 Hypothesis testing

The hypothesis testing conducted in the study aimed to assess the significance of differences between pasteurized and non-pasteurized masau juice in terms of quality, taste, and color. The hypotheses were formulated and subjected to statistical analysis to determine its validity. Hypothesis testing serves as a critical tool for evaluating the impact of pasteurization on masau juice quality, taste, and color. By formulating the hypotheses and subjecting it to rigorous statistical analysis, the study aimed to elucidate the significance of differences between pasteurized and non-pasteurized juice samples. The hypothesis suggested that there would be a significant difference between pasteurized and non-pasteurized masau juice in terms of quality, taste, and color. Statistical analysis conducted to evaluate this hypothesis corroborated the observed sensory and color disparities between pasteurized and non-pasteurized juice samples. The hypothesis was supported by the statistical findings, affirming that pasteurized masau juice differed significantly from its non-pasteurized counterpart in sensory attributes and color characteristics. These results provided empirical evidence of the influence of pasteurization on juice quality and lent support to the notion that thermal processing affects the sensory perception and visual appeal of masau juice. In summary, hypothesis testing played a pivotal role in elucidating the effects of pasteurization on masau juice quality, taste, and color. The study underscored the importance of considering pasteurization effects in the development and assessment of masau juice products. These findings have implications for ensuring consumer acceptance and satisfaction with pasteurized masau juice offerings.

5.1.3 Optimum conditions for pasteurization

A pasteurization duration of 15 minutes was selected based on its efficacy in microbial reduction and its potential to preserve sensory and nutritional quality. Extending the pasteurization period beyond 15 minutes did not yield significant improvements in microbial safety, suggesting that shorter durations may suffice for achieving desired microbiological outcomes. By selecting 72 ˚C for 15 minutes as the optimal pasteurization conditions, producers can strike a balance between microbial safety and product quality. This temperature-time combination offers effective pathogen inactivation while minimizing adverse effects on flavour, color, and nutrient content. However, it is important to note that the optimal pasteurization conditions may vary depending on factors such as juice composition, initial microbial load, and processing equipment. Therefore, continuous monitoring and optimization of pasteurization protocols are necessary to ensure consistent product quality and safety.

Future research endeavours could explore alternative pasteurization methods or processing parameters to further refine the optimization process. Techniques such as pulsed electric fields or high-pressure processing may offer viable alternatives for achieving microbial safety while preserving sensory and nutritional attributes. Furthermore, ongoing research efforts could delve deeper into understanding the specific mechanisms underlying the effects of pasteurization on masau juice quality. Investigating the kinetics of heat-induced reactions and the degradation pathways of heat-sensitive compounds would provide valuable insights into optimizing pasteurization protocols. Additionally, exploring novel preservation techniques, such as non-thermal technologies or natural antimicrobial agents, could offer alternative approaches to ensuring microbial safety while preserving sensory and nutritional attributes. These innovative methods may offer advantages in terms of efficiency, sustainability, and consumer acceptability. Moreover, conducting sensory studies with a broader range of consumer panels could provide a more comprehensive understanding of how pasteurization affects consumer perception and preference. By incorporating diverse demographic groups and cultural perspectives, researchers can ensure that pasteurized masau juice meets the preferences of a wider audience. Furthermore, investigating the stability and shelf life of pasteurized masau juice under different storage conditions would be crucial for ensuring product quality and safety over time. Understanding the factors that influence product stability, such as packaging materials and storage temperatures, can inform recommendations for optimal storage practices.

5.2 Summary

The masau fruit is valued for its distinct flavour and nutritional makeup, which makes it a desirable option for juice manufacturing. In the processing chain, pasteurization is a crucial step to guarantee safety and prolong the juice's shelf life. The pasteurization procedure for masau fruit juice was optimised to preserve its excellent qualities and guarantee microbial safety. The objective of this research was to examine how various pasteurization factors, including temperature and duration, affect the physicochemical characteristics, nutritional value, and sensory aspects of masau juice. Fresh masau fruits were collected, washed, and dried to remove excess moisture. After that, the fruits were physically inspected, graded, and juiced in accordance with a method created at Bindura University of Science Education. After that, the juice was pasteurised in a water bath for varying lengths of time (15, 20, and 30 minutes) and at various temperatures (55, 65, 72, 84, and 95 ˚C). Juice samples were also prepared for a control group, which did not undergo pasteurization. In order to assess the effects of the various pasteurization procedures, the experimental and control groups were both kept in constant storage settings and observed over time for changes in pH, colour, appearance, and sensory characteristics. The study examined the impact of pasteurization on the sensory and nutritional quality of masau juice. Longer pasteurization times resulted in darker colouring, a reduction in flavour richness, and maybe even the breakdown of heat-sensitive substances, especially at higher temperatures. The sensory qualities of the juice were noticeably altered by higher temperatures, even if they improved microbiological safety. The results highlighted the difficulty of striking a balance during pasteurization optimisation between sensory preservation and microbiological safety. To reduce the influence on sensory qualities while maintaining microbiological safety, future study could concentrate on investigating alternate pasteurization techniques or technologies, which would improve masau juice's overall acceptability. The process of pasteurization is an essential measure to guarantee the microbiological safety of masau juice. However, it poses certain difficulties in maintaining the juice's nutritional and sensory attributes. It is feasible to achieve microbial inactivation while maintaining desired sensory qualities by carefully choosing the right pasteurization parameters, such as temperature and duration. This well-rounded strategy can improve the final pasteurised masau juice product's overall quality and consumer acceptability.

5.3 Recommendations for future research

Future research could explore alternative pasteurization methods or combinations of temperature and time to optimize microbial safety and sensory quality of masau juice. Additionally, investigating the impact of different preservatives and processing techniques on juice stability and shelf life would further enhance our understanding of masau juice preservation.

1. Exploration of Alternative Preservation Techniques

Future research endeavours could explore alternative preservation techniques beyond traditional thermal pasteurization. Non-thermal technologies such as high-pressure processing, pulsed electric fields, and ultraviolet light treatment offer promising avenues for achieving microbial safety while minimizing the impact on sensory and nutritional quality. Investigating the efficacy of these methods in preserving masau juice attributes could provide valuable insights for industry adoption.

2. Characterization of Heat-Resistant Microorganisms

Understanding the heat resistance profiles of specific microbial strains present in masau juice is essential for designing effective pasteurization protocols. Future research could focus on characterizing the heat resistance of common spoilage organisms and pathogens associated with masau fruit. This knowledge could inform the development of targeted pasteurization strategies to ensure adequate microbial safety.

3. Optimization of Pasteurization Parameters

Further optimization of pasteurization parameters, such as temperature, time, and holding conditions, is warranted to achieve the desired balance between microbial safety and product quality. Conducting systematic studies to evaluate the effects of varying pasteurization conditions on masau juice attributes would facilitate the identification of optimal processing parameters tailored to specific product requirements.

4. Investigation of Natural Preservatives

Exploring the potential of natural preservatives, such as plant-derived antimicrobial compounds or essential oils, could offer sustainable alternatives to synthetic preservatives. Future research could investigate the efficacy of these natural ingredients in inhibiting microbial growth in masau juice while preserving its sensory and nutritional properties. Additionally, studying the synergistic effects of combining natural preservatives with mild heat treatment could enhance the overall safety and shelf life of masau juice.

5. Consumer Preference Studies

Conducting consumer preference studies across diverse demographic groups and cultural contexts would provide valuable insights into the sensory preferences and acceptance of pasteurized masau juice products. Future research could employ sensory evaluation techniques, consumer surveys, and focus group discussions to understand consumer perceptions of pasteurized masau juice and identify factors influencing purchase decisions.

6. Evaluation of Shelf Life and Storage Conditions

Investigating the shelf life and stability of pasteurized masau juice under different storage conditions is essential for ensuring product quality and safety throughout its intended shelf life. Future research could assess the effects of temperature, packaging materials, and storage atmospheres on the sensory, nutritional, and microbial attributes of masau juice over time. This knowledge could inform recommendations for optimal storage practices and packaging solutions to extend the shelf life of pasteurized masau juice products.

5.4 Conclusions

In conclusion, pasteurization is essential for ensuring the microbial safety of masau juice, although it poses challenges in preserving its sensory and nutritional qualities. By carefully selecting pasteurization conditions, such as temperature and duration, it is possible to achieve a balance between microbial safety and sensory attributes, thereby enhancing the overall quality of masau juice.

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Appendices

Appendix A: ANOVA

Analysis of variance for different weeks at 55 ˚C

2 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Week	1	6.661	6.661	657.9	1.86e-10	***
Residuals	10	0.101	0.010			

4 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
W	1	6.845	6.845	417	1.75e-09	***
Residuals	10	0.164	0.016			

6 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
W	1	6.845	6.845	417	1.75e-09	***
Residuals	10	0.164	0.016			

Analysis of variance for sensory attributes of masau juice at 65 ˚C pasteurization

2 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Wee	1	2.0000	2.0000	34.68	0.000153	***
Residuals	10	0.5767	0.0577			

4 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Wk	1	2.1013	2.1013	31.11	0.000235	***
Residuals	10	0.6754	0.0675			

6 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
E	1	2.205	2.2050	30	0.00027	***
Residuals	10	0.735	0.0735			

Analysis of variance of sensory attributes for masau juice pasteurized at 72 ˚C

2 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Week	1	2.5313	2.5313	52.96	2.67e-05	***

```
Residuals   10 0.4779  0.0478
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4 weeks

```
             Df Sum Sq Mean Sq F value   Pr(>F)
Week          1 2.7613  2.7613   49.72 3.49e-05 ***
Residuals    10 0.5554  0.0555
```

6 weeks

```
            Df Sum Sq Mean Sq F value   Pr(>F)
Week         1 2.7613  2.7613   49.72 3.49e-05 ***
Residuals   10 0.5554  0.0555
```

Analysis of variance of sensory attributes for masau juice pasteurized at 84 ˚C

2 weeks

```
            Df Sum Sq Mean Sq F value  Pr(>F)
week         1  2.645  2.6450   50.38 3.3e-05 ***
Residuals   10  0.525  0.0525
```

4 weeks

```
             Df Sum Sq Mean Sq F value  Pr(>F)
Week          1 2.4200  2.4200   50.77 3.2e-05 ***
Residuals    10 0.4767  0.0477
```

6 weeks

```
             Df Sum Sq Mean Sq F value  Pr(>F)
K             1 2.4200  2.4200   50.77 3.2e-05 ***
Residuals    10 0.4767  0.0477
```

Analysis of variance for sensory attributes of masau juice pasteurized at 95 ˚C over storage duration (2, 4 and 6 weeks)

2 weeks

```
            Df Sum Sq Mean Sq F value  Pr(>F)
Week         1 2.4200  2.4200   50.77 3.2e-05 ***
Residuals   10 0.4767  0.0477
```

4 weeks

```
             Df Sum Sq Mean Sq F value  Pr(>F)
Week          1 2.4200  2.4200   50.77 3.2e-05 ***
Residuals    10 0.4767  0.0477
```

6 weeks

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Week	1	7.22	7.220	343.8	4.49e-09	***
Residuals	10	0.21	0.021			

Analysis of variance for sensory evaluation of unpasteurized masau juice

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Week	1	7.220	7.220	71.72	7.13e-06	***
Residuals	10	1.007	0.101			