DEVELOPMENT OF ANTIOXIDANT-RICH FRUIT BEVERAGE

Dissertation submitted by

Ms. ARYA. B

(REG NO. VM23FPT007)

ST. TERESA'S COLLEGE (Autonomous), ERNAKULAM
Mahatma Gandhi University, Kottayam, Kerala

In partial fulfilment of the requirement for the award of degree of

MASTER OF VOCATION
IN
FOOD PROCESSING TECHNOLOGY

Under the Guidance of

Mrs. AMUDHA SENTHIL
Sr. Principal Scientist
Department of Traditional Food and Applied Nutrition

Co Guide

Mr. ANANT CHINTAMANI GAHIRE
Scientist
Department of Fruit and Vegetable Technology

CSIR - CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE

MYSURU-570020

APRIL 2025

ST.TERESA'S COLLEGE, (AUTONOMOUS)
ERNAKULAM

Re- Accredited by NAAC in the 4th cycle with grade A++ and CGPA 3.57
Ranked 41st in National Institutional Ranking Framework (NIRF 2023)

ANNEXURE I

UNDERTAKING BY THE SELECTED STUDENT
FOR PROJECT WORK AT CSIR-CFTRI

The project students and their college / staff members shall have no claim whatsoever on the results of the project work. CSIR-CFTRI retains all Intellectual Property Rights in patents, designs, software, copyright and publications, if any that may be generated during the course of project work. In the event of any dispute arising, the decision of Director, CSIR-CFTRI, is final and is binding on the student(s) and college. (Sl. No. 17 of Annexure I)

The college certificate in the project report prescribed by college / University shall categorically reflect that the place of project work is CSIR-CFTRI and acknowledge the name of the staff member who has guided. (Sl. No. 18 of Annexure I)

The successful execution of the project work by the students is solely under the guidance of scientific/technical guide of CFTRI. **No interference from the College/University is entertained.**

We have read the prescribed rules and regulations (Sl. Nos. 1 to 18 of Annexure I) and we agree to abide by them during our project work at CFTRI, Mysore and to the Conduct and Discipline mandate of the Institute.

22/9/2024
ARYA. B

ST. TERESA'S COLLEGE AUTONOMOUS
ERNAKULAM-682 011

Dr. ALPHONSA VIJAYA JOSEPH
PRINCIPAL
ST. TERESA'S COLLEGE
Autonomous
ERNAKULAM

DECLARATION

I ARYA. B, hereby declare that the report on the project entitled '**DEVELOPMENT OF ANTIOXIDANT-RICH FRUIT BEVERAGE**' submitted by me to St. Teresa's College (Autonomous), Ernakulam, affiliated to Mahatma Gandhi University, Kottayam, Kerala, for the partial fulfilment of the requirement for the award of degree of M.Voc in Food Processing Technology is the record of the original work carried out by me under the guidance of Mrs. AMUDHA SENTHIL, Principal Scientist, Department of Traditional Food and Applied Nutrition, and with the co-guidance of Mr. ANANT CHINTAMANI GAHIRE, Scientist, Department of Fruit and Vegetable Technology, CSIR-Central Food Technological Research Institute, Mysuru, Karnataka. I further declare that this project work has not been submitted elsewhere for the award of any other degree. The observations and results of this work recorded in this report are completely known to me and found to be true.

Place: Mysuru **Arya. B**

Date:

ACKNOWLEDGEMENT

I express my deepest gratitude and sincere salutations to CSIR-Central Food Technological Research Institute, Mysore, Karnataka and Dr. Sridevi Annapurna Singh, Director of CSIR-CFTRI, for providing me the opportunity and facilities to undertake this work. I extend my profound gratitude to Dr. Revathy Baskaran, Chief Scientist and Head, Human Resource Development and her team members for their constant help in carrying out this work

I am grateful to Dr. Attar Singh Chauhan, Head of the Traditional Food and Applied Nutrition Department, CSIR-CFTRI, Mysuru. It is with great pleasure and respect that I express my sincere appreciation to my advisor and mentor, **Mrs. Amudha Senthil**, Senior Principal Scientist, Department of Traditional Foods and Applied Nutrition, CSIR-CFTRI, Mysuru. I also extend my heartfelt thanks to the Traditional Food and Applied Nutrition Department for providing the necessary resources, facilities, and a supportive environment for conducting research and executing this project.

I am immensely thankful to **Mr. Anant Chintamani Gahire,** Scientist**,** Department of Fruit and Vegetable Technology, CSIR-CFTRI, Mysuru for his guidance and support in shaping this work. My sincere thanks to Dr. Pradeep Singh Negi, Chief Scientist, Department of Fruit and Vegetable Technology, CSIR-CFTRI, Mysuru.

I would like to convey my heartfelt thanks to Ms. Teena Thomas, PhD Scholar, Ms. Vincy S, Project Associate and my lab mates for their generous help, support and encouragement which helped me with the successful completion of this work.

My heartfelt gratitude goes to St. Teresa's College (Autonomous), Ernakulam, for providing all the necessary resources to help us complete our project work.

I would also like to extend my thanks to Ms. Sherin Mary Simon, Head of the Department of Food Processing Technology at St. Teresa's College (Autonomous), Ernakulam, as well as our class tutor, Ms. Pinku Maria and other faculty members for their constant support.

Lastly, and most importantly, I extend my deep sense of gratitude to my parents, family and friends for their unwavering support, understanding, and encouragement throughout this project. Their faith in me kept me motivated during difficult times, without them, none of this would have been possible. Their constant support was instrumental in overcoming obstacles and reaching significant milestones, and their confidence in my abilities has been a driving force behind this project's success.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPANSION
AFB 1	Untreated Antioxidant-rich Fruit Beverage
AFB 2	UV-treated Antioxidant-rich Fruit Beverage
DPPH	2,2-Dipheny l-1-picrylhydrazyl
FAO	Food and Agriculture Organization
FAV	Fruits and Vegetables
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PET	Polyethylene Terephthalate
pH	Potential of Hydrogen
RT	Room Temperature
RTS	Ready To Serve
TA	Titratable Acidity
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TSS	Total Soluble Solids
UV	Ultraviolet
VRBA	Violet Red Bile Agar
WHO	World Health Organization

ABSTRACT

Antioxidant-enriched fruit beverages have received attention for their role in promoting health and reducing the risk of lifestyle-related diseases, largely due to their ability to neutralize harmful free radicals. The present study aims to develop an antioxidant-rich fruit beverage from amla and orange with the addition of ginger essential oil. Amla and orange are both abundant in phenolic compounds, flavonoids, and vitamin C with strong antioxidant properties. To study the effectiveness of UV-C treatment as a non-thermal preservation technique, two variations were assessed: AFB 1 (untreated) and AFB 2 (UV-treated). A 60-day storage study of the fruit beverage under refrigerated conditions (4-6°C) was done to analyse the changes in the beverage's physicochemical properties, antioxidant properties and microbial quality over a period of time.

Physicochemical and nutritional characteristics, including pH, titratable acidity, total soluble solids, ascorbic acid content, antioxidant activity (DPPH inhibition), total phenolic content (TPC), flavonoid levels, reducing sugars, and total invert sugars, were monitored at 15-day intervals. The results showed a steady decrease in bioactive components over a period of time, however ascorbic acid, phenolics, and flavonoids were better retained in the UV-treated sample (AFB 2). The findings also revealed that UV-C treatment effectively reduced microbial contamination. It showed a delayed onset of microbial growth, while the untreated sample exhibited microbial growth on 30^{th} day. Thus, the UV-C treated sample (AFB 2) showed better shelf stability compared to the untreated sample (AFB 1).

To evaluate consumer acceptance, the Hedonic test was conducted involving a diverse group of participants which showed a strong positive response on the antioxidant-rich fruit beverage before its storage. Approximately 96% of respondents rated the beverage within the “Like Category”, with a significant number expressing a strong preference. As the stored fruit beverage initiated microbial growth on 30^{th} day for untreated (AFB 1) and end of 60^{th} day for treated sample (AFB 2), the samples were not subjected for further consumer acceptance study.

The present study demonstrates that UV treatment is a successful non-thermal technique that extends the fruit beverage's shelf life by preserving its microbiological quality, physicochemical characteristics, and antioxidant properties. This study indicates that the developed RTS beverage had a shelf life of 30 days for AFB 1(Untreated sample) and 60 days for AFB 2 (UV-treated sample) under refrigerated conditions.

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

Fruit is defined as the edible product of a plant or tree which consists of a seed and its envelope. Fruit is either naturally sweet or normally sweetened before eating. Fruits are developed from flower or flower parts or inflorescences. They are rich sources of antioxidants, which play a major role in preventing oxidative damage, inflammation, and chronic diseases. The antioxidant capacity of fruits is due to the presence of bioactive components which include phenolic acids, flavonoids, carotenoids, and anthocyanins (Slavin & Lloyd, 2012).

Fruits with high antioxidant content belong to the family *Rosaceae* (strawberry, raspberry, blackberry), *Ericaceae*(blueberry), *Punicaceae*(pomegranate), *Grossulariaceae*(blackcurrant), *Juglandaceae* (walnut). Fruits such as amla, grape, orange, pomegranate, pineapple, lemon, grapefruit, date, and kiwi are rich in antioxidant properties. Among these fruits, berries have a high content of phytochemicals like flavonoids, tannins, stilbenoids, phenolic acids, and lignans have the highest antioxidant properties among these fruits (Jideani et al.,2021).

The nutritional composition of fruits includes water about 60%, and protein about <1% on most fruit tissues. Fat content may vary depending on varieties but most of them have a lipid concentration below 1% except avocadoes, olives, and nuts. The dry mass of avocadoes and olives fat comprises about 35% - 70%. The abundant acids present in fruits are citrate (berries & tomato), malate (pome and stone fruit species), and tartrate (grapes). The sugar-to-acid ratio plays an important role in fruit taste. Some vitamins are stabilized due to the presence of organic acids in fruits and prevent phenolic compounds' oxidation. Carbohydrate content varies from 50% to 80%. The most common simple sugars present in fruits are glucose and fructose and sucrose is the disaccharide. The dietary fibres in fruits include cellulose, Cellulosic Longitudinal Glycans (CLG), pectin, lignin, starch, and Non-Digestible Oligosaccharides (NDO). Fruits are excellent sources of vitamins and minerals but their concentration may vary depending on the species, cultivars, environmental conditions, and cultural practices (Vincente, 2014).

Fruits are easily perishable due to their high moisture content. Mechanical, physicochemical, and microbial losses are the main causes of their deterioration. The spoilage of fruits is caused by abiotic factors, including pH, water activity, transpiration, ethylene production, harvesting, and processing procedures. Biotic factors include preharvest insect infestation damage and postharvest damage by microbes. Interval tissue invasion, physical changes in produce, rapid softening of the produce, shrinkage, and decay, are major modes of spoilage in fruits which decrease the shelf life of fruits (Y.H. Hui, 2006). To extend the shelf life of fruits, edible films and coatings designed by using lipids, proteins, and polysaccharides are used. Fruits are also processed into ready-to-serve (RTS) beverages using minimal processing procedures such as pretreatment, filtering, clarifying, pasteurizing, cooling, and storage. Fruit juices are made by combining fruit pulp with the addition of sugar, acids, and pectin, and with or without preservatives. Non-alcoholic and non-carbonated beverages encompass natural fruit juices, sweetened juices, ready-to-serve beverages, nectar, cordial, squash, crush, syrup, fruit juice concentrate, and fruit juice powder (Chettri et al., 2023).

The major types of fruit beverages include:

- Prepackaged drinks that are ready to serve (RTS beverages)
- Fruit nectars and syrups
- Diluted drink.

Due to the rising demand for nutritional beverages, different combinations of fruits are added for the development of naturally coloured and nourishing drinks with increased shelf life. The objective of the study is to develop antioxidant-rich fruit beverages from Amla, and orange with the addition of honey and citric acid The stability and shelf life of the beverages are compared with and without preservatives.

1.1. READY TO SERVE BEVERAGES (RTS)

The fruits and vegetables are easily perishable due to their high moisture content, so they have a very short shelf life. They are processed into Ready to Serve (RTS) beverages for preservation. RTS beverages are beverages made with varying amounts of fruits and vegetables together with water, sugar, and other ingredients. Fruits containing vitamins, and minerals are less consumed due to their bitterness and astringency, to mask this bitterness these fruits are mixed with other fruits and vegetables to improve the flavour, and nutritional properties, and to extend shelf life. (Rathinasamy et al., 2021).

RTS beverages typically contain 10% fruit, 10% TSS, and 0.3% acid. It is prepared from pure, unfermented fruit juice and intended for undiluted use. It also contains carbohydrates and water with or without pulp. A juice drink or juice cocktail is defined as a juice blend with additional components in the 2001 Code of Federal Regulations. Fruit nectars can be prepared using either pure juices or liquids that have been diluted with sugar syrup. RTS beverages often contain a single fruit, such as an orange, peach, or apple, but they can also be made by combining the pulps and juices of several different fruits. Government guidelines, industry specifications, and other mandatory and optional requirements continue to prepare the blended fruit juices with the least amount of fruit. All of these standards adhere to FAO/WHO's Codex Alimentarius (Food Standard Program) recommendations to ensure international trade (Vojir et al., 2012).

Natural RTS drinks are prized for their therapeutic qualities, tasty flavour, refreshing nature, and nutritional worth. Consequently, it is believed that combining natural RTS beverages is an ideal method to preserve and use fruits. According to the current review, natural RTS drinks with high sensory acceptability and improved shelf life are produced through blending and enrichment. Such RTS beverages serve as tasty appetizers and may lower the risk of illness. During storage, changes are noted in the physiochemical characteristics of RTS beverages, including pH, acidity, and total soluble solids. Fruits like grapes, gooseberries, litchi, pineapple, apples, oranges, and others are used to make a variety of RTS beverages mixed with whey protein to increase their nutritious content.

1.2. ANTIOXIDANT-RICH FRUIT BEVERAGES AND THEIR SIGNIFICANCE

Antioxidant-rich fruit beverages are a type of functional beverage made from fruits that are high in compounds that can protect the body from damage caused by free radicals. Free radicals are unstable molecules that can contribute to ageing and various diseases. Fruits and vegetables (FAV) are vital for health due to their antioxidant content, which synergistically combats chronic diseases like heart disease, stroke, cancer, and diabetes. Numerous studies confirm these benefits, with low FAV intake significantly contributing to global health issues. The FAO/WHO recommends a daily intake of 400-500g of FAV to prevent diseases and micronutrient deficiencies, highlighting their crucial role in preventing nutritionally related non-communicable diseases.

The health benefits of fruits and vegetables are largely due to their antioxidant content. These antioxidants, which neutralize harmful free radicals, include well-known vitamins like A, C, and E, as well as carotenes and glutathione. Beyond these, a wide range of other compounds like alkaloids, terpenoids, sulfur-containing substances, and various phenolic and polyphenolic compounds also contribute to the protective effects of fruits and vegetables by minimizing oxidative damage. Of all fruits, berries are the richest in antioxidants, with dog rose leading the pack over other varieties like currants, cherries, and strawberries. This high antioxidant capacity in berries stems from their abundance of beneficial plant compounds, including flavonoids, tannins, stilbenoids, phenolic acids, and lignans (Jideani et al., 2021).

Food and beverage industries now commonly add vitamin C, extracted from fruits, to products like juices and wines. Furthermore, they're shifting towards using natural colourings from fruits and vegetables instead of artificial ones (Food and Agriculture Organization, n.d.). While berries are exceptional antioxidant sources, processing them into products like jams diminishes their total phenol content, leading to a decrease in their overall antioxidant capacity compared to fresh berries (Nicoli, Anese, & Parpinel, 1999). Despite the availability of some fruits and vegetables as staple foods in tropical regions, global consumption consistently falls short of recommended daily nutritional requirements. To increase their availability, they are processed into various fruit beverages including fruit juices, health drinks, dietary supplements, etc. This would promote increased consumption, potentially leading to a significant reduction in degenerative diseases worldwide. Additionally, fortification with vitamins, minerals, probiotics, and other bioactive compounds further enhances their health benefits, making them an attractive option for individuals seeking functional nutrition (Maurya et al., 2023).

Expanding the consumption of these antioxidant-rich fruit beverages could have a profound impact on public health by potentially reducing the prevalence of degenerative diseases, including cardiovascular conditions, diabetes, and neurodegenerative disorders (Salehi et al., 2020; Sharma et al., 2022). Furthermore, the promotion of fruit-based beverages supports sustainable agricultural practices by increasing the demand for fresh produce and reducing post-harvest losses. With continuous advancements in food technology, innovative processing methods such as cold-pressing, freeze-drying, and minimal heat treatments are being developed to retain the maximum nutritional value of these beverages, ensuring that consumers receive optimal health benefits with every serving (Zhang et al., 2023).

1.3. UV TREATMENT ON FRUIT BEVERAGES

Ultraviolet (UV) radiation constitutes a small segment of the electromagnetic spectrum, which also includes radio waves, microwaves, infrared radiation, visible light, X-rays, and gamma radiation. UV radiation spans wavelengths from 100 to 400 nm and is divided into UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm). Ultraviolet-C is known for its germicidal properties against microorganisms like bacteria, viruses, protozoa, yeasts, moulds, and algae with the highest germicidal effectiveness observed between 250 and 270 nm. Consequently, the 254 nm wavelength is commonly employed for disinfecting surfaces, water, and various liquid food products, such as fruit juice**.**

As a non-thermal disinfection technique, ultraviolet therapy is carried out at low temperatures. When UV-C radiation is used as a non-thermal method, the following benefits are associated with it: certain organic contaminants can be eliminated; no off taste or odour is formed when treating water; no known toxic or significant non-toxic byproducts are formed during the treatment; and the treatment uses very little energy in comparison to thermal pasteurization processes. In contrast to juices treated with UV light, which often retain their colour and scent, fruit juice that is thermally pasteurized or sterilized tends to change colour and lose some of its vitamins and fragrances during the heating process (Keyser et. al,2008).

Furthermore, integrating UV-C irradiation with other non-thermal technologies, such as high-pressure processing (HPP), has demonstrated synergistic effects in maintaining juice quality. A study on "Nanglae" pineapple juice indicated that the combined UV-C and HPP treatment preserved carotenoids and protein levels similar to those in fresh juice over a 91-day storage period, while conventional heat treatment resulted in significant losses of these nutrients. Moreover, ascorbic acid levels were better preserved in the combined treatment compared to heat-treated juice (Sánchez-Moreno et al., 2023).

The purpose of UV-C irradiation treatment is to preserve the natural nutritional components of food while extending its shelf life and lowering health risks related to microorganisms. Two methods can be employed to initiate the photochemical reactions involved in basic UV-C irradiation mechanisms: (a) Direct reactions: When a molecule absorbs a photon of light, it can undergo a chemical reaction and undergo a state change. The quantum yield and fluence of incident photographs determine the magnitude of a chemical reaction. With a radiant energy of 112.8 kcal/Einstein, UV-C light at 257.3 nm has the potential to alter the O-H, C-C, C-H, C-N, H-N, and S-S bonds if it is absorbed. (b) Photosensitized: Photo-oxidation is the most prominent kind of photosensitizing response. In most instances, photosensitizers are excited from their ground state to a singlet excited state that lasts for a short time before converting to a triplet state that mediates the process for a long time. There are two main ways that the triplet sensitizer can react further: energy-transfer reactions or processes involving the transfer of hydrogen or electrons. In the meantime, two methods are used to quantify the effects on food quality: (a) juice characteristics; and (b) sensory. Measurements of pH, vitamins, polyphenols, colour, and antioxidant activity are among the physical and chemical components of the former. In contrast, the latter comprises a sensory assessment of the food's taste, smell, colour, texture, or organoleptic attributes (Shah et al., 2016).

1.4. OBJECTIVES OF THE STUDY

- To develop an antioxidant-rich fruit beverage from amla and orange juice incorporated with ginger essential oil.
- To analyse and evaluate the physicochemical properties, total phenolic content, flavonoid content, antioxidant activity, microbiological quality and consumer acceptance of the product.
- To conduct the shelf-life study of the prepared product with and without UV treatment under refrigerated conditions.

1.5. SCOPE

The purpose of this study is to develop an antioxidant-rich functional beverage by blending amla (*Phyllanthus emblica*) and orange juice, with the addition of ginger essential oil. Amla is well known for its high vitamin C, polyphenols, and flavonoids, while oranges contribute essential vitamins, minerals, and natural sweetness, making them complementary to each other in the beverage. Ginger, widely recognized for its bioactive compounds such as gingerols and shogaols, is expected to enhance the beverage's functional properties by providing additional antioxidant, anti-inflammatory, and antimicrobial benefits. By combining these three natural ingredients, the study aims to formulate a beverage that not only offers enhanced nutritional value but also aligns with consumer preferences for natural and health-promoting drinks.

The scope of this research extends to a comprehensive evaluation of the beverage's physicochemical properties, including pH, Total Soluble Solids, Titratable Acidity, and stability during storage. Additionally, a detailed nutritional analysis will be conducted to determine its antioxidant activity, flavonoid content, and total phenolic content, which are key indicators of its potential health benefits. This study also includes a consumer acceptance evaluation conducted using a 7-point hedonic scale to assess key sensory attributes such as taste, aroma, texture, and overall preference. The evaluation aims to measure consumer perceptions and satisfaction to determine the product's acceptability. Microbiological analysis will also be carried out to ensure the beverage's safety and shelf stability. The ultimate goal of this study is to develop a functional beverage that caters to the growing consumer demand for natural and beneficial dietary options, offering both enhanced nutrition and functional properties.

CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. FRUITS

2.1.1. AMLA

Figure 1: Amla (Source: Tevarak, 2016)

Amla belongs to the family of *Phyllanthaceae.* It is known as *Phyllanthus emblica*, Indian gooseberry, or Aonla. India and tropical and subtropical regions of Pakistan, Uzbekistan, Sri Lanka, South East Asia, China, and Malaysia are the native lands of Amla (Khan, 2009; Baliga et al., 2011). Tannins, alkaloids, and phenols are the chemical constituents of amla. It plays an important role in the prevention of cancer, diabetes, liver treatment, ulcers, anaemia, and heart trouble and is widely used in Ayurvedic medicine preparation (Baliga & Dsouza, 2011). By combining amla with other plants, it is used to treat the common cold and fever, as a diuretic, laxative, liver tonic, stomachic, restorative, antipyretic, and hair tonic; to prevent ulcers and dyspepsia (Krishnaveni & Mirunalini, 2010).

Table 1: Taxonomic classification of amla

Kingdom	Plantae
Division	Angiosperms
Class	Dicotyledonae
Order	Malpighiales
Family	Phyllanthaceae
Genus	Phyllanthus
Species	*Phyllanthus emblica L.*

(Source: Christenhusz et al., 2016)

Varieties: Banarasi, Chakaiya, and Francis (Hatijhool) are major amla cultivars in India. Banarasi is most cultivated in Varanasi, Uttar Pradesh with a straight growth habit and three branchlets per node. The flesh has a soft texture with a moderate amount of fibre which is semi-transparent. The negative impact of this variety is, that it is prone to fruit loss and its shelf life is shorter than other varieties. Chakaiya is a variety with a tall, vertical growth pattern of greenish colour. The fruit of chakaiya is small to medium, flattened, fibrous, and hard flesh. The variety Francis is favoured for the production of value-added products like candy, powder, and juices. It is a large fruit, flattened, oval, and has a greenish-yellow colour with smooth skin. It is unfit for preservation since it is prone to fruit necrosis. Due to the limitations of these

varieties, the Narendra Dev University of Agriculture and Technology has produced a range of varieties namely Krishna (NA-4), Kanchan (NA-5), Narendra Aonla-6 (NA-6), Narendra Aonla-7(NA-7), and Narendra Aonla-10 (NA-10). Banarasi was used in the selective breeding of Krishna (NA-4). Chakaiya was used for developing Kanchan (NA-5), and Narendra Aonla (NA-6). Francis cultivar was used in the open pollination of Narendra Aonla (NA-7). Banarasi is used for the random selection of Narendra Aonla-10 (NA-10) (Sawantt et al., 2022).

Nutritive value: Amla is widely recognized for its nutritive benefits. Amla contains 28% of the tannins that constitute the entire plant. Emblicanin A and B, are two hydrolyzable tannins with antioxidant qualities found in the fruit. Gallic acid, corilagin, rosin, and geraniin were among the phytochemicals found via activity-directed fractionation. There are alkaloids like phyllantine and phyllantidine as well as flavonoids like quercetin. In addition to this, it mostly consists of carbohydrates, amino acids, and other substances. The highest concentration of vitamin C (478.56 mg/100 mL) is found in its fruit juice. With 200–900 mg of vitamin C per 100 g of edible amount, it is considered one of the richest sources of mineral and polyphenol-rich food.

Table 2: Nutritional composition of Amla

Carbohydrate	14.1
Proteins	0.5
Fat	0.1
Fibers	3.7
Mineral matter	0.7
Calcium	0.05
Phosphorus	0.02
Iron	1.5mg/100g
Vitamin C	600mg/100g
Nicotinic acid	0.2mg/100g
Moisture	81.2

(Source: Kulkarni & Ghurghure, 2018)

Health benefits: Amla's antioxidant properties help to protect against chronic diseases, such as heart disease, cancer, and cognitive decline. It helps in the prevention of various cancers including breast, uterus, pancreas, stomach, and liver cancers. Studies have shown that Emblicanin A and B in amla fruit exhibit potent antioxidant and anti-cancer properties. High blood pressure can be controlled by the consumption of amla fruit powder. The mineral, chromium in amla has an antidiabetic effect which reduces blood sugar levels. Combining amla powder with milk or amla juice with honey relieves constipation. Amla juice also eases asthma, cough, and other respiratory disorders. Currently, amla is extensively utilized to strengthen the immune system. Amla includes vital nutrients that help prevent a wide range of health issues because of its potent antioxidant and biological properties. It may be used as a food additive or in the biopharmaceutical and nutraceutical fields. Amla is regarded as a herbal solution that is safe and has no adverse side effects (Kulkarni & Ghurghure, 2018).

2.1.2. ORANGE

Figure 2: Orange (Source: bergamont, 2024)

Orange (*Citrus sinensis*) belongs to the family Rutaceae. The term orange was derived from the Sanskrit word 'narang'. Typically, each orange contains 11 individual pieces. The fruit has high water content, is indehiscent, and normally has a size range between 4 cm to 12 cm. It is a widely grown fruit crop having a global production of about 120 million tons. They are mainly cultivated in tropical and subtropical climates. Oranges are hesperidium that is a type of berry with a peelable rind that varies in size, colour, shape, and juice quality. The bioactive components in oranges are effective against cancer, cardiac diseases, diabetes, and arthritis, and also have anti-bacterial, antifungal, anti-inflammatory, antioxidant, anti-asthmatic, and anti-hypertensive properties.

Table 3: Taxonomic classification of orange

Kingdom	Plantae
Division	Magnoliophyta
Class	Dicotyledons
Order	Rosidae
Family	Rutaceae
Genus	Citrus
Species	*Sinensis*

(Source: Parle & Chaturvedi, 2012)

Varieties: The main varieties of oranges include Mosambi, Malta (common), Malta (blood red), and Sathgudi. Mosambi are light yellowish orange in color with prominent streaks on the rind. The peeling of the rind is difficult and the pulp has a light yellow and sweet juice. Fruits of Malta (common) variety have an orange-yellow color, and smooth surface and the thickness of the rind is medium. The pulp is orange in color with good flavor. The Malta (Blood red) variety has a yellow color with scarlet blush. The rind is relatively thin, tight, and glossy having sweet pulp with abundant red colored juice. Sathgudi fruits are smooth with attractive color, and shape and have orange-colored juice with good flavor (Parle & Chaturvedi, 2012).

Nutritive value: Oranges are a highly nutritious fruit, offering a wealth of essential vitamins and minerals. They are best known for their high vitamin C content, which supports immune function and skin health, with a medium-sized orange providing more than the daily recommended intake. Oranges are also a good source of dietary fiber, aiding digestion and promoting gut health, as well as potassium, which helps regulate blood pressure and supports heart health. Additionally, they contain small amounts of vitamin A, folate, and antioxidants like flavonoids and carotenoids, which protect the body from oxidative stress. Low in calories, oranges are a hydrating and healthy snack option, providing a natural source of energy and a wide range of nutrients.

Table 4: Nutritional composition of orange per 100g

Carbohydrates	11.75 g
Protein	0.94 g
Dietary fibre	2.40 g
Vitamin C	53.20 g
Total fat	0.12 g
Vitamin A	225 IU
Iron	0.10 mg
Magnesium	10 mg
Calcium	40 mg
β-carotene	71 µg

(Source: Richa et al., 2023)

Health Benefits: The consumption of oranges provides various health benefits which include antioxidant, anti-carcinogenic, anti-ulcer, anti-bacterial, anti-diabetic, anti-fungal, and anti-inflammatory properties. The vitamin C content in oranges prevents free radical generation and thereby acts as an antioxidant. It also plays a crucial role in maintaining a healthy immune system. The bioactive components like vitamin C, carotenoids, and flavonoids in oranges reduce blood cholesterol and act as a cardioprotective. Various cancers like mouth, skin, lung, breast, stomach, and colon cancer are reduced by the presence of limonene in oranges. Hesperidin and its flavone analog, diosmin in oranges also have anticancer properties. The risk of lung cancer can be lowered by the action of β-cryptoxanthin. Due to its antibacterial properties' orange peel, leaves, and flowers are used for the treatment of various ailments. Bioflavonoids help in the regulation of glucose regulatory enzymes and maintain the blood sugar level (Parle & Chaturvedi, 2012).

2.2. GINGER ESSENTIAL OIL

Ginger, or Zingiber officinale, is an underground rhizome belonging to the 47–53 genera and 1200–1400 species of the Zingiberaceae family (Raji et al., 2002). Ginger is one of the earliest spices used by humans and is naturally rich in antioxidants. It ranks among the top five foods high in antioxidant content (Hadi et al., 2020). Bioactive substances, including phenolic and terpenic components, are abundant in its rhizome. Together with terpenes like zingiberene, γ-terpinene, β-bisabolene, α β-sesquiphellandrene, α-farnesene, and curcumene, the plant also includes phenolic chemicals such gingerols, shogaols, paradols, zingerone, and catechins. It also contains other healthy substances including β-carotene and ascorbic acid (Aleem et al. (2020).

The essences may be referred to as volatile oils or ethereal oils because of their chemical composition, which makes them volatile at room temperature. To extract this critical proportion of the plant material, a variety of methods have been used, including water, steam distillation, microwave application, and liquid carbon dioxide. Ginger essential oil is well-known for its anti-inflammatory, antibacterial, and antioxidant qualities. It has demonstrated moderate to strong inhibitory effects on fungi, including Fusarium moniliforme and Aspergillus flavus. It is useful in both traditional and modern medicine because it also has analgesic, antiplatelet, and anti-ulcer qualities. The recovery of ginger's essential oils is dependent on the plant's origin and variety, culture, harvesting humidity, extraction techniques, and, to a lesser extent, plant age. Although ginger essential oils are yellow, their colour, scent, and flavour vary depending on where they were first cultivated. Fresh ginger that has been sliced and oven-dried at 50AB (SOD) has the highest levels of volatile oil, protein, calcium, and magnesium, claim Famurewa and Emuekele (2011). By using the hydro distillation process, Gurdip Singh et al. (2008) extracted the essential oil of ginger (Zingiber officinale) and then exposed the oil to GC-MS analysis. Sultan et al. (2005) studied the composition of essential oils using GC with a flame ionization detector and extracted the essential oil of Thai and Chinese ginger using steam hydro distillation (Kamaliroosta et al., 2013).

2.3. PROCESSING OF FRUIT JUICE

The processing of fruit juice involves several meticulously planned steps to ensure the final product is safe, delicious, and has a long shelf life. It begins with receiving and inspecting high-quality, mature fruits, followed by thorough washing to remove any contaminants. The fruits are then sorted to remove any decayed ones, and subsequently crushed, ground, or disintegrated depending on the type of fruit. Optional enzyme treatment can be applied to improve juice yield and quality. The juice is then extracted from the fruit pulp using various equipment, and strained or filtered to remove suspended particles. Optional clarification can be done to completely remove any remaining suspended material. De-tartarisation may be applied to raisin juice to eliminate potassium bi-tartrate. The juice is preserved using methods like pasteurization, chemical preservatives, or freezing. It is then filled into sterilized bottles, sealed, and pasteurized again to ensure safety. Finally, the labelled bottles are stored in a cool, dry place until they are distributed (Srivastava & Kumar, 2020).

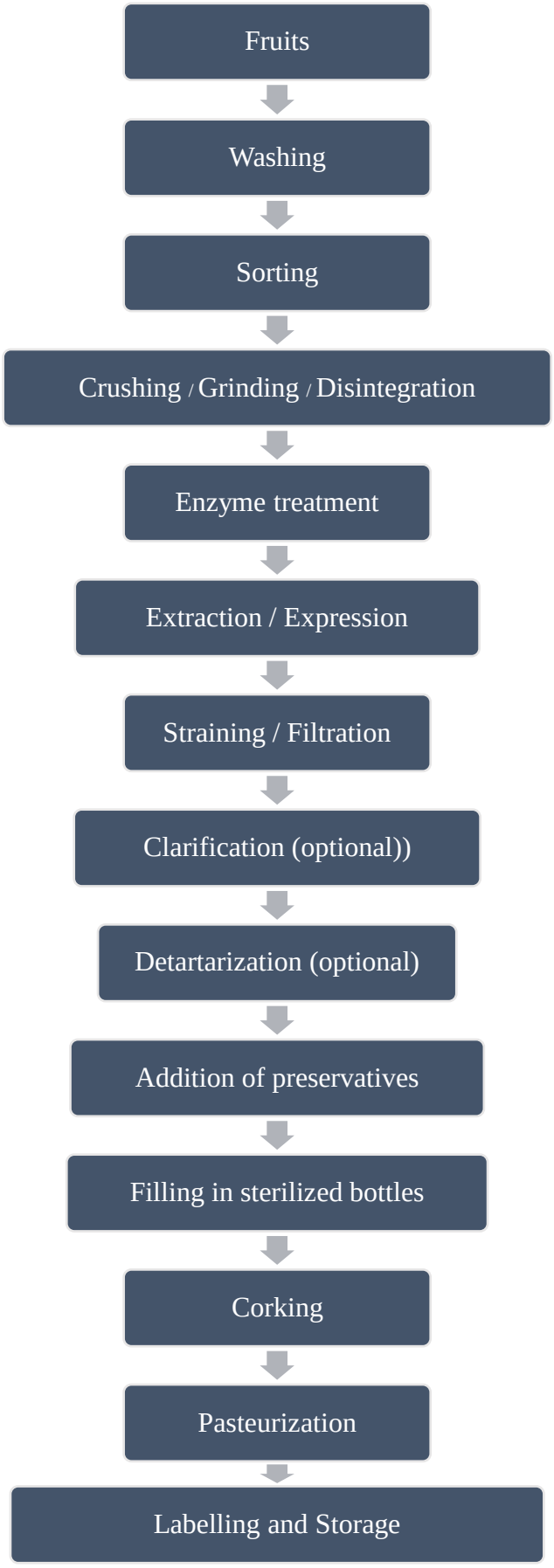


Figure 3: Flow diagram for fruit juice processing. (Source: Srivastava & Kumar, 2020).

2.4. FUNCTIONAL BEVERAGES

A functional beverage is a non-alcoholic beverage that has unconventional substances in its composition to offer particular health advantages beyond basic nourishment. Energy drinks, ready-to-drink teas, sports, and performance drinks, enhanced fruit drinks, soy beverages, and improved water are a few of the product categories introduced to the market as functional beverages (Bigliardi & Galati, 2013). Any chemical that can prevent other molecules from oxidizing is an antioxidant. Since they accomplish this by oxidizing themselves, antioxidants are frequently reducing agents like polyphenols, ascorbic acid, or thiols. The word "oxidative stress" refers to an imbalance that favours oxidants over antioxidants, which may cause adverse effects. Oxidative stress may result in diseases that include cancer, atherosclerosis, Parkinson's disease, and Alzheimer's disease (Morillas-Ruiz,2014).

Consuming a beverage derived from fruits or vegetables that includes a range of bioactive chemicals shown to provide various health benefits. Fruit juices are now a staple of the modern diet as a result. The demand of consumers for healthy beverages boosts both the acceptance of nutrient-dense beverages and their openness to adopting new different and unusual flavours. Phenol impacts the capacity to scavenge radicals and possesses strong anti-mutagenic, anti-cancer, and anti-inflammatory properties. Minerals can support biological systems' regular operation and guard against negative consequences including reduced cellular immunity and changes in behaviour and cognition. One of the antioxidant nutrients that helps fortify the body's defences against possible harm from free radicals is vitamin C (Lim & Rabeta, 2018)

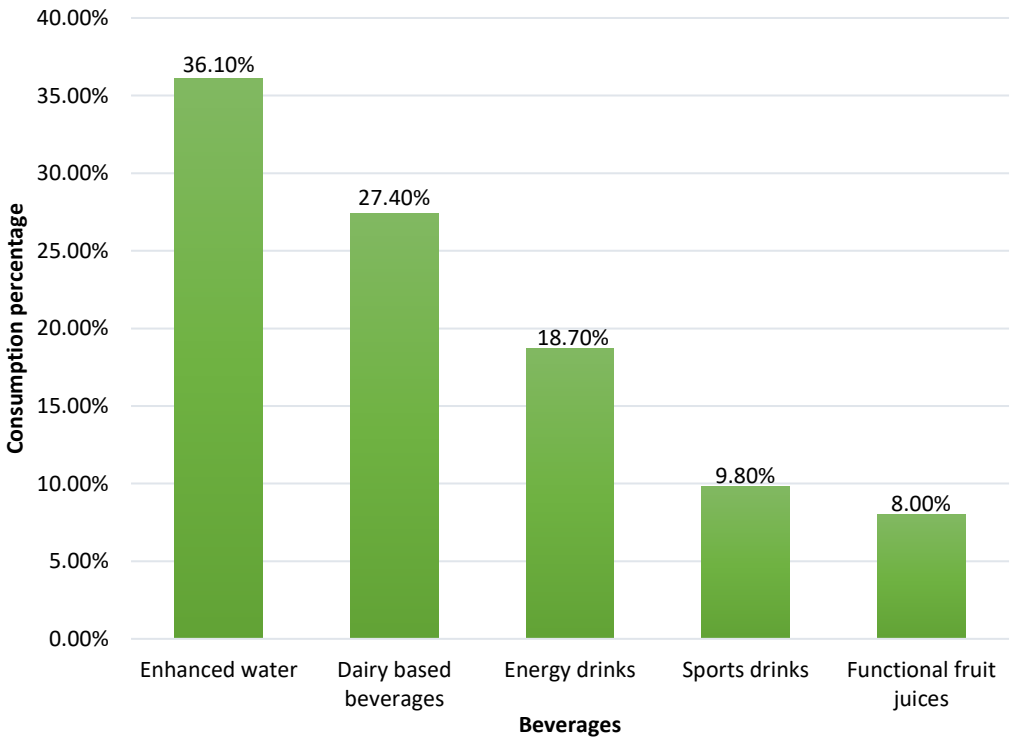


Figure 4: Worldwide market of some selected popular beverages.

(Source: B Anuradha, 2019)

The global market for functional beverages is now dominated by sugar-free drinks, energy drinks, dairy-based beverages, improved water, and functional fruit juices (Anuradha, 2019). The production and consumption of functional beverages have increased significantly due to their significant benefits to disease prevention and health promotion, rising urbanization, the middle class, the rise in dual-income households, and growing health concerns. With a $131.47 billion valuation, Asia-Pacific held the largest share of the worldwide functional beverage market in 2022. The need for functional foods varies among European nations. The market has expanded steadily and profitably, holding a 16 percent share of worldwide sales. The United Kingdom is the largest source of income, with 20% of total revenue (Arthur R, 2021).

2.5. ANTIOXIDANT-RICH BEVERAGES FROM DIFFERENT SOURCES

An antioxidant-rich beverage with no added sugar was developed from mahua (*Madhuca indica)* that are rich in sugars, vitamins and minerals and amla (*Emblica officinalis*). Mahua juice prepared from mahua flowers consists of natural sugar which was combined with amla juice which is rich in vitamin C content to produce a beverage with antioxidant activity. Eighteen blends were made by combining three different concentrations of mahua juice (20˚B, 40˚B and 60˚B) and amla juice. The TSS, pH, titratable acidity (TA), total phenol content, sugar profile and organoleptic properties of the blends are evaluated. The most accepted blend was the blend with 40˚B. It contains sugars, specifically glucose and fructose, but no sucrose. 15.94 ± 0.68 mg GAE/ml was the TPC value and it showed 91.22% DPPH radical scavenging activity (Patel et al., 2014).

Sports drink was prepared from coconut water and pineapple juice. Smooth cayenne, MD2 and sugar loaf were the pineapple varieties used in the preparation of pineapple drink. Formulations were designed in order to pick a sample with a high energy value, brix, and a percentage of carbohydrates that facilitates easy stomach emptying. The MD2 pineapple variety (55% coconut water: 45% pineapple juice), which is available all year round, was chosen for the formulation based on these qualities. The final sports drink product that was chosen had a brix of 6.8, 5.61% carbohydrate, and 161.67 mg of vitamin C per 100 millilitres. The sports drink had a pH of 3.80 and a 12-month shelf life (Asante & Donyinah, D. 2010).

Apple juice was blended with carrot and ginger to produce a healthy beverage. Consuming apple juice may improve memory because it increases acetylcholine levels in the brain. Boron is one of the many minerals and nutrients found in apple juice that may help maintain strong bones. Apples are rich in antioxidants, potassium, and vitamin C. By reducing lipid peroxidation and enhancing general antioxidant status, carrot juice may help prevent cardiovascular disease. These benefits are independent of cardiovascular risk. As per scientific studies, the antioxidant properties of ginger may alleviate nausea and vomiting brought on by motion sickness, cancer therapy, pregnancy, and surgery. According to the evaluation panel, health beverages made with an 85:15:2.5 ratio of apple, carrot, and ginger juice had the best organoleptic qualities and scored the highest in colour and appearance, consistency, flavour and taste, and overall acceptability (8.5, 8.3, 8.1, and 8.3 respectively) (Mahanandia, Singh, & Saini, 2022).

A new antioxidant beverage was prepared from green tea and apple. Making a new beverage with high antioxidant power and long-term stability at room temperature by blending apple and green tea extracts and analyzing the antioxidant activity, composition, organoleptic characteristics, and stability status during storage was the objective of the study. To increase the antioxidant content, lemon juice was added which is a rich source of vitamin C and flavonoids. TSS and pH were assessed as quality measures. At the beginning pH was 3.18 and the average TSS value was 10.70 Brix. During the storage time, these values remained constant but due to Maillard's reaction products and the formation of condensed tannins there is a decrease in the amount of ascorbic acid and total phenolic content. The antioxidant efficacy only dropped by 9.74% (Rubio-Perez et al., 2014).

Ready-to-serve beverage based on apples and whey was developed, even though apples are rich in proteins they contain less mineral content so to compensate for this whey, the byproduct obtained during the production of channa, paneer, cheese consists of almost all the water-soluble nutrients like lactose, proteins, vitamins and minerals. Whey has various health benefits including prevention of cancer, cardiovascular, arthritis also anti-bacterial and anti-viral properties. To make it more appetizing herbs/spices extract (*jaljeera)* extract was also used. In addition to enhancing the beverage's sensory quality, the addition of whey and jaljeera extract increased its nutritional value in terms of calcium content (15.64 mg/100 mL), total proteins (0.29%), ascorbic acid (10.57 mg/100 mL), and total phenols (37.86 mg/100 mL) (Sharma et al., 2019).

Soy whey was incorporated with orange juice to increase the protein content of the beverage. Various soy whey-orange juice beverage formulations were made by boiling whey to 45°C and adding sugar and orange juice. The protein percentage in orange juice is 0.45%; protein content rises from 0.58% to 1.65% when soy whey concentration was increased from 20% to 50%. It was shown that the pH of the beverage also increased with the addition of soy whey in orange juice. When soy whey levels increase, the TSS of orange-based beverages containing soy whey declines. Soybeans are rich in genistein, glycetin, and daidzein, which have strong antioxidant and other biological activities. Orange juice is also rich in ascorbic acid, carotenoids, and other phenolics, which is why it also exhibits strong antioxidant activity (Punoo, Rather, & Muzaffar, 2023).

Functional beverage was developed from different fruit juices (pineapple, oranges, carrots) with the *Hibiscus sabdariffa* extracts (HSE). A high number of proteins and nutrients beneficial for human health are found in Hibiscus sabdariffa extracts (HSE). It has been observed that the calyces' aqueous extract has a high acidity and a low sugar content. The carotenoids that occur in carrots are abundant and well-known for their neuroprotective, antioxidant, and cognitive-development-enhancing properties. Rich sensory qualities, ascorbic acid content, and nutritional value make oranges and pineapples popular. They may also have a preventive effect against several degenerative disorders. The ideal beverage blend was determined to be one that had 40% pineapple, 16.5% carrot, 17.2% orange, and 26.3% HSE. This beverage included 512.82 mg of total phenols (GAE 100 g−1), 3.37 mg of vitamin C (g−1), and 51.34% DPPH inhibition. Increased HSE ratios were primarily responsible for the formulation's improved antioxidant response (Ogundele et al., 2016)

2.6. DIFFERENT METHODS USED FOR EVALUATION OF ANTIOXIDANT ACTIVITY

The antioxidant activity of beverages can be determined by various methods including DPHH activity, and FRAP method, by measuring total phenolic content and reducing power.

- In DPPH activity, 0.1 mmol/L DPPH ethanolic solution was added to 3 mL of each beverage sample. After vigorously shaking the mixture and allowed to stand it in the dark for thirty minutes, the absorbance was measured at 517 nm using an ultraviolet-visible (UV-vis) spectrophotometer. The percent DPPH activity was calculated using the equation:

$$\text{DPPH (\%)} = \frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of control}}$$

- FRAP Analysis: A FRAP solution was made by mixing 20 mmol/L ferric chloride solution, 10 mmol/L TPTZ, and acetate buffer (pH 3.6) in a 10:1:1 ratio with 40 mmol/L HCl solution. The 100 µL beverage sample was then placed in centrifuge tubes with 900 µL of warm (37°C) FRAP solution. After agitating and incubating the tubes for 40 minutes at 37°C, the absorbance at 595 nm was estimated using a UV-vis Spectrophotometer to track the decline.

- Total phenolic content: The Folin-Ciocalteu method was used to measure total phenolics colorimetrically. 1.5 mL of the Folin-Ciocalteu reagent (previously diluted ten times) was combined with 300 µL of beverage samples and left to stand at 25°C for five minutes. Following the addition of 3 mL of sodium bicarbonate solution (60 g/L) to the mixture, the samples were incubated at 25°C for 90 minutes. A UV-vis spectrophotometer was then used to detect absorbance at 725 nm. The calibration curves derived from calculating the absorbance of the gallic acid standard at five concentrations (25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, and 125 µg/mL) were used to determine the total phenolic content (TPC). Gallic acid equivalents (GAE)/100 mL of beverage samples were used to calculate the results.

- The beverage samples reduction power was calculated by mixing 1 mL beverage sample was with 1% potassium ferricyanide and an equal volume of 0.2 mol/L phosphate buffer (pH 6.6). Following a 20-minute incubation period at 50°C, 2.5 mL of 10% tricarboxylic acid (TCA) was added, and the samples were centrifuged for 10 minutes at 5,000 rpm. After that, 0.5 mL of a 0.1% ferric chloride solution was combined with 4 mL of supernatant, and the mixture was allowed to stand for 10 minutes. At 700 nm, the absorbance was measured with a UV-vis spectrophotometer (Punoo, Rather, & Muzaffar, 2023).

2.7. ANTIOXIDANTS AND THEIR HEALTH BENEFITS

Fiber, polyphenols, flavonoids, conjugated isomers of linoleic acid, D-limolene, epigallocatechin, gallate, soy protein, isoflavanones, vitamins A, B, C, E, tocopherols, calcium, selenium, chlorophyllin, alipharin, sulphides, catechin, tetrahydrocurcumin, seasamol, glutathione, uric acid, indoles, thiocyanates, and protease inhibitors are the most abundant dietary substances found in fruits and vegetables that function as antioxidants. These substances may function as cardio-protective or anticancer agents alone or in combination through a number of different methods.

Polyphenols present in fruits and vegetables are secondary plant metabolites which have both desirable and undesirable properties. Due to the presence of tannins, it was considered as antinutrient since it has negative effect on human metabolism. At present, polyphenols are recognised for the majority of antioxidant activity in comparison with ascorbic acid in fruits. Flavonoids and phenolic compounds present in grapes are known for their anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, antiallergic, antithrombic and antioxidative effects. Another rich source of antioxidants vitamins, fibre and various phenolic compounds are berries. Most of the berries contain similar or higher levels of phenolic acids and flavonoids than other fruits. Berry extracts are effective in the treatment of urinary tract infections. Large citrus fruits and vegetables contain limonoids which helps in the prevention of human cancers and atherosclerosis. Phenolic acids and flavonoid present in apples are shown to be effective against colon and liver cancer cells.

Carotenoids like β-carotene, lycopene, lutein, and zeaxanthin are also recognized for their antioxidant activity. Vitamin C, vitamin E, and β-carotene are together referred as antioxidant vitamins. Carotenoids reduce the risk of breast cancer. It also helps to prevent the formation of N-nitroso compounds which are cancer causing compounds from nitrates and nitrites present in preserved meat and certain water supplies. Since carotenoids consist of provitamin A activity such as α-carotene and β-carotene, it has been effective against various types of cancers, oxidative stress and chronic diseases. Lycopene present in tomatoes positively interferes with oxidative damage and prevents carcinogenesis and atherogenesis. Vitamin E is the lipid-soluble antioxidant. The tocopherols synthesized by plants produce tocopheroxyl radical by reacting with lipid peroxyl radicals, thus prevents atherosclerosis, heart diseases, prostate and colon cancers. Vitamin C is a water-soluble antioxidant that reduces the risk of lung, oesophageal, and pancreatic cancers (Kaur & Kapoor, 2001).

Flavonoids are bioactive compounds which are secondary metabolites of plants having polyphenolic structure. They are linked to numerous health benefits including antioxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic effects. Enzymes like xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide can be inhibited by flavonoids. It comprises of several subgroups including flavones, flavonols, isoflavones, anthocyanins, chalcones. Numerous flavonoids have developed as bioactive substances with pharmacological, antibacterial, and insecticidal qualities that alter proteins or nucleic acids. Because of this, flavonoids are useful as pharmaceuticals in medicine and as insecticides in agriculture. Cellular damage brought on by oxidative stress is related to a number of illnesses, including diabetes, cancer, cardiovascular disease, neurodegenerative diseases, and aging. Numerous biological molecules are susceptible to oxidative stress, and proteins and DNA molecules are particularly susceptible to cellular harm. In order to prevent these free radicals

from damaging cells, antioxidants disrupt the processes that produce radicals and promote the activity of endogenous antioxidants (Panche et al., 2016).

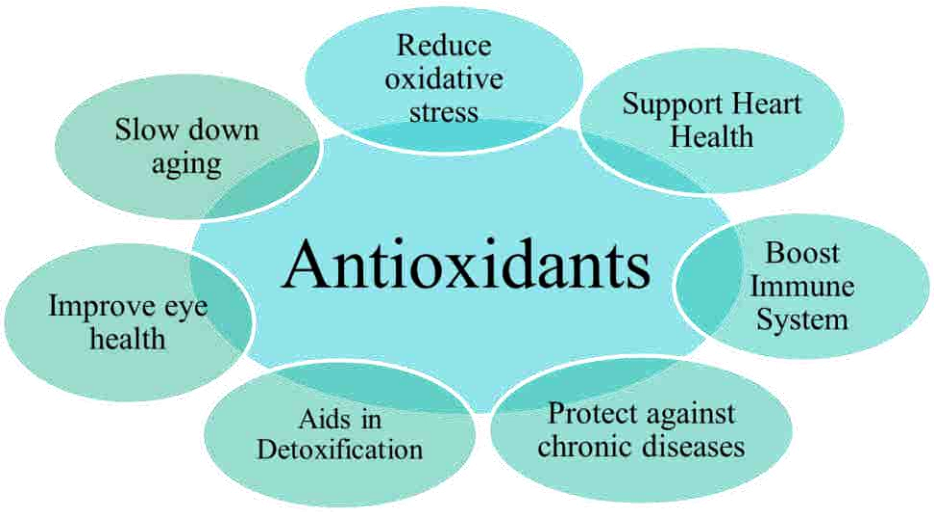


Figure 5: Health benefits of antioxidants

2.8. METHODS FOR ENHANCING ANTIOXIDANT CONTENT

Fruit extracts were used to boost the antioxidative potential and polyphenol content of beverages. 80% ethanol was used to extract the fruit, and distillation under lower pressure was employed to thicken it. The apple, orange, and grapefruit beverages were enhanced with extracts. For all applied fruit extracts, the fortification of evaluated beverages led to an increase in total polyphenol content and antioxidative activity. The red grapefruit beverage had the best antioxidant qualities among the beverages made, whereas the orange beverage had the best organoleptic evaluation. According to the results of the sensory evaluation, the inclusion of lingonberry extract (found in grapefruit beverages) and Japanese quince fruit extract (found in apple and orange beverages) was chosen above the other samples (Tarko et al., 2015).

The antioxidant activity of fruit beverages can also be increased with the addition of antioxidant-rich ingredients like herbs and spices. To increase the antioxidant activity of an antioxidant beverage made with cucumber and pomegranates and to give it a natural preservation effect, extracts of herbs and spices, such as mint, lemongrass, ginger, and cinnamon, were added either separately or in combination, at a comparable dose of 2%. Accordingly, the drink with ginger extract preserved its storage quality since it had the strongest potential to scavenge free radicals (49 to 67%) concerning its highest levels of polyphenol and flavonoids (1203 ± 64 mg GAE/L and 178 ± 8 mg TE/L, respectively). On the other hand, a drink made with lemongrass had the highest ascorbic acid content, measuring 22 ± 1 mg/100 mL (Fatima et al., 2024).

The application of reverse osmosis during the process can increase the antioxidant activity of fruit beverages. RO is a membrane separation technique that involves applying a hydraulic pressure greater than the solution's osmotic pressure in a way that allows water to permeate from a high to a low solute concentration. Fruit juice bioactives can be concentrated using this method, which minimizes the harm caused by heat evaporation of water and preserves the fruit's nutritional value and flavour. The application of RO in the concentration of numerous fruits shows great potential, and this method partially encourages dehydration, which raises

TSS, including phenolic bioactivity. This method has been applied to a variety of fruit juices, including grape and orange (Gunathilake et al., 2014).

The food industry frequently uses thermal treatments, such as pasteurization and sterilization, to preserve functional beverages because of their capacity to destroy bacteria and deactivate enzymes. Sterilization and pasteurization are processes that involve high temperatures, which frequently result in chemical and physical changes that alter sensory qualities and lower the concentration or bioavailability of certain nutrients. Therefore, non-thermal treatments such as HHP, HIPEF, US, pulsed light, irradiation, oscillating magnetic fields, and low-temperature plasma are considered to be more efficient in preserving quality attributes in food products than heat treatments. The main bioactive compounds present in these products are well retained with the application of non-thermal treatments (Morales-de la Peña et al., 2016).

2.9. EFFECT OF UV TREATMENT IN EXTENDING THE SHELF LIFE

Ultraviolet (UV) radiation is a small part of the electromagnetic spectrum, which also includes radio waves, microwaves, infrared radiation, visible light, X-rays, and gamma radiation. UV radiation ranges from 100 to 400 nm and is divided into UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm). UV-C is notable for its germicidal properties against microorganisms such as bacteria, viruses, protozoa, yeasts, moulds, and algae, with the highest effectiveness observed between 250 and 270 nm. As a result, the 254 nm wavelength is commonly used for disinfecting surfaces, water, and various liquid food products like fruit juice.

As a non-thermal disinfection technique, UV therapy is conducted at low temperatures. When UV-C radiation is employed in non-thermal methods, the following advantages are noted: elimination of certain organic contaminants; no off taste or odour when treating water; no known toxic or significant nontoxic byproducts formed; and minimal energy usage compared to thermal pasteurization processes. In contrast to juices treated with UV light, which typically retain their colour and scent, thermally pasteurized or sterilized fruit juice often changes colour and loses some vitamins and fragrances during heating (Keyser et al., 2007).

The purpose of UV-C irradiation treatment is to preserve the natural nutritional components of food while extending its shelf life and reducing health risks associated with microorganisms. Two methods can initiate the photochemical reactions involved in basic UV-C irradiation mechanisms: (a) Direct reactions: When a molecule absorbs a photon of light, it undergoes a chemical reaction and a state change. The quantum yield and fluence of incident photons determine the magnitude of the chemical reaction. UV-C light at 257.3 nm, with radiant energy of 112.8 kcal/Einstein, can alter the O-H, C-C, C-H, C-N, H-N, and S-S bonds if absorbed. (b) Photosensitized reactions: Photo-oxidation is the most notable kind of photosensitizing response. Typically, photosensitizers are excited from their ground state to a singlet excited state that lasts briefly before converting to a longer-lasting triplet state that mediates the process. The triplet sensitizer can react further through energy-transfer reactions or processes involving hydrogen or electron transfer.

Two methods are used to measure the effects on food quality: (a) juice characteristics and (b) sensory attributes. The former includes measurements of pH, vitamins, polyphenols, colour, and antioxidant activity. The latter involves sensory assessments of the food's taste, smell, colour, texture, or organoleptic attributes.

The effects of UV treatment on fruit beverages are multifaceted. First and foremost, it leads to substantial microbial inactivation. For example, studies have shown that UV-C treatment can extend the shelf life of orange juice by up to 7 days by reducing microbial activity. This reduction in microbial load helps prevent spoilage and maintains the beverage's safety for consumption. Additionally, UV treatment impacts enzymatic activities within the beverages. Enzymes such as polyphenol oxidase and peroxidase are responsible for processes like browning and ripening, which can affect the quality and appearance of fruit beverages. UV-C treatment has been observed to delay these processes, thus helping to maintain the colour, texture, and firmness of the beverages. One of the significant advantages of UV treatment over thermal methods is its minimal impact on the physicochemical properties of the beverages. Parameters such as pH, colour, and texture remain relatively stable after UV treatment. For instance, in a study involving orange juice, there was a slight reduction in Vitamin C content, but the overall nutritional value remained largely unchanged. This is in contrast to thermal methods, which can cause significant changes in flavour, colour, and nutritional content.

UV treatment is also energy-efficient compared to thermal methods. It consumes less energy and does not require the high temperatures associated with pasteurization, making it a more sustainable option. Furthermore, UV treatment helps preserve the natural flavour of the beverages, which is often altered during thermal processing (Karim Shah et al., 2016).

UV-C radiation has a wide range of applications and can be effectively utilized to lower the microbial burden in various single-strength fruit juices and nectars. It is crucial to optimize the parameters for various liquids treated in order to guarantee the greatest possible reduction of the microbial load without compromising the product's flavour. The use of this non-chemical cold pasteurization technique to eliminate bacteria, viruses, yeasts, and moulds as well as food spoilage is becoming more and more popular. A further advantage for the consumer is that fruit juices can be produced without the need of preservatives by employing UV-C light alone to eradicate germs (Koutchma, 2019).

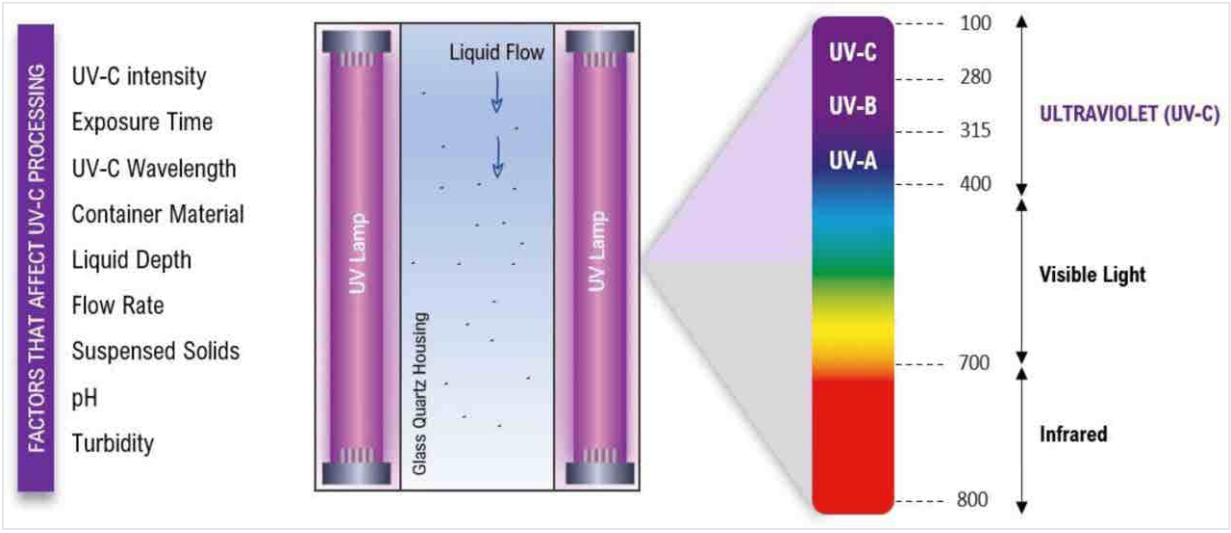


Figure 6: Reactor chamber of UV-C processing for fluid food and the main factors that influence the process. (Source: Tchonkouang et al., 2023)

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present research was conducted to develop an antioxidant-rich fruit beverage from amla and orange juice with added ginger essential oil. The beverage was formulated on a laboratory scale in three different ratios (50:50, 40:60, 30:70) and its Vitamin C content along with physicochemical parameters such as pH, Total Soluble Solids (TSS) and Titratable Acidity (TA) were analysed before and after pasteurisation. Subsequently, the beverage was produced on a larger scale and stored in Polyethylene Terephthalate (PET) bottles for shelf-life study. A subset of the PET bottles containing the beverage was exposed to UV-C treatment to assess the effect of UV-C treatment on fruit beverages. The samples were also assessed for their antioxidant activity, total phenolic content, flavonoid content, and other physicochemical properties, as well as microbial quality and consumer acceptance over a period of 60-day storage. This section details the resources used for the research, including ingredients, materials, and equipment.

3.1. MATERIALS

3.1.1. Raw Materials

a) Orange (Mandarin), Amla, and Ginger

Orange (Mandarin), amla, and ginger of optimum maturity were procured from the local market of Mysore. Orange and amla were the primary ingredients. Ginger was extracted into essential oil and added to the juice for flavour enhancement.

b) Honey

Honey bought from the local market, was added to the juice as a natural sweetener.

c) Citric acid

Food-grade citric acid was used in the beverage due to its preservative, flavour-enhancing and antioxidant properties.

3.1.2. Tools and Equipment

- Weighing balance: For measuring the amount of raw materials.
- Knife: For cutting the raw materials into small pieces.
- Crusher: To obtain a fine paste.
- Muslin cloth: For extracting and filtering the juice.
- Boiling machine: To pasteurize the mixture on a large scale.
- Thermometer: For measuring the temperature of the mixture during pasteurization.
- Screw type extractor: For extracting the juice on a large scale.
- Measuring cups: For measuring the amounts of extracted juice and water.
- Clevenger Apparatus: To extract ginger essential oil
- Bottles: PET bottles (Polyethylene Terephthalate bottles) were used for storage of the final product.

3.2. PLAN OF WORK

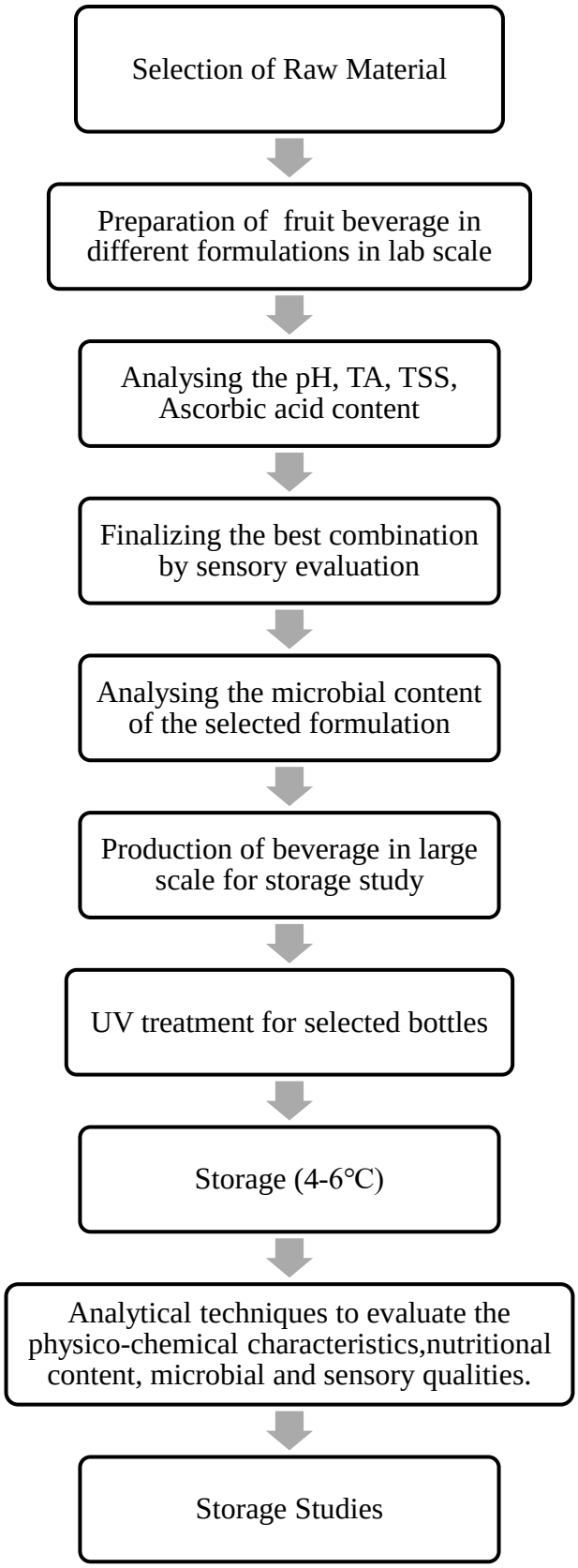


Figure 7: Flowchart for the Work Plan

3.3. PREPARATION OF INGREDIENTS

3.3.1. Extraction of fruit juices

Extraction of Amla and Orange juice was done separately using a screw-type juice extractor which involves the use of a rotating screw mechanism within a cylindrical chamber to apply pressure on the fruit, effectively separating the juice from the pulp. The process began with the thorough washing of oranges and amla to remove any dirt or contaminants. Oranges were peeled to eliminate the outer skin, while amla was cut into smaller pieces. The prepared fruits were then fed into the hopper of the screw-type extractor, where they were gradually moved forward by the rotating screw. As the fruit advances through the chamber, it undergoes gradual compression due to the decreasing gap between the screw and the cylindrical wall. This pressing action effectively crushes the fruit, forcing the juice to come out through perforations in the chamber. The extracted juice was collected in a stainless-steel vessel below, while the remaining pulp, containing skin, seeds, and fibres, was expelled separately. To improve the quality of the juice, it was filtered using a muslin cloth.

Figure 8: Screw-type juice extractor and juice outlet

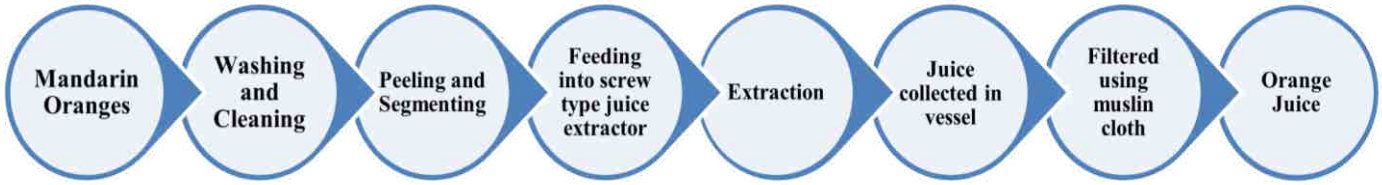


Figure 9: Flowchart of orange juice extraction

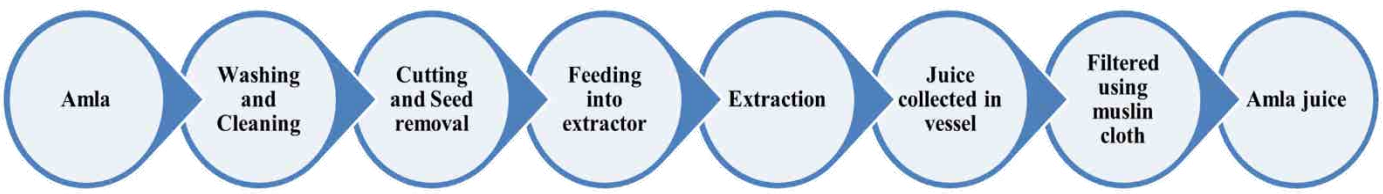


Figure 10: Flowchart of amla juice extraction

3.3.2. Extraction of ginger essential oil

Hydro-steam Distillation Method

Extraction of oil by using the Clevenger apparatus is a hydro steam distillation process, and the oil present in the ground sample was distilled with water, both were condensed and filled into the Clevenger oil trap. The oil floats on the surface of the water and it can be collected in a separator or receiver.

Fresh ginger (500 g) rhizomes were soaked in water for 2 hrs to remove the adhered mud from the surface. Cleaned and washed ginger was sliced and then transferred into the distillation flask. Water (3 litres) and a few boiling pellets were added to it and mixed by swirling. The flask was connected for 5 hours until there was no increase in the oil content over a period of 1 hour. The essential oil in the trap was measured and finally dried over anhydrous sodium sulphate to get a clear oil content. The collected essential oil was measured and reported as mL of essential oil / 100g of sample. The separated oil was collected in a vial, sealed with parafilm, and stored in the refrigerator.

Calculation:

Yield percentage of Essential oil content was calculated using the formula:

$$\text{Essential oil yield \%} = \frac{\text{Volume of collected oil (mL)}}{\text{Weight of sample taken}} \times 100$$

Figure 11: Extraction of Ginger essential oil by Hydro-steam distillation Method

3.4. PRODUCTION OF ANTIOXIDANT-RICH FRUIT BEVERAGE

A specific proportion of amla and orange juice was blended with water, followed by the addition of honey and citric acid. To enhance the flavour, ginger essential oil was incorporated. The mixture was then subjected to pasteurization at 85°C for 2-3 minutes. The beverage was then hot-filled into pre-sterilized PET bottles. UV-C treatment was applied to a subset of bottles to assess its effect on shelf life, and the bottles were stored in refrigerated conditions (4-6°C).

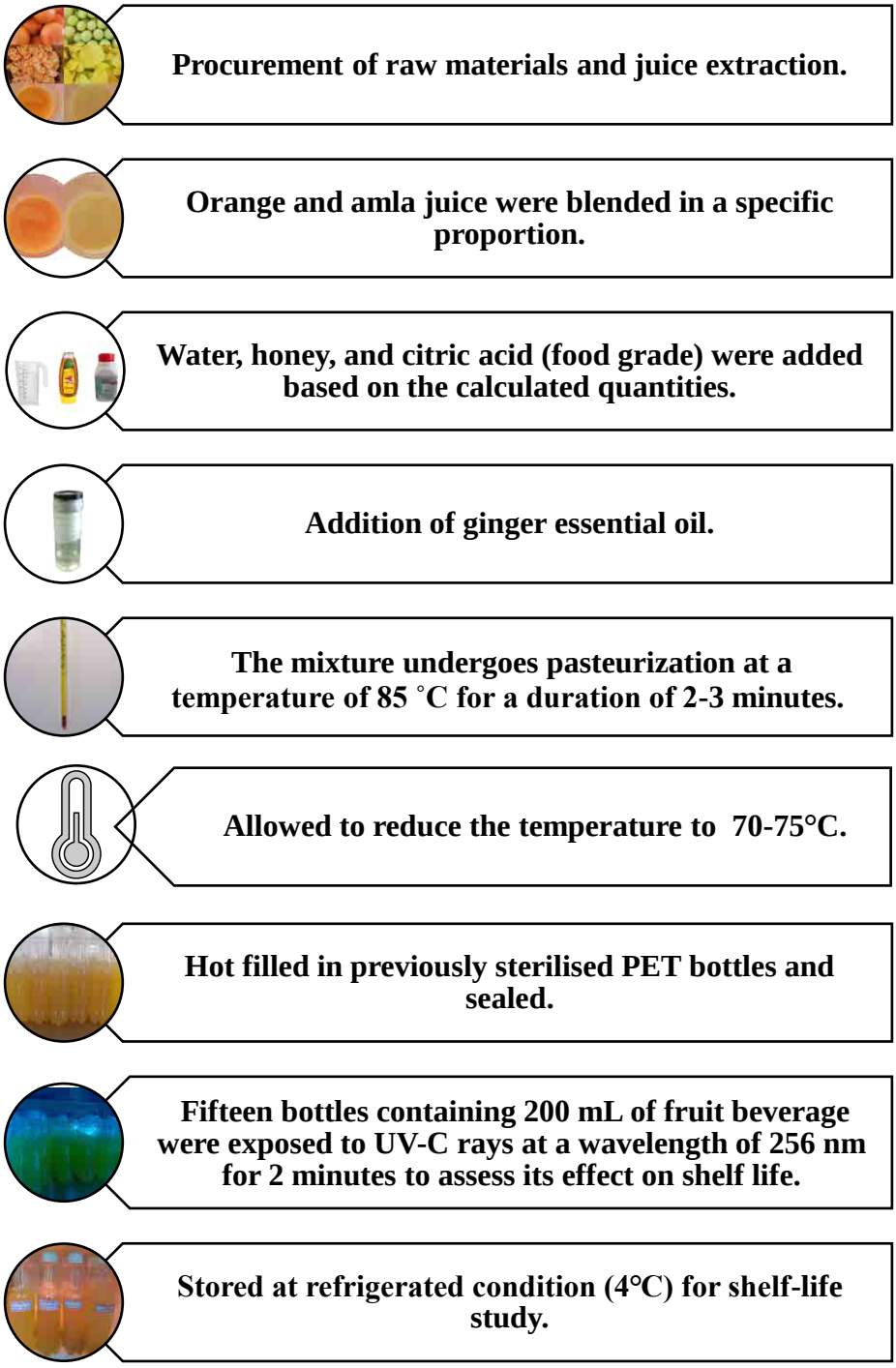


Figure 12: Flowchart of Production of Antioxidant-rich Fruit Beverage

3.5. pH MEASUREMENT

A statistical tool that tracks the hydrogen-ion activity in water-based solutions and determines their acidity or alkalinity, expressed as pH, is a pH meter, which measures pH values from 0.0 to 14.0 units. A solution is deemed basic if its pH is greater than 7.0 and acidic if its pH is less than 7.0. A neutral pH is 7.0. By monitoring the electrical potential between two electrodes—a reference electrode and a measuring electrode, a pH meter determines the pH of a solution. The pH of the solution was determined by the interaction between the measuring electrode and the hydrogen ions (H^+) in the solution when the electrodes were submerged in it.

Procedure

The pH meter was standardised using a buffer solution and then the pH of the sample was determined.

Figure 13: pH metre

3.6. TOTAL SOLUBLE SOLIDS (TSS)

The TSS value is the quantity of sugar and soluble minerals present in fruits and vegetables. Total Soluble Solids (TSS) of the fruit beverage were measured using a hand refractometer ranging from 0º – 32º Brix and the results were expressed as "ºBrix".

Figure 14: Hand Refractometer

3.7. TITRATABLE ACIDITY

5 mL of fruit juice was taken in a 100 mL measuring cylinder. The juice was diluted with 95 mL of distilled water and shaken well. A 10 mL aliquot of the diluted juice was transferred to a conical flask, and 90 mL of distilled water was added. The mixture was titrated with 0.1 N NaOH using phenolphthalein as an indicator, and the endpoint was determined by the appearance of a pink colour. The percentage of anhydrous ascorbic acid was then calculated based on the titration result.

$$\% \text{ acidity} = \frac{\text{titre} \times \text{Normality of alkali} \times \text{volume madeup} \times \text{Equivalent wt.of acid}}{\text{Volume of sample taken for estimation} \times \text{Wt. or volume of sample taken}} \times 100$$

Figure 15: Estimation of titratable acidity

3.8. ASCORBIC ACID (VITAMIN C)

2,6-Dichlorophenol-Indophenol - Visual titration method

The dye, which is blue in alkaline solution and red in acid solution, was reduced by ascorbic acid to a colourless form. The reaction was quantitative and practically specific for ascorbic acid in solution in the pH range 1-3.5.

Reagents

1. **3% Metaphosphoric acid** (HPO_3): Prepared by dissolving the sticks or pellets of HPO_3 in distilled water.
2. **Ascorbic acid standard**: 100 mg of L-ascorbic acid was weighed accurately and made up to 100 mL with 3% HPO_3. 10 mL of the solution was diluted to 100 mL with 3% HPO_3. (1mL = 0.1 mg of ascorbic 3acid).
3. **Dye solution**: 50 mg of the sodium salt of 2,6-dichlorophenol-indophenol was dissolved in approximately 150 mL of hot glass-distilled water containing 42 mg of sodium bicarbonate. The solution was then cooled and diluted to 200 mL with glass-distilled water. It was stored in a refrigerator and standardized every day.

Standardization of dye

5 mL of ascorbic acid solution and 5 mL of HPO_3 were taken. A micro burette was filled with the dye solution. The ascorbic acid solution was titrated with the dye solution until a pink color persisted for 15 seconds. The dye factor, which represents the mg of ascorbic acid per mL of the dye, was determined using the formula:

$$\text{Dye factor} = \frac{0.5}{\text{titre value}}$$

Preparation of Sample

Fruit juice: 10 mL of the sample was taken and made up to 100 mL with 3% HPO3. The mixture was then filtered or centrifuged.

Assay of extract

A 3 mL aliquot of the HPO_3 extract of the sample was titrated with the standard dye until a pink endpoint persisted for at least 15 seconds. An initial rapid titration was performed to estimate the titre, followed by a more precise titration where most of the dye was added initially, and the remaining amount was added carefully. The sample aliquot was chosen to ensure the titre value fell within the range of 3 to 5 mL.

Calculation

$$\text{mg of ascorbic acid per 100 g/mL} = \frac{\text{titre} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{aliquot of extact taken} \times \text{volume of sample taken}}$$

Figure 16: 2,6-Dichlorophenol-Indophenol - Visual titration method

3.9. ESTIMATION OF REDUCING SUGAR

Sample Preparation

10 g of sample was accurately weighed into 3 different conical flasks. 100 mL of water was added to each conical flask, and the mixture was stirred properly. The sample solution was then transferred to a 250 mL volumetric flask. 5 mL of neutral lead acetate was added, and the mixture was shaken and allowed to stand for 10 minutes. Subsequently, 5 mL of potassium oxalate was added. 20% NaOH was added dropwise using a phenolphthalein indicator until a faint pink colour was achieved. The volume was then made up to 250 mL, and the solution was mixed and filtered through a Whatman filter circle.

Note: If the solution was not faint pink when adding it to the burette:

i. For reducing sugar, 20% NaOH was added dropwise.
ii. For total sugar, 1:1 NaOH was added dropwise.

Preliminary Titration

5 mL of Fehling's A and 5 mL of Fehling's B were pipetted into a 250 mL conical flask and mixed. 10 mL of distilled water was added, and the solution was heated to boiling. Once boiling, 3 drops of methylene blue indicator were added. The sugar solution was then dispensed from the burette dropwise until the colour changed to brick red.

Final Titration

5 mL of Fehling's A and 5 mL of Fehling's B were pipetted into a 250 mL conical flask and mixed. The titre value or volume from the preliminary titration, minus 0.05 to 1 mL, was added to the flask. The mixture was heated to boiling, and as it boiled, the colour changed to brick red. 3 drops of methylene blue indicator were added, and the titration was completed within 1 minute by adding sugar solution dropwise until the endpoint was reached, marked by a brick red colour with no hint of purple or blue.

$$\% \text{ Reducing Sugar} = \frac{0.066 \times 250 \text{ volume of dilution}}{\text{titre value} \times \text{weight of sample}} \times 100$$

3.10. ESTIMATION OF TOTAL INVERT SUGAR

50 mL of the clarified and diluted solution was pipetted into a 250 mL volumetric flask, and 10 mL of 1:1 HCl was added. The flask was left at room temperature for 24 hours to allow for inversion. After 24 hours, the solution was neutralized with a concentrated NaOH solution (prepared by dissolving 5 g of NaOH in 5 mL of water) using phenolphthalein as an indicator. The solution was then diluted to 250 mL, and the total inverted sugar content was determined by titrating it as reducing sugar.

Preliminary Titration

5 mL of Fehling's A and 5 mL of Fehling's B were pipetted into a 250 mL conical flask, and 50 mL of distilled water was added. A sufficient amount of sample solution (approximately 30 mL) was added to almost completely reduce Fehling's solution. The mixture was heated to boiling on a hot plate and boiled for 15 seconds, during which a colour change was observed. 3 drops of methylene blue indicator were then added, and the titration was completed by adding the sample solution dropwise until the indicator was completely decolourized. The volume of sample solution required was recorded.

Final Titration

5 mL of Fehling's A and Fehling's B were pipetted into a flask. A burette was filled with the sample solution. The estimated volume of the sample solution from the preliminary titration, minus 0.05 to 1 mL, was added to Fehling's solution. The mixture was boiled for 2 minutes, and then 3 drops of methylene blue indicator were added. The titration was completed by adding the sample solution dropwise until the indicator was completely decolourized, resulting in a brick-red endpoint.

$$\% \text{ Total Sugar} = \frac{0.066 \times 250 \text{ dilution} \times 250}{\text{titre value} \times \text{weight of sample}} \times 100$$

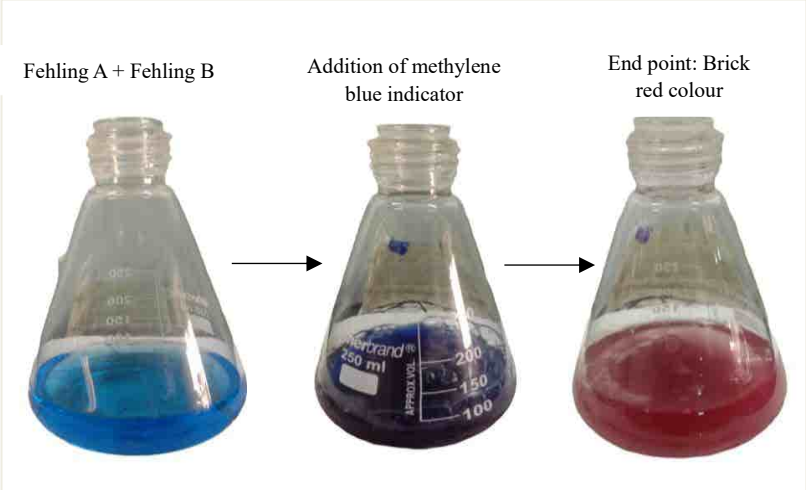


Figure 17: Different stages of Reducing Sugar and Total Invert Sugar Estimation

3.11. ANTIOXIDANT ACTIVITY

An 'antioxidant' is defined as a substance that inhibits free radicals and reactions promoted by oxygen.

It is well known that the antioxidant activity of plant extracts containing polyphenols and flavonoid components exhibits a wide range of biological effects due to their capacity to be donors of hydrogen atoms or electrons and to capture free radicals. Antioxidant activity was estimated by the DPPH method (Hossain, 2011) with modification. The analytical methods done during this investigation are free radical scavenging activity by DPPH method.

Free radical scavenging activity using DPPH assay

The presence of antioxidants was evaluated by using DPPH in different extracts by different solvents. Free radical scavenging of extracts was tested against an ethanolic solution of α, α -diphenyl -β- picrylhydrazyl (DPPH). The degree of discolouration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517 nm has been used as a measure of antioxidant activity (Blois, 1958).

Materials

- 2, 2-diphenyl-1-picrylhydrazyl radical, (4.9 mg of DPPH dissolved in 25 ml of ethanol).
- Ascorbic acid (AA): 1mg/mL.
- Ethanol (Analytical grade, Qualigens).
- UV -Visible Spectrophotometer

Procedure

The method of Brand-Williams et al., (1995) based on the reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solutions was applied with some modifications. DPPH (0.5mM) solution in ethanol was prepared at dark. Different concentrations equivalent to 100,200,300,400 and 500 ppm of fruit beverage extract were taken, and the total volume was made up to 2 mL with ethanol, followed by the addition of DPPH solution (1mL). The free radical scavenging activity was evaluated, by measuring the absorbance at 515 nm after 30 min of the incubation at 37 °C. The assay was standardised to get constant radical scavenging activity with an increase in extract concentration. Ascorbic Acid (AA) was used as standard. The radical scavenging activities of the tested samples were expressed as a percentage inhibition of DPPH.

Calculation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

3.12. DETERMINATION OF TOTAL PHENOLIC COMPOUNDS (TPC)

Folin-Ciocalteu Method

The total phenolic content of the fruit beverage was determined by spectrophotometric method. The Phenol group reacts with the Folin-Ciocalteu reagent to give blue coloured complex. The reagent has phosphomolybdate blue. Sodium carbonate maintains alkali conditions to increase the rate of reaction. The intensity of the blue colour developed is proportional to the concentration of phenolic compound present in the sample. The absorption is measured at 765 nm using a spectrophotometer after incubating for 30 minutes at room temperature.

Materials

1. 20% Sodium carbonate.
2. Folin-Ciocalteu reagent (1: 10)
3. Standard gallic acid: 100mg was dissolved in 100 mL distilled water.
4. Working: 100µl of stock made up to 1mL (100µg/ml).

Procedure

Different aliquots of standard gallic acid solution (200µl-1000µl) were taken whose concentrations ranged from 20µg-100µg. 6 ml of distilled water was added. 0.5ml of Folin-Ciocalteu reagent and 20% Sodium carbonate (1.5 mL) were added to all the tubes. The volume was made up to 10 mL with distilled water, mixed well and incubated at room temperature for 30 mins. The colour developed was read spectrophotometrically at 760nm against a reagent blank. A standard graph was plotted and the percentage of the phenolic compound was calculated using the standard graph. 50 & 100µl of each sample (solvent extracts) were taken and the procedure was followed as the standard and concentration of phenolic compounds were calculated using the standard graph. (Slinkard and Singleton, 1977).

3.13. DETERMINATION OF TOTAL FLAVONOID CONTENT(TFC)

Aluminium chloride colorimetric assay

Principle

A colorimetric assay using aluminium chloride was reported by Woisky and Salatino (1998) to detect flavonoids. In the test, aluminium chloride reacts with flavonoids to create a stable colour complex. The reactions of both the C-4 keto and the C-3 or C-5 hydroxyl groups initiated a stable complex of acid, while a few acid-labile complexes could be caused by the reaction of ortho hydroxyl in the A and B rings of a flavonoid. These flavonoid complexes offer maximum absorption at 510 nm. The content of the complex is proportional to its colour intensity.

Reagents

- 10% Aluminium chloride ($AlCl_3$)
- 5% Sodium Nitrite ($NaNO_2$)
- 1M Sodium Hydroxide (NaOH): 0.4 g of NaOH dissolved in 10 ml distilled water.
- Quercetin (1 mg/ml) was used as the standard in the TFC.

Procedure

To generate a standard curve, 3 mg of Quercetin was dissolved in deionized water and the volume was adjusted to 6 mL. Aliquots (20, 40, 60, 80, 160, and 200µl) of the 6 mL quercetin solution were transferred to test tubes and 0.5 mL of 5% sodium nitrite was added. The solution mixture was incubated at room temperature (RT) for 5 minutes, following which 0.5 mL of

10% aluminium chloride was added and incubated at RT for 6 minutes, 2 mL of 4% sodium hydroxide was added and the volume was adjusted to 10 mL using deionized water, the solution mixture was then approximately mixed and incubated at RT for 15 minutes. TFC was determined by measuring the absorbance at 510 nm using a UV spectrophotometer.

3.14. COLOUR MEASUREMENT

Colour of fruit beverage was measured using Hunter Lab colour measuring system (Minolta Lab scan XE, USA) by reflectance. The L*, a* and b* values were recorded using illuminant 'C' with 10 ° observer angle and 1" slit width. CIE, 1976 (L* a* b*) colour coordinates, represent L* for lightness or brightness of the sample and a* indicates red for positive value and green for negative value and b* indicates yellow for positive value and blue for negative values. The values were obtained with the help of inbuilt software provided with the instrument (Hunter, 1975) in Fig. 17.

Figure 18: Colour Measuring System

3.15. MINERAL ESTIMATION

Reagents and Standard solutions

Calibration standards were prepared for the analysis of the fruit beverage. A Multistandard IV solution, containing Ag, Al, B, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, and Zn, along with additional standard solutions of Si, As, P, Sb, and Mo at a concentration of 1000 ppm, were used to prepare the calibration solutions. These standard solutions were diluted to obtain calibration standards within the expected concentration range of the test elements.

Sample Preparation

The sample preparation was performed using wet digestion. A 2 mL portion of the fruit beverage was combined with 18 mL of 2% nitric acid. After 24 hours, the samples were diluted with distilled water purified by Fisher Chemical (HPLC grade) and filtered before MP- AES analysis.

Analysis of Samples

The quantitative analysis of the fruit beverage was performed on MP-AES (Microwave Plasma Atomic Emission Spectroscopy) (Savic et al., 2015).

3.16. CONSUMER ACCEPTANCE

The hedonic test was adopted to assess consumer acceptance of Antioxidant-rich Fruit Beverage. A 7-point hedonic scale ranging from "Like very much" to Dislike very much" with "Neither like nor dislike" as a midpoint was employed for this purpose. Respondents who participated in the study included staff, researchers and students from the institute. They ranged in age between 20 and 58 years of age. Each respondent was served about 20-25ml of RTS beverage in 3-digit coded glass beakers. The samples were chilled and served.

Scale	Description
1	Like Very Much
2	Like Moderately
3	Like Slightly
4	Neither Like nor Dislike
5	Dislike Slightly
6	Dislike Moderately
7	Dislike Very Much

Figure 19: 7- Point Hedonic Scale

3.17. MICROBIAL ANALYSIS

All chemicals required for the medium used in this study were procured from Hi Media Private Limited., Mumbai, India. The following three media were prepared according to the manufacturer's instructions.

3.17.1. Plate Count Agar (PCA)

Plate count agar was used to detect the total bacterial count. Plate count agar was prepared as per the instructions given in the bottle (i.e.) 23.5gm of PCA of media in 1000 mL distilled water. In the present study, 5.87 g of media dissolved in 250 mL of distilled water was taken in a conical flask, mixed well, plugged with cotton and sterilized by using an autoclave at 121℃ (15 lbs pressure) for 20 minutes. It was cooled to 45 - 50℃, mixed well and poured into pre-

sterilized petri plates at room temperature under laminar airflow. With the help of sterile micropipettes, 1mL of the sample was plated immediately on respective media aseptically and rotated gently to ensure uniform mixing of the sample with agar and allowed the plates to settle before incubation. Control plates were maintained without the addition of sample. After the media was set, the plates were immediately kept for incubation. The colonies developed after the incubation period at 37℃ for 24 hours were counted and expressed as CFU/mL.

Figure 20: Plate Count Agar Media

3.17.2. Potato Dextrose Agar (PDA)

Potato Dextrose agar (PDA) was used to detect yeast and mould count. PDA agar was prepared as per instruction given in the bottle (i.e.) 39 g of PDA media dissolved in 1000 mL distilled water. In the present study, 9.75 g of PDA media dissolved in 250 mL of distilled water was taken in a conical flask, mixed well, plugged with cotton and sterilized by using an autoclave at 121℃ (15 lbs pressure) for 20 minutes. It was cooled to 45 -50℃, mixed well and poured into pre-sterilized petri plates at room temperature under laminar airflow. With the help of sterile micropipettes, 1 mL of the sample was plated immediately on the respective media aseptically and rotated gently to ensure uniform mixing of the sample with agar and allow the plates to settle before incubation. Control plates were maintained without the addition of sample. After the media was set, the plates were immediately kept for incubation. The colonies developed after the incubation period at 30℃ for 48 to 72 hours were counted and expressed as CFU/mL.

Figure 21: Potato Dextrose Agar Media

3.17.3. Violet Red Bile Agar (VRBA)

VRBA media was used to detect coliform**.** The media was commercially available and prepared as per the instructions given in the bottle. In the present study, 10.3 g of media and 1 g Nutrient agar were dissolved in 250 ml of distilled water within a conical flask, mixed well and plugged with a cotton plug. This media does not require an autoclave. It needs to be boiled for some time, cooled to 45 -50°C and immediately poured onto sterile petri plates at room temperature under laminar airflow. With the help of sterile micropipettes, 1 mL of the sample was plated immediately on the respective media aseptically and rotated gently to ensure uniform mixing of the sample with agar and allow the plates to settle before incubation. Control plates were maintained without the addition of sample. After the media was set, the plates were immediately kept for incubation. The colonies developed after incubation period at 37°C for 24 to 48 hours were counted and expressed as CFU/mL.

Figure 22: Violet Red Bile Agar Media

3.18. STORAGE STUDIES

Storage studies of the antioxidant-rich fruit beverage with and without UV treatment were carried out for a period of 60 days under refrigerated conditions (4-6°C). These samples were withdrawn periodically and analyzed for their physico–chemical parameters like pH, titratable acidity, TSS, Ascorbic acid content, reducing and total sugar estimation, colour measurement and microbiological analysis. The antioxidant activity, total phenolic content, and total flavonoid content were also determined. To understand the sensory appeal of antioxidant-rich fruit beverage a consumer acceptance study was also done.

CHAPTER 4
RESULT AND DISCUSSIONS

4. RESULT AND DISCUSSIONS

The goal of this study was to develop an antioxidant-rich fruit beverage from amla and orange juice with the addition of ginger essential oil, analyse its physicochemical and antioxidant properties, and assess the effect of UV treatment during storage. This chapter presents and discusses outcomes from the study during production and storage.

4.1. EVALUATION OF FRUIT BEVERAGE PRODUCTION

4.1.1. Analysis of fruit juice

The antioxidant-rich fruit beverage was primarily formulated using amla and orange juice. Raw materials of optimal maturity and quality were selected and processed into juice. The TSS, TA, pH, and Ascorbic Acid content of the orange and amla juice before and after pasteurization (85℃) are shown in Table 5.

Table 5: Physicochemical properties of amla and orange juice

AMLA JUICE	**Before pasteurisation**	**After pasteurisation**
pH	2.85	2.92
TSS	6˚Brix	8˚Brix
TA	0.5984	0.4224
mg of ascorbic acid per 100 ml	300 mg	156.25 mg
ORANGE JUICE	**Before pasteurisation**	**After pasteurisation**
pH	4.5	4.52
TSS	9˚Brix	12˚Brix
TA	0.1408	0.1408
mg of ascorbic acid per 100 ml	15 mg	12.25 mg

The beverage was initially prepared using three different ratios of amla and orange juice; 50:50, 40:60 and 30:70 represented as AO 1, AO 2, and AO 3 respectively. The TSS, TA, pH, and Ascorbic Acid content before and after heat treatment are presented in Table 6.

Table 6: Physicochemical properties of fruit beverage in different formulations

AO 1(50:50)	**Before pasteurisation**	**After pasteurisation**
pH	2.91	2.88
TSS	7˚Brix	7˚Brix
TA	0.088	0.1408
mg of ascorbic acid per 100ml	20 mg	17.5 mg
AO 2 (40:60)	**Before pasteurisation**	**After pasteurisation**
pH	3.05	3.02
TSS	9˚Brix	12˚Brix
TA	0.1408	0.1584
mg of ascorbic acid per 100 ml	17.5 mg	15 mg
AO 3 (30:70)	**Before pasteurisation**	**After pasteurisation**
pH	3.12	3.07
TSS	9˚Brix	14˚Brix
TA	0.1584	0.1584
mg of ascorbic acid per 100 ml	15 mg	10 mg

After thoroughly assessing the physicochemical properties and sensory qualities of various formulations, formulation AO 2, which contains a specific ratio of orange and amla juice (40:60), was identified as the most acceptable one. Consequently, this formulation was selected for large-scale production and a comprehensive storage study to evaluate its stability, quality retention, and overall acceptability over time.

4.1.2. Extraction efficiency of fruit juices in large-scale production

Good quality oranges and amla were washed, peeled, cleaned, and cut into small segments before being processed in a screw-type juice extractor. This method efficiently separated the juice from the pulp and seeds. The juice yield was 60% for oranges and 76.85% for amla, as indicated in Table 7.

Table 7: Fruit juice yield

Fruit	Initial wt.	Juice volume	Yield %
Orange (Mandarin)	4 Kg	2.40 litres	60%
Amla	3.5 Kg	2.69 litres	76.85%

4.1.3. Extraction yield of Ginger essential oil

Ginger essential oil was obtained through hydro-steam distillation using a Clevenger apparatus, a widely recognized method for extracting volatile oils from plant materials. The distillation process yielded an essential oil content equivalent to 0.4% of the total weight of the ginger used as shown in Table 8. This percentage represents the efficiency of the extraction process and the concentration of essential oil present in the raw material.

Table 8: Ginger Essential oil yield

Wt. before cleaning	Wt. after cleaning	% Yield
700 g	500.64 g	0.40%

4.1.4. Production of antioxidant-rich fruit beverage

The large-scale production of the selected fruit beverage formulation was carried out in the pilot plant, following the processing steps outlined in Figure 12. A portion of the bottled beverage underwent UV-C treatment at a wavelength of 256 nm for 2 minutes before being stored under refrigerated conditions (4–6°C). The untreated fruit beverage was represented as AFB 1 and the UV-treated ones as AFB 2. A storage study was conducted to compare the shelf life of UV-treated beverage with those that did not receive UV treatment to assess the effect of UV treatment on the shelf life of fruit beverages.

Figure 23: AFB 1 (untreated)

Figure 24: AFB 2 (UV treated)

4.2. EFFECT ON pH

The pH of the fruit beverage is directly proportional to the inherent acids in the fruits used for preparation and indirectly depends on the organic acids added during product formulation. The pH of the samples shown in Table 9 indicates a gradual decline over the 60 days, reflecting a consistent acidification trend. AFB 1 (Untreated) started at 2.93 and decreased to 2.88 by the 30th day, while AFB 2 (UV Treated) began at 3.04 and declined to 2.85. This slight decrease in pH suggests ongoing biochemical changes, potentially due to organic acid production resulting from the degradation of ascorbic acid (Purewal et al., 2022).

Table 9: pH of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	2.93	2.9	2.88	_	_
AFB 2 (UV-treated)	3.04	2.98	2.94	2.91	2.85

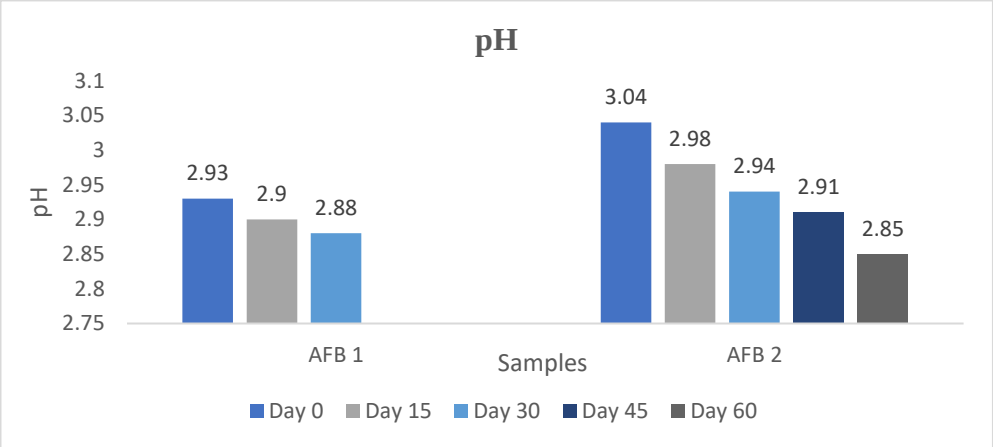


Figure 25: pH variations in product during storage

4.3. EFFECT ON TOTAL SOLUBLE SOLIDS (TSS)

The total soluble solids (TSS) in fruit juice is a critical parameter that reflects the sweetness and overall quality of the fruit beverage. TSS is measured in degrees Brix and represents the percentage of soluble solids, primarily sugars, in the fruit beverage. The total soluble solids (°Brix) of both AFB 1 (Untreated) and AFB 2 (UV Treated) remained constant at 9° Brix throughout the 60-day study period as shown in Table 10. This stability suggests that no significant sugar degradation, fermentation, or dilution occurred during storage. The consistency in °Brix values indicates that the UV treatment method did not affect the soluble solid content of the sample.

Table 10: TSS of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	9˚ Brix	9˚ Brix	9˚ Brix	_	_
AFB 2 (UV-treated)	9˚ Brix	9˚ Brix	9˚ Brix	9˚ Brix	9˚ Brix

4.4. EFFECT ON TITRATABLE ACIDITY (TA)

Table 11 represents the titratable acidity of both AFB 1 (Untreated) and AFB 2 (UV Treated) increased over the 60-day storage period, indicating a gradual accumulation of acidic compounds. AFB 1 (Untreated) started at 1.17 and rose to 1.62 by 30th day, while AFB 2 (UV Treated) began at 1.15 and increased to 1.58 by 60th day. The increase in acidity suggests ongoing biochemical changes, likely due to organic acid production (Purewal et al., 2022). The slightly lower acidity in AFB 2 (UV Treated) compared to AFB 1 (Untreated) may indicate that UV treatment slowed down microbial growth or enzymatic reactions responsible for acid formation.

Table 11: TA of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	1.17	1.40	1.62	_	_
AFB 2 (UV-treated)	1.15	1.23	1.40	1.40	1.58

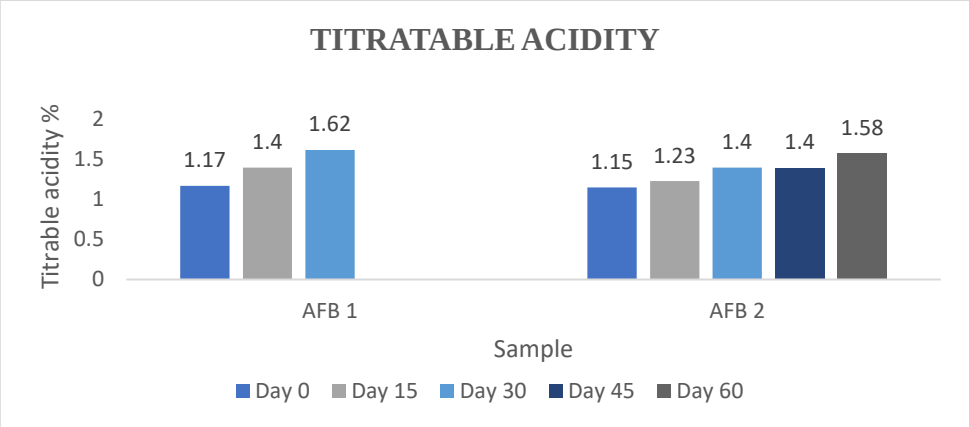


Figure 26: TA variations in product during storage

4.5. EFFECT ON ASCORBIC ACID

The ascorbic content was analysed using 2,6-dichlorophenol-indophenol (DCPIP) titration method. The ascorbic acid content in both AFB 1 (Untreated) and AFB 2 (UV Treated) showed a gradual decline over the storage period as shown in Table 12, indicating vitamin C degradation. AFB 1 (Untreated) initially had a higher ascorbic acid content (25 mg) but decreased to 12.24 mg by 30th day, whereas AFB 2 (UV Treated) started at 16.7 mg and declined gradually to 10.02 mg. The slower degradation in AFB 2 (Untreated) suggests that UV treatment helped to slow down the loss of ascorbic acid, possibly by reducing oxidation process. However, the decline in both samples is expected due to natural oxidation and environmental exposure.

Table 12: Ascorbic acid content in untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	25 mg	17.5 mg	12.24 mg	_	_
AFB 2 (UV-treated)	16.7 mg	15 mg	13.91 mg	11 mg	10.02 mg

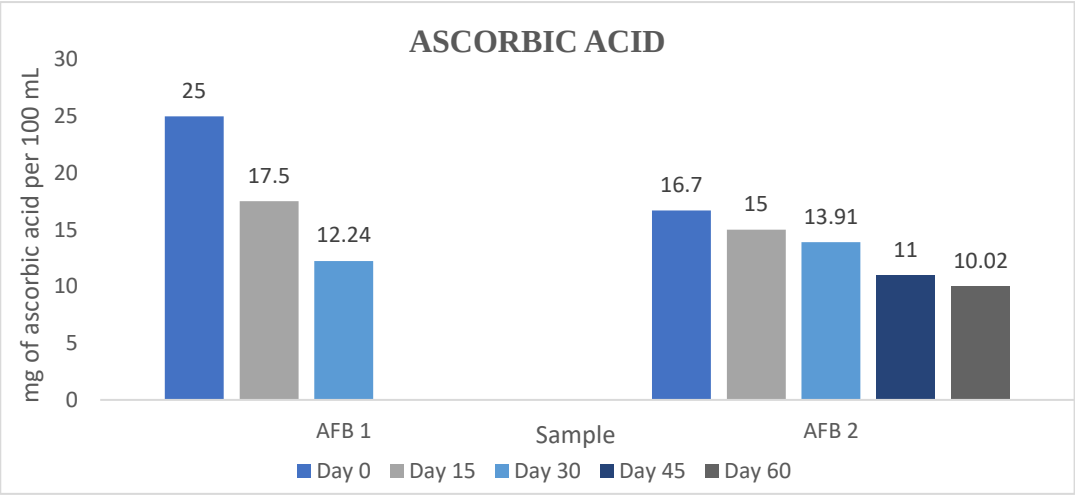


Figure 27: Ascorbic acid content variations in product during storage

4.6. EFFECT ON REDUCING SUGAR

The reducing sugar may rise due to the acidic hydrolysis of non-reducing sugars thereby producing reducing sugars with the increase in storage time (Zia et al., 2019). The percentage of reducing sugars in both AFB 1 (Untreated) and AFB 2 (UV Treated) showed a gradual increase over the storage period as shown in Table 13. AFB 1 (Untreated) increased from 9.29% on the 0^{th} day to 10.5% by 30^{th} day, while AFB 2 (UV Treated) started at 9.4% and rose to 11.37%.

Table 13: % Reducing sugar of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	9.29%	9.94%	10.5%	_	_
AFB 2 (UV-treated)	9.4%	9.93%	11.02%	11.34%	11.37 %

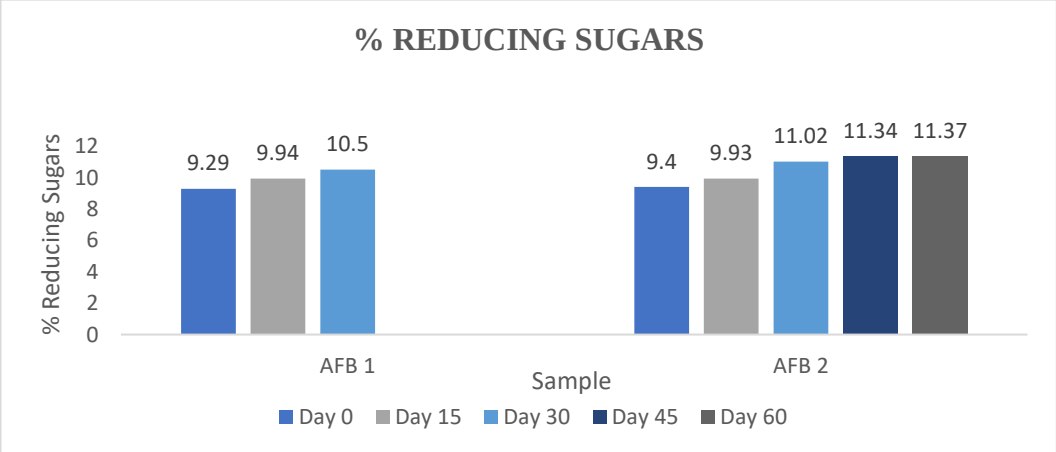


Figure 28: % Reducing Sugar variations in product during storage

4.7. EFFECT ON TOTAL INVERT SUGAR

The solubilisation of pulp components and the hydrolysis of polysaccharides, such as pectin and starch materials, are responsible for the increase in total sugar over the storage period During storage, polysaccharides may undergo enzymatic breakdown into simpler sugars, which results in increased total sugar content (Faizi et al., 2019; Hossain et al., 2016). The total invert sugar percentage in both AFB 1 (Untreated) and AFB 2 (UV Treated) gradually increased over the storage period as shown in Table 14, indicating ongoing sugar conversion. AFB 1 (Untreated) increased from 10.69% on 0^{th} day to 11.7% by the 30^{th} day, while AFB 2 (UV Treated) showed a slightly higher initial value of 11.53%, reaching 12.09% by the end of the study. The rise in invert sugar suggests enzymatic hydrolysis of sucrose into glucose and fructose.

Table 14: % Total Invert sugar of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	10.69%	11.51%	11.7%	_	_
AFB 2 (UV-treated)	11.53%	11.57%	11.64%	11.97%	12.09 %

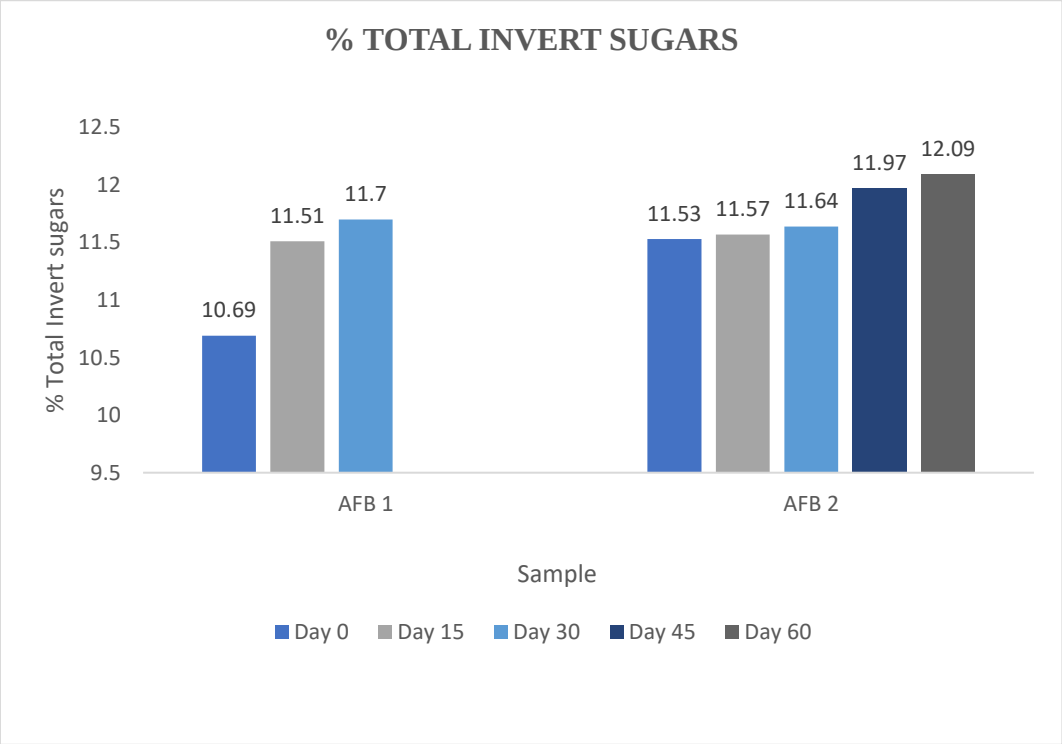


Figure 29: % Total Invert Sugar variations in product during storage

4.8. EFFECT ON ANTIOXIDANT ACTIVITY

Figure 30 represents the effect of storage time on the % DPPH inhibition activity of the samples. AFB 1 (Untreated) and AFB 2 (UV-treated) showed differences in the percentage of DPPH inhibition values as shown in Table 15. AFB 2 demonstrated a relatively slower rate of decrease compared to AFB 1. This suggests that AFB 2 may possess more stable bioactive compounds or better resistance to oxidative degradation. During the storage period, a decreasing trend was observed in the % DPPH inhibition activity of studied samples. The decrease in antioxidant properties during storage may be due to the potential oxidation of bioactive components under suitable conditions (Dar et al., 2016).

Table 15: % DPPH inhibition activity of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	81.62%	81.41%	75.67%	_	_
AFB 2 (UV-treated)	79.9%	79.59%	76.27%	73.42%	72.79%

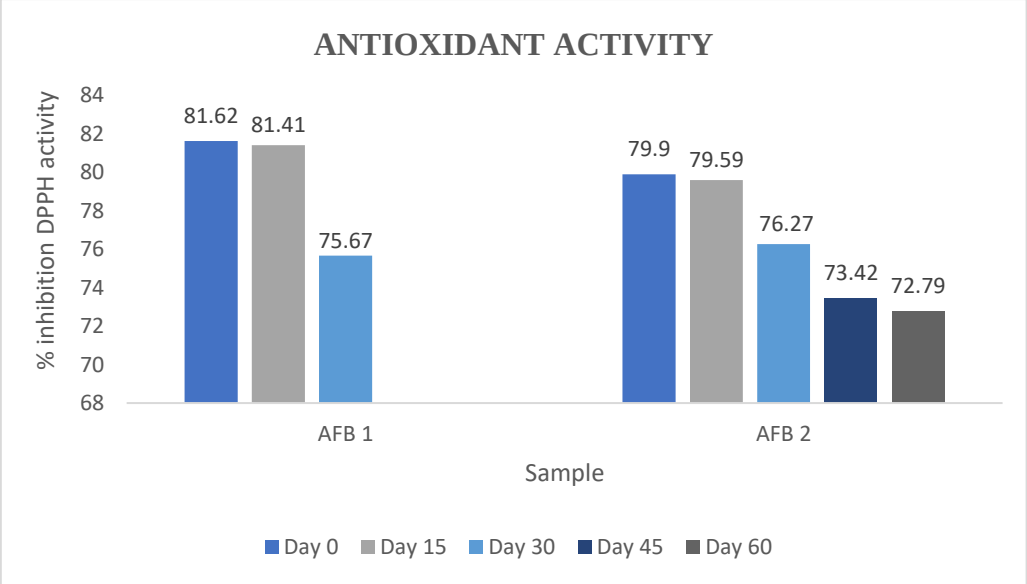


Figure 30: % DPPH inhibition activity variations in product during storage

4.9. EFFECT ON TOTAL PHENOLIC CONTENT (TPC)

As shown in Table 16, the total phenolic content of both AFB 1 (Untreated) and AFB 2 (UV Treated) samples decreased progressively during the storage period, indicating a gradual degradation of phenolic compounds over a period of time. AFB 1 (Untreated) dropped from 52.61 to 47.24 on 30th day, while AFB 2 (UV Treated) declined from 52 to 39.82 on 60th day. The reduction in phenolic content may be attributed to oxidative degradation, enzymatic activity, or interactions with other components during storage. AFB 2 (UV Treated) retained a higher level of phenolics compared to AFB 1 (Untreated) throughout the study, suggesting that UV treatment may have played a protective role in slowing down phenolic degradation.

Table 16: TPC (mg GAE/100ml) of untreated (AFB 1) and UV-treated (AFB 2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	52.61	49.72	47.24	_	_
AFB 2 (UV-treated)	52	50.04	48.65	43.43	39.82

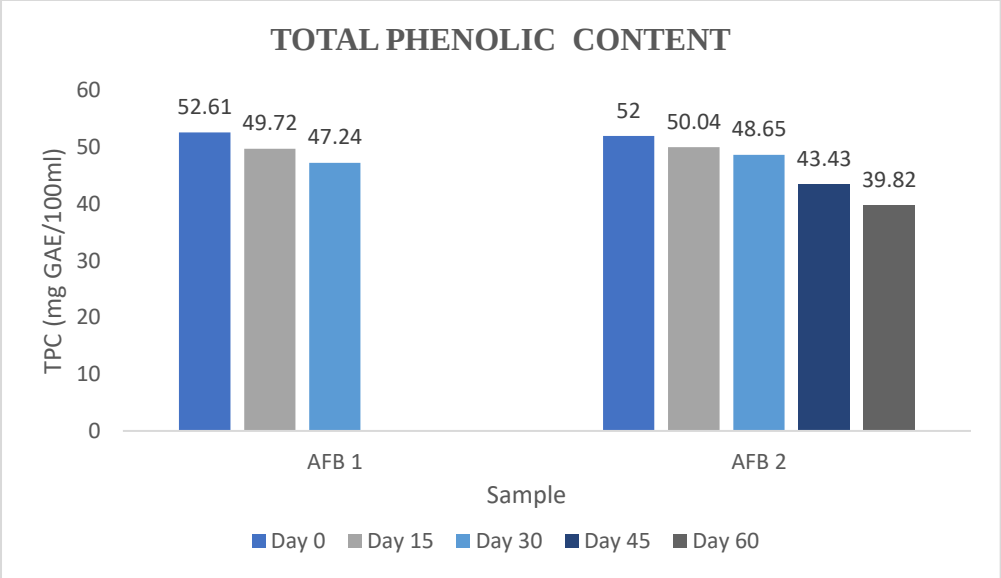


Figure 31: TPC (mg GAE/100ml) variations in product during storage

4.10. EFFECT ON TOTAL FLAVONOID CONTENT (TFC)

Table 17 shows the total flavonoid content in both AFB 1 (Untreated) and AFB 2 (UV- treated) samples which gradually declined throughout the storage period, indicating the degradation of flavonoid compounds over a period of time. AFB 1 (Untreated) initially measured 17.36, decreasing to 16.66 by 30th day, whereas AFB 2 (UV Treated) exhibited a slightly slower reduction, from 17.39 to 15.32 on 60th day. This decline can be attributed to oxidative degradation, enzymatic reactions, or interactions with other compounds during storage. However, the relatively higher flavonoid retention in AFB 2 (UV Treated) indicates that UV treatment may have contributed to delaying flavonoid degradation, potentially by inhibiting enzymatic oxidation.

Table 17: Flavonoid content (QE µg/100 mL) of untreated (AFB1) and UV-treated (AFB2) fruit beverage during storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60
AFB 1 (Untreated)	17.36	16.82	16.66	_	_
AFB 2 (UV-treated)	17.39	17.16	16.94	16.38	15.32

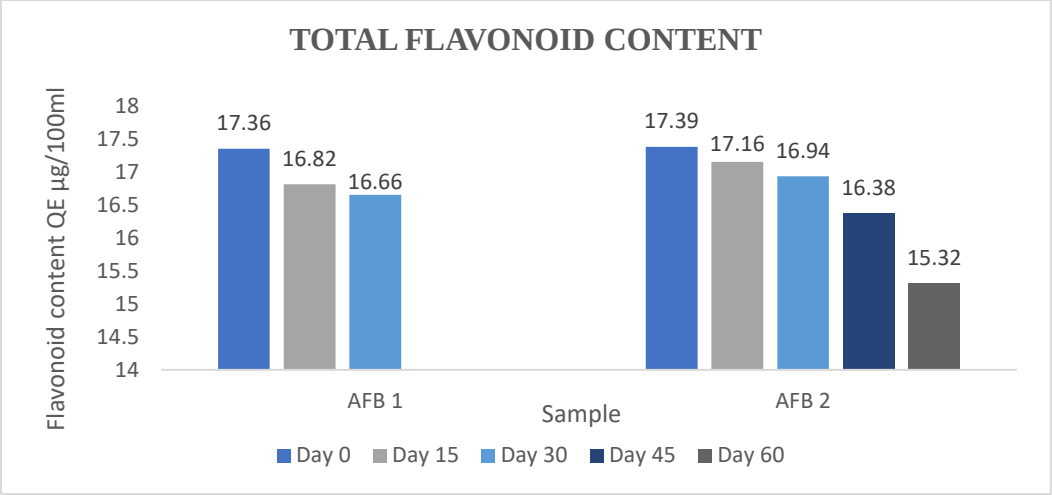


Figure 32: Flavonoid content (QE µg/100mL) variations in product during storage

4.11. COLOUR MEASUREMENT

One of the most crucial sensory characteristics for determining a product's quality and freshness is colour. Fruit qualities (such as variety, ripeness, and growth circumstances), juice extraction technique, and treatment or processing all have an impact on the sample colour. As shown in Tables 18 and 19, the colour parameters (L*, a*, b*) of both AFB 1 (Untreated) and AFB 2 (UV Treated) changed over the storage period. In AFB 1, the L* value (lightness) decreased from 20.50 to 19.08, while b* (yellowness) reduced from 9.35 to 7.43, suggesting darkening and loss of yellow tones. The a* values remained negative with minor variation, indicating a stable greenish tint. Similarly, AFB 2 showed a decline in L* from 19.77 to 16.99 and a slight reduction in b* from 7.38 to 6.77.

Table 18: Colour measurement of AFB 1 (Untreated) fruit beverage during storage

AFB 1 (Untreated)	L*	a*	b*
Day 0	20.50 ± 0.04	- 1.32 ± 0.02	9.35 ± 0.09
Day 15	19.87 ± 0.01	- 1.25 ± 0.23	7.56 ± 0.36
Day 30	19.08 ± 0.26	- 0.94 ± 0.01	7.43 ± 0.12

Table 19: Colour measurement of AFB 2 (UV-treated) fruit beverage during storage

AFB 2 (UV-treated)	L*	a*	b*
Day 0	19.77 ± 0.10	- 0.85 ± 0.04	7.38 ± 0.08
Day 15	19.05 ± 0.21	- 0.92 ± 0.03	7.21 ± 0.40
Day 30	18.95 ± 0.09	- 1.07 ± 0.02	7.20 ± 0.06
Day 45	18.56 ± 0.04	- 1.08 ± 0.00	7.16 ± 0.04
Day 60	16.99 ± 0.11	- 1.03 ± 0.00	6.77 ± 0.04

4.12. MINERAL ESTIMATION

The mineral content in a mixed beverage of amla and orange is influenced by the individual mineral profiles of these fruits. Amla is known for its high ascorbic acid content, while oranges are rich in β-carotene and total carotenoids. These fruits, when combined, can create a beverage with a diverse mineral and antioxidant profile. The mineral content of such a beverage would likely include potassium, calcium, magnesium, and trace elements like copper and zinc, which are common in fruit juices (Puri, 2016) (Goran et al., 2009).

Table 20 represents the mineral content in AFB 1 and AFB 2 during the period of storage. The major minerals including Ca, K, Na, Mg, and P and minor minerals like Fe, Zn, and Cu were analysed using Microwave Plasma Atomic Emission Spectroscopy. The results indicate that the mineral content in AFB 2 (UV-treated) has higher value for Ca, Mg, Na and Fe when compared to AFB 1 (Untreated).

Table 20: Comparison of Mineral Content (ppm) in Untreated (AFB 1) and UV-Treated (AFB 2)

Minerals	AFB 1 (ppm)	AFB 2 (ppm)
Ca	5.72	8.34
K	9.53	8.44
Na	7.51	7.73
Mg	2.66	3.08
P	5.86	5.08
Fe	0.1	0.2
Zn	0.08	0.08
Cu	0.02	0.03

4.13. EFFECT ON MICROBIAL QUALITY

Fruit beverages are highly susceptible to microbial invasion, which reduces the shelf of beverages. Food safety is an essential component of food quality. Some foods have natural antioxidants and antimicrobial properties and can be stored effectively for a long period without any changes. (Shadwaj & Pandey, 2011).

Table 21 shows that microbial analysis of AFB 1 (untreated) and AFB 2 (UV-treated) revealed significant differences in microbial growth over time. In AFB 1, microbial growth was found on 30^{th} day, with bacterial count 2×10^2 CFU/mL and yeast and mould counts reaching 3×10^2 CFU/mL. No coliforms (VRBA) were detected. In contrast, AFB 2 (UV-treated) showed no microbial growth up to 45^{th} day, suggesting UV treatment effectively inhibited microbial growth. These findings suggest that UV treatment has the potential to extend the shelf life and improve the microbial safety of fruit-based beverages by reducing bacterial and fungal contamination. This delay in microbial proliferation in AFB 2 highlights the effectiveness of UV treatment as a non-thermal preservation method, which can be especially beneficial for retaining the nutritional and sensory qualities of fruit-based beverages.

Table 21: Microbial Load (CFU/ml) of Untreated (AFB 1) and UV-Treated (AFB 2) Fruit Beverage During Storage

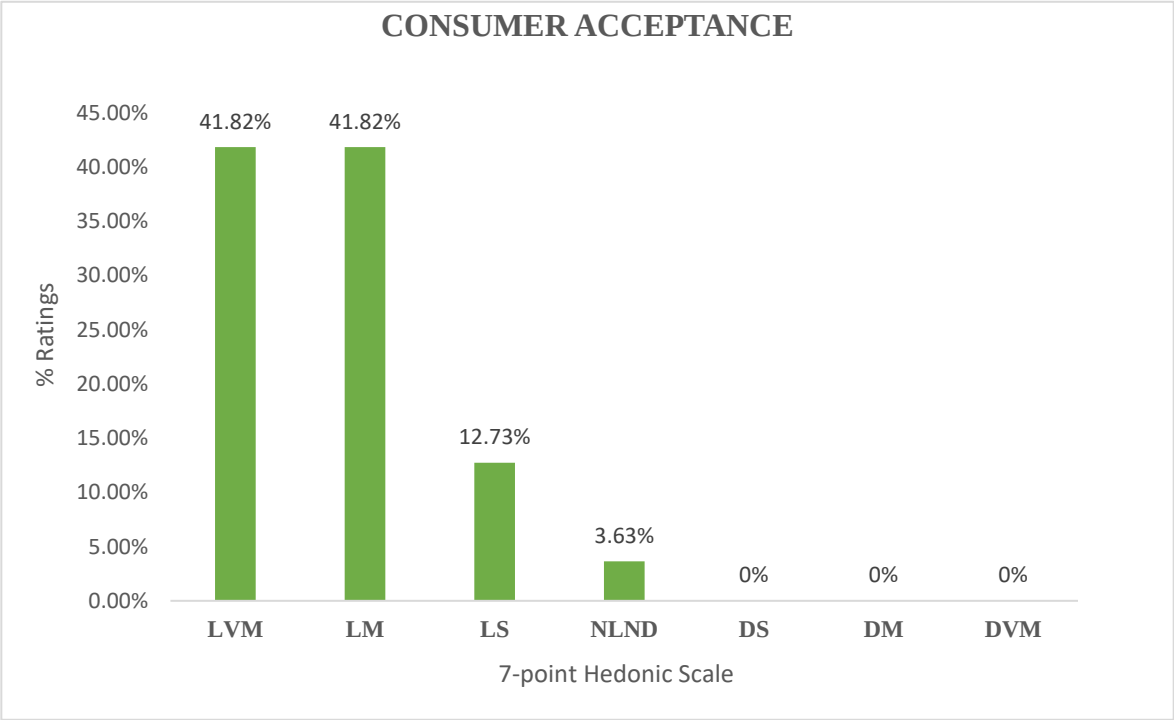
AFB 1 (Untreated)			
Days	**PCA (CFU/ml)**	**PDA (CFU/ml)**	**VRBA (CFU/ml)**
Day 0	Nil	Nil	Nil
Day 15	Nil	Nil	Nil
Day 30	2×10^2	3×10^2	Nil
AFB 2 (UV-treated)			
Days	**PCA (CFU/ml)**	**PDA (CFU/ml)**	**VRBA (CFU/ml)**
Day 0	Nil	Nil	Nil
Day 15	Nil	Nil	Nil
Day 30	Nil	Nil	Nil
Day 45	Nil	Nil	Nil
Day 60	3×10^2	1×10^2	Nil

4.14. CONSUMER ACCEPTANCE

The Hedonic test was conducted to assess consumer acceptance of the antioxidant-rich fruit beverage in the initial stage. Over 55 respondents, including staff and students from various departments at CSIR-CFTRI, Mysuru aged between 20 and 58 years, participated in the study. The results revealed that 41.82% of respondents rated it as "Like Very Much," while another 41.82% rated it as "Like Moderately." Additionally, 12.73% of participants gave a rating of "Like Slightly." Only 3.63% of respondents placed the beverage in the "Neither Like nor Dislike" category. Overall, 96% of respondents rated the beverage within the "Like" category, while only 3.64% fell into the "Neither Like nor Dislike" category.

Respondents noted that the product had a distinct ginger flavour with a slightly sour aftertaste. Some suggested increasing the sweetness for better balance. The colour and consistency were deemed appropriate. Overall, the beverage was described as refreshing, though many preferred a sweeter taste, and it was perceived as a good health drink.

The prepared samples were planned for consumer acceptance study at the initial and end of the storage period. However, the AFB 1 (untreated) fruit beverage initiated microbial growth at the end of 30th day and the AFB 2 (UV-C treated) sample on 60th day. Hence both the samples were not subjected for further consumer acceptance study.



LVM: Like Very Much, **LM**: Like Moderately, **LS**: Like Slightly, **NLND**: Neither Like nor Dislike, **DS**: Dislike slightly, **DM**: Dislike Moderately, **DVM**: Dislike Very Much

Figure 33: Consumer acceptance study of Antioxidant-rich fruit beverage

CHAPTER 5
CONCLUSION

5. CONCLUSION

Based on the research study conducted on the development of antioxidant-rich fruit beverage using amla and orange juice with the addition of ginger essential oil and the evaluation of the effect of UV-C treatment on such fruit beverage, the following conclusions were drawn:

- **Successful Development of a Functional Beverage**: The combination of Amla and orange juice resulted in a nutrient-dense beverage rich in vitamin C, phenolics, and flavonoids, all of which contribute significantly to antioxidant activity. The formulation meets the growing demand for health-oriented, functional beverages that offer additional physiological benefits beyond basic nutrition.
- **Significant Impact of UV Treatment**: UV treatment, used as a non-thermal preservation technique, proved to be highly effective in maintaining the overall quality of the beverage. It not only enhanced the shelf life but also preserved sensitive bioactive compounds better than the untreated counterpart. The UV-treated beverage (AFB 2) showed reduced degradation in antioxidants and better microbial quality for 60 days of storage.
- **Superior Retention of Nutritional Properties**: Over the 60-day storage period, the UV-treated sample consistently retained higher levels of ascorbic acid, total phenolic content, and flavonoids when compared to the untreated sample. This helped maintain the beverage's antioxidant potential.
- **Enhanced Microbial Stability**: Microbial analysis indicated that AFB 2 (UV-treated) remained free from bacterial and fungal contamination up to 45^{th} day, whereas AFB 1 (untreated) showed microbial growth at the end of 30 days. This highlights the effectiveness of UV light in delaying spoilage and ensuring food safety during storage.
- **Preservation of Essential Minerals**: Mineral content analysis confirmed that both AFB 1 and AFB 2 retained important dietary minerals such as calcium, magnesium, potassium, phosphorus, and iron. The mineral content in AFB 2 (UV-treated) has higher value for Ca, Mg, Na and Fe when compared to AFB 1 (Untreated).
- **Better Colour Stability**: The colour parameters (L*, a*, b*) of both untreated (AFB 1) and UV-treated (AFB 2) fruit beverages showed a gradual decline during the 60-day storage period, indicating slight degradation in visual quality over time. However, the UV-treated sample (AFB 2) exhibited better colour stability, with relatively slower decreases in lightness (L*) and chromatic values (a* and b*), compared to the untreated beverage.
- **High Consumer Acceptability**: The hedonic sensory evaluation, involving a diverse group of participants aged 20–58 years, showed a strong positive response over the consumer acceptance of the antioxidant-rich fruit beverage in the initial stage. Approximately 96% of respondents rated the beverage within the "Like" category, with a significant number expressing strong preference. This confirms the product's commercial viability and consumer acceptance. The prepared samples were planned for consumer acceptance study at the initial and end of the storage period. However, the AFB 1 (untreated) fruit beverage initiated microbial growth at the end of 30^{th} day and the AFB 2 (UV-C treated) sample on 60^{th} day. Hence both the samples were not subjected for further consumer acceptance study.

In conclusion, this research study successfully developed a nutrient-rich functional beverage and analysed the effectiveness of UV-C treatment in preserving its quality and nutritional properties. This study indicates that the developed RTS beverage had a shelf life of 30 days for AFB 1(Untreated sample) and 60 days for AFB 2 (UV-treated sample) under refrigerated conditions. The UV-treated sample showed superior retention of antioxidants, enhanced microbial stability, and better colour stability. The study highlights the potential of UV-C treatment as a non-thermal preservation technique for developing safe, healthy, and appealing functional beverages that meet the growing demand for health-oriented products.

CHAPTER 6
REFERENCE

6. REFERENCE

- Afam I. O. Jideani, Henry Silungwe, Thakhani Takalani, Adewale O Omolola, Henry O Udeh & Tonna A Anyasi (2021) Antioxidant-rich natural fruit and vegetable products and human health, International Journal of Food Properties, 24:1, 41-67, DOI: 10.1080/10942912.2020.1866597
- Aleem, M., Khan, M. I., Shakshaz, F. A., Akbari, N., & Anwar, D. (2020). Botany, phytochemistry and antimicrobial activity of ginger (Zingiber officinale): A review. International Journal Herbal Medicine, 8(6), 36–49. https://doi.org/10.22271/ f lora.2020.v8.i6a.705
- Anuradha, M. (2019). *Functional beverages: Trends and opportunities*. Journal of Food Science and Technology, 56(4), 1456–1468.
- Arthur, R. (2021) Unprecedented Growth for Asia Beverage Market; Beverage Daily: West Sussex, UK.
- Asante-Donyinah, D. (2010). *Product development of sports drink using coconut water and pineapple juice* (Master's dissertation, Kwame Nkrumah University of Science and Technology, Kumasi). Department of Biochemistry and Biotechnology.
- Baliga, M. S., & Dsouza, J. J. (2011). "Amla (*Emblica officinalis Gaertn*.), a wonder berry in the treatment and prevention of cancer." *European Journal of Cancer Prevention, 20*(3), 225-239. DOI: 10.1097/CEJ.0b013e328345a836
- Bigliardi, B., & Galati, F. (2013). Innovation trends in the food industry: The case of functional foods. *Trends in Food Science & Technology, 31*(2), 118-129. https://doi.org/10.1016/j.tifs.2013.03.006
- Chettri, S., Sharma, N., & Mohite, A. M. (2023). Edible coatings and films for shelf-life extension of fruit and vegetables. *Biomaterials Advances, 154*, 213632. https://doi.org/10.1016/j.bioadv.2023.213632
- Fatima, K., Tehseen, S., Ashfaq, F., Bilal, A., Zubair Khalid, M., Khalid, W., & Madilo, F. K. (2024). Development and storage stability studies of functional fruit and vegetable based drinks incorporated with polyphenols extracted from herbs and spices. *International Journal of Food Properties, 27*(1), 381–399. https://doi.org/10.1080/10942912.2024.2317740.
- Food and Agriculture Organization. (n.d.). *Food fortification technology*. Retrieved from https://www.fao.org/4/w2840e/w2840e03.htm
- Gunathilake, K. D. P. P., Yu, L. J., & Rupasinghe, H. P. V. (2014). Reverse osmosis as a potential technique to improve antioxidant properties of fruit juices used for functional beverages. *Food Chemistry, 148*, 335–341. https://doi.org/10.1016/j.foodchem.2013.10.061
- Hadi, S. T., Hussien, H. D., Abed, M. A., & Alhadithi, H. J. (2020). Chemical analysis of ginger rhizomes and sensory and microbial evaluation of ginger juice during storage. *International Journal of Pharmaceutical Quality Assurance*, *11*(4), 1-4.
- Hui, Y. H. (Ed.). (2006). *Handbook of fruits and fruit processing*. Blackwell Publishing. https://doi.org/10.1002/9780470277737

- Jideani, A. I. O., Silungwe, H., Takalani, T., Omolola, A. O., Udeh, H. O., & Anyasi, T. A. (2021). Antioxidant-rich natural fruit and vegetable products and human health. *International Journal of Food Properties, 24*(1), 41-67 https://doi.org/10.1080/10942912.2020.1866597
- Kamaliroosta, Z., Kamaliroosta, L., & Elhamirad, A. H. (2013). *Isolation and identification of ginger essential oil.* Journal of Food Biosciences and Technology, 3, 7380.
- Karim Shah, N. N. A., Shamsudin, R., Abdul Rahman, R., & Mohd Adzahan, N. (2016). Fruit juice production using ultraviolet pasteurization: A review. *Beverages, 2*(3), 22. https://doi.org/10.3390/beverages2030022
- Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables – The millennium's health. *International Journal of Food Science and Technology, 36*(7), 703725.
- Keyser, M., Müller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2007). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Food Microbiology Research Group, Department of Biotechnology, University of the Western Cape*.
- Khan, K. H. (2009). "Roles of *Emblica officinalis* in medicine–A review." *Botanical Research International, 2*(4), 218-228. Available at: https://www.researchgate.net/publication/26844670
- Koutchma, T. (2019). *Ultraviolet light in food technology: Principles and applications* (2nd ed.). CRC Press.
- Krishnaveni, M., & Mirunalini, S. (2010). "Therapeutic potential of *Phyllanthus emblica* (Amla): The ayurvedic wonder." *Journal of Basic and Clinical Physiology and Pharmacology, 21*(1), 93-105. DOI: 10.1515/JBCPP.2010.21.1.93
- Kulkarni, K. V., & Ghurghure, S. M. (2018). *Indian gooseberry (Emblica officinalis): Complete pharmacognosy review*. International Journal of Chemistry Studies, 2(2), 5-11. Retrieved from www.chemistryjournal.in
- Lim, W.S., & Rabeta, M.S. (2017). Development of functional beverage from Sapodilla (Manilkara zapota L.) fruit. *Food Research*, 2(2), 163–170. https://doi.org/10.26656/fr.2017.2(2).227
- Mahanandia, B. K., Singh, S. S., & Saini, N. (2022). Development and quality assessment of health beverage from apple juice blended with carrot and ginger. *The Pharma Innovation Journal, 11*(9), 1563-1567. Retrieved from www.thepharmajournal.com
- Maurya, V. K., Shakya, A., McClements, D. J., Srinivasan, R., Bashir, K., Ramesh, T., Lee, J., & Sathiyamoorthi, E. (2023). *Vitamin C fortification: need and recent trends in encapsulation technologies*. https://www.frontiersin.org/journals/nutrition/articles/10.3389/fnut.2023.1229243/full
- Morales-de la Peña, M., Welti-Chanes, J., & Martín-Belloso, O. (2016). Application of novel processing methods for greater retention of functional compounds in fruit-based beverages. *Beverages, 2*(2), 14. https://doi.org/10.3390/beverages2020014
- Morillas-Ruiz, J. M. (2014). Polyphenols and antioxidant activity in functional beverages. *International Journal of Food Sciences and Nutrition, 65*(7), 774-779.

- Nicoli, M. C., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science & Technology, 10*(3), 94-100. Retrieved from https://pmc.ncbi.nlm.nih.gov/articles/PMC9194584/
- Ogundele, O. M. A., Awolu, O. O., Badejo, A. A., Nwachukwu, I. D., & Fagbemi, T. N. (2016). Development of functional beverages from blends of *Hibiscus sabdariffa* extract and selected fruit juices for optimal antioxidant properties. *Food Science & Nutrition, 4*(5), 679–685. https://doi.org/10.1002/fsn3.331
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5*, e47. https://doi.org/10.1017/jns.2016.41
- Parle, M., & Chaturvedi, D. (2012). *Orange: Range of Benefits*. *International Research Journal of Pharmacy, 3*(7), 59-63. Retrieved from www.irjponl
- Patel, M., Jarrwal, S., Naik, M., Naik, M., Saxena, D., & Sinha, S. N. (2014). *Development of antioxidant rich beverage from Mahua (Madhuca indica) and Amla (Emblica officinalis)*. Journal of Scientific & Industrial Research, 75(1), 35-19
- Punoo, H. A., Rather, J. A., & Muzaffar, A. (2023). Development of soy whey fortified orange juice beverages: Their physicochemical, rheological, antioxidant, and sensory properties. *Exploratory Foods and Foodomics, 1*, 206–220. https://doi.org/10.37349/eff.2023.00016
- Purewal, S. S., Kamboj, R., Sandhu, K. S., Kaur, P., Sharma, K., Kaur, M., Salar, R. K., Punia, S., & Siroha, A. K. (2022). Unraveling the effect of storage duration on antioxidant properties, physicochemical and sensorial parameters of ready to serve Kinnow-Amla beverages. *Applied Food Research, 2*(1), 100057. https://doi.org/10.1016/j.afres.2022.100057
- Raji, Y., Udoh, U. S., Oluwadara, O. O., Akinsomisoye, O. S., Awobajo, O., & Adeshoga, K. (2002). Anti-flammatory and analgesic properties of the rhizome extract of Zingiber officinale. *African Journal of Biomedical Research*, *5*(3).
- Rathinasamy, M., Ayyasamy, S., Velusamy, S., & Suresh, A. (2021). Natural fruits based ready to serve (RTD) beverages: a review. Journal of Food Science and Technology, 1-7.
- Richa, R., Kohli, D., Kothakota, A., Vishwakarma, D., Richa, S., Sirohi, R., Mishra, A., Kabdal, B., Kumar, R., & Naik, B. (2023). *Citrus fruit: Classification, value addition, nutritional and medicinal values, and relation with pandemic and hidden hunger.* Journal of Agriculture and Food Research, 100718. https://doi.org/10.1016/j.jafr.2023.100718
- Rubio-Perez, J. M., Vidal-Guevara, M. L., Zafrilla, P., & Morillas-Ruiz, J. M. (2014). A new antioxidant beverage produced with green tea and apple. *International Journal of Food Sciences and Nutrition, 65*(5), 552-557. https://doi.org/10.3109/09637486.2014.893282
- Salehi, B., Sharopov, F., Martorell, M., Rajkovic, J., Ademiluyi, A. O., Sharifi-Rad, M., & Sharifi-Rad, J. (2020). Natural antioxidants from some fruits, seeds, foods, natural products, and associated biochemistry: An overview. *Frontiers in Bioengineering and Biotechnology, 8*, 1-23.
- Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., & De Ancos, B. (2023). Synergistic effect of UV-C irradiation and high-pressure processing on quality parameters of

"Nanglae" pineapple juice during storage. *Frontiers in Sustainable Food Systems, 7*, 979943. https://doi.org/10.3389/fsufs.2023.979943

- Savic, R. S., Petrovic, M. S., Stamenkovic, J. J., & Petronijevic, B. Z. (2015). *The presence of minerals in clear orange juices*. *4*(2), 71–78. https://doi.org/10.5937/SAVTEH1502071S
- Sawantt, S. S., Lee, B., Song, J., & Seo, H.-J. (2022). The Indian Gooseberry (Emblica officinalis) Industry and Cultivation in India. *Journal of the Korean Society of International Agriculture, 34*(3), 199–204. https://doi.org/10.12719/KSIA.2022.34.3.199
- Shah, A. K., Shamsudin, R., Abdul Rahman, R., & Adzahan, N. M. (2016). UV-C irradiation as a non-thermal treatment for food preservation. *International Food Research Journal, 23*(4), 1381-1392.
- Sharma, K., Mahato, N., Cho, M. H., & Lee, Y. R. (2022). Converting citrus wastes into value-added products: Economic and environmentally friendly approaches. *Nutrition, 7980*, 110892.
- Sharma, R., et al. (2019). Development and quality of apple-whey based herbal functional ready-to-serve beverage. *Journal of Applied and Natural Science, 11*(2), 291-298. https://doi.org/10.31018/jans.v11i2.2047
- Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition, 3*(4), 506–516. https://doi.org/10.3945/an.112.002154
- Srivastava, R. P., & Kumar, S. (2020). *Fruit and vegetable preservation: Principles and practices* (3rd ed., p. 145). CBS Publishers & Distributors.
- Tarko, T., Duda-Chodak, A., Semik, D., & Nycz, M. (2015). The use of fruit extracts for production of beverages with high antioxidative activity. *Potravinarstvo, 9*(1), 280–28https://doi.org/10.5219/480
- Vincente, A. R., Manganaris, G. A., Ortiz, C. M., Sozzi, G. O., & Crisosto, C. H. (2014). Nutritional quality of fruits and vegetables. In Postharvest handling (pp. 69-122). Academic press.
- Vojir, F., Schübl, E., & Elmadfa, I. (2012). The origins of a global standard for food quality and safety: Codex Alimentarius Austriacus and FAO/WHO Codex Alimentarius. *International Journal for Vitamin and Nutrition Research, 82*(3), 223–227. https://doi.org/10.1024/0300-9831/A000115
- Zhang, H., Zhang, C., & Wang, X. (2023). Novel food processing technologies for retaining nutrition of fruit and vegetable products: A review. *Food and Bioprocess Technology, 16*(3), 543-559.