

DEVELOPMENT AND NUTRITIONAL ANALYSIS OF INSTANT IDLY PODI USING *S.TRILOBATUM*, *C.HALICACABUM* AND *M.MADERASPATANA*

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Clinical Nutrition & Dietetics

by

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**DEPARTMENT OF CLINICAL NUTRITION & DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade A++ by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of ANGALAESWARI D (41736001) AND NITHYA SHREE P (42736010) who carried out the project entitled “**DEVELOPMENT AND NUTRITIONAL ANALYSIS OF INSTANT IDLY PODI USING *S.TRILOBATUM*, *C. HALICACABUM* AND *M. MADERASPATANA***” under our supervision from October 2023 to April 2024.

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Submitted for Viva – voce Examination held on 27.04.24

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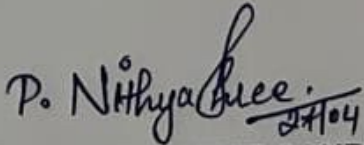
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DECLARATION

We, Nithya Shree P (41736010) and Angalaeswari D (41736001) hereby declare that the project entitled **“DEVELOPMENT AND NUTRITIONAL ANALYSIS OF INSTANT IDLY PODI USING S. TRILOBATUM, C. HALICACABUM AND M. MADERASPATANA”** done by us under the guidance of **Ms. Roshni .M** at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

PLACE: CHENNAI


SIGNATURE OF THE CANDIDATES

DECLARATION

We, Angalaeswari D(41736001) and Nithya Shree P(41736010) hereby declare that the project entitled "**DEVELOPMENT AND NUTRITIONAL ANALYSIS OF INSTANT IDLY PODI USING S. TRILOBATUM, C. HALICACABUM AND M. MADERASPATANA**" done by us under the guidance of Ms. Roshni .M at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27. 04. 2024

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ABSTRACT

Ready to eat food products are very desirable and mostly popular food commodity for consumer due to their ease of preparation, storage, consumption. Majority of the consumers demand for convenience foods which satisfy their hunger, adding bulk to the diet but lacks important nutrients like proteins, vitamins, minerals etc. It can lead to high blood pressure, obesity, diabetes & heart disease. Also there is a higher chance of upper respiratory infection caused by most relevant groups of respiratory viruses include rhinovirus, influenza, etc. Various natural plants said to have medicinal values that helps in treatment of such UR symptoms. Those medicinal plants are of great benefit if taken regular in our diet. This research is an attempt to resurrect South Indian therapeutic herbal recipes which we are fast being forgotten. The main aim of this study to develop and evaluate the nutritional analysis of an instant idly podi using medicinal leaves *Solanum trilobatum*, *C.halicacabum* and *M.maderaspatana*. The macro nutrient composition of the product revealed that it comprises of 353kcal/100g of energy, 61.8g/100g of carbohydrates, and 21.6g/100g of protein and 2.10g/100g of fat. The micro nutrient composition of the product observed that it comprises of 11.7g/100g of dietary fibre, 763mg/100g of sodium, 664mg/100g of calcium and 7.12mg/100g of iron. Using hedonic scale, the overall acceptability of the idly Podi was 8 respectively. Therefore, incorporating underutilized traditional medicinal leaves into daily convenient food in the form of Idly Podi not only prevents higher chance of upper respiratory infection but also proved for its high quality with nutritive value.

Keywords: Ready to eat foods, Medicinal leaves, *S.trilobatum*, *C.halicacabum* and *M.maderaspatana*

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LIST OF ABBREVIATIONS

C. halicacabum	- Cardiospermum Halicacabum
DL	- Detection Limit
G	- Gram
Kcals	- Kilo calories
Mg	- Milligram
MPs	- Medicinal Plants
M. maderaspatana	- Mukia Maderaspatana
RTC/RTS	- Ready to Cook/ Ready to Serve
RTE	- Ready to Eat
S. trilobatum	- Solanum Trilobatum
TC	- Total Carbohydrates
TDF	- Total Dietary Fibre
TF	- Total Fat
URTIS	- Upper Respiratory Tract Infections
WHO	- World Health Organization

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CHAPTER 1

INTRODUCTION

In today's world, the evolving globalization, increasing consumer awareness on education and health and increase in per capita income have an impact on the perception of food among people. This has led to change in the lifestyle and food purchase pattern of people of developing nations like India. Majority of the consumers demand for convenience foods like ready-to-cook, ready-to-eat and ready-to-drink foods which satisfy their hunger, adding bulk to the diet which on the other hand, lacks important nutrients like proteins, vitamins and minerals (Sindhu., 2019). Due to busy life style and work pressure on people, they prefer an easy and low time consuming cooking methods and quick cooked products.

The RTE Foods or Instant food products, which originated in Japan with Instant noodles, had its beginning in India in 1980's, found today in the kitchen shelves. Due to innovation and technology, the people have new foods processing vessels, equipment and tools but still people are in search of new techniques to speed up the cooking process in order to cope up with mechanical life, as they do not have sufficient time to cook food in the conventional methods (KRISHNAKUMAR., 2017).

Ready to eat food products is a category of food which comprises of packed food products which is used in direct consumption without any cooking where ready to cook food products need some preparations like heating, thawing, and boiling before consumption. (Kumar P, 2019). Ready to eat food products are very acceptable and a popular food commodity for consumer due to their ease of preparation, storage and consumption.

In the modern days where the life is at fast rapidity with the time very valuable to every person, people desire to save the time and to spare it on the other works.

Consumers are in the main interested in RTE and REC snacks in latest 5 years that is quickest developing meals zone due to their convenience. (Patel D, 2017) Consumer interest was raised in these food products due to their attractiveness, reasonable price, taste, appearance and texture. Different types of food comes

under RTE products such as sweet, salted fried, canned food, Fast food, baked food, dried or preserved food, extruded food, etc.

Now a day's ready to eat/serve, ready to cook product demand is increased among young consumers which are easily targeted by RTE manufacturing companies due to convenience level, texture and pleasant taste during whole shelf life of products.

Also, they capture consumer interest mainly due to its wide availability, convenience and snacking habit of consumer (Patheeparambil, 2020). Different types of convenience food are made from cereals, pulses and millets including puffed and flake millets, pasta, noodles, baked products, extruded product, fermented food and weaning food (Jaybhaye RV, 2014). These types of foods are good for supplying instant energy and nutrient where its main purpose is partially satisfying hunger (Kumar P, 2019).

Chronic administration of these agents may be associated with an increased risk of hypertension, obesity, hyperglycemia, and cardiovascular disease. Also there is a higher chance of upper respiratory infection (Running nose, Sore throat, Cold and Cough) caused by most relevant groups of respiratory viruses include rhinovirus, influenza etc..

Incorporating foods with medicinal properties into the daily diet alongside regular meals can enhance both nutrition and well-being. Medicinal value foods often contain essential vitamins, minerals, and antioxidants that support various bodily functions and may even provide specific health benefits, ranging from improved digestion to strengthened immune function. By consciously adding these nutrient-dense options to the daily diet, can create a more balanced and health-promoting eating pattern.

The holy Bhagwad Geeta says "Annaath Bhavanthi Bhoothani" meaning all beings is evolved from food. Food is one of the basic necessities of man and has been a part of man's existence on this earth. Traditional Indian foods are recognized as functional foods because of the functional components present it, such as body healing chemicals, antioxidants, dietary fibres and probiotics which are healthy and more nutritious and also helps in weight management controls blood sugar and provides enormous amount of immunity to our body (Radha, 2021).

The consumption of such traditional food provides good preventive measure to control many diseases (Choudhary, 2021). Number of medicinal plants, historically used for over a thousand years named rasayana is found in natural arrangements of Indian conventional fitness care systems. In Indian structures of medication maximum practitioners formulate and dispense their very own recipes.

The World Health Organization (WHO) has indexed 21,000 plants, which can be used for medicinal functions across the World. Among those 2,500 species are in India, out of which a hundred and fifty species are used commercially on a reasonably massive scale. (D.Thamayandhi, 2011)

Medicinal plants are a gift from nature since they supply us with a variety of health benefits. These medicinal plants have been utilised in India for millennia for their medical characteristics and are still used today. Ayurveda, siddha, unani, and ethnomedicine are only a few of the traditional medical systems of India. This knowledge of medicine was lost owing to the influence of western culture on us in the past, but is now reappearing as their relevance has been recognised, and the lack of side effects is also an essential factor in traditional medicine. According to a World Health Organization (WHO) research, more than 80% of people in underdeveloped countries rely on traditional medicines derived from plants for their basic health needs (WHO, 2022).

The South Indian traditional foods are considered as Siddha system of medicine, which helps in a natural way to achieve physical and mental wellness (Anbu, 2019). In Tamil Nadu they're the usage of many kinds of conventional recipes like Thuthuvalairasam, Karpuravallirasam, Ponnakannikeeraiporiyal, Nattukozhi soup, vazhathandu soup, Tulsi tea, Ingithuvaiyal, Sukkumalli coffee, Milagupaal etc., to therapy and control the diseases in order to maintain the good health (priyadharshini., 2019)

Vegetables hold significant position in balanced diet. Green leafy vegetables are being used since ancient period as source of food as they contain many nutrients and minerals which are helpful in maintaining human health. Leafy vegetables play important role in alleviating food security and they are very important in the diet of

many people. They contribute protein, mineral, vitamins, fiber and other nutrients which are usually in short supply in daily diets. (Solanke and Awonorin, 2021)

Quercetin, a bioflavonoid found in green leafy vegetables has antioxidant and anti-inflammatory activity, thus displays unique anticancer properties. The high level of vitamin K in greens plays an important role for the production of osteocalcin, a protein essential for bone health. Lutein, lycopene zeaxanthin, and carotenoids found in dark green leafy vegetables are concentrated in the eye lens and macular region of retina and play a protective role in the eye by preventing both cataract and age related macular degeneration. (Craig, 2010)

Traditional medicines must be used in the manufacture of current medical preparations, and 'Phytomedicines' are therefore a link between traditional and modern medicine. Individuals and communities in many underdeveloped nations rely heavily on medicinal plants for their wellbeing. Medicinal plants, on the other hand, are thought to be safer and are used to treat a variety of maladies. (Indumathi D. , 2022).

Consumers today are more concerned about their health; they focus on consuming products that strengthen their immune system. Foods rich in protein and fiber are now preferred primarily by consumers to maintain their health and keep them away from many types of diseases such as cardiovascular disease, diabetes, weight gain, etc. (Kumar, 2015). Traditional foods with medicinal values helps in preventing higher chance of upper respiratory infection (Running nose, Sore throat, Cold and Cough) caused by most relevant groups of respiratory viruses include rhinovirus, influenza etc.. However, there is an absence of readily available food products in the market that incorporates underutilized traditional medicinal leaves into convenient daily dietary form. Therefore, from the above facts the current study aims to develop and analyse the nutrients of new product named “instant idly podi” incorporated with the medicinal leaves such as *Solanum trilobatum*, *Cardiospermum halicacabum* and *Mukia maderaspatana*.

CHAPTER 2

REVIEW LITERATURE

The review of literature pertaining to the study entitled Development and nutritional analysis of idly powder using medicinal leaves is discussed under the following headings.

2.1. Introduction of Ready to eat food products

2.2. Medicinal leaves and its importance

2.2.1. *S. trilobatum* and its importance

2.2.2. *C. halicacabum* and its importance

2.2.3. *M. maderaspatana* and its importance

2.1. Introduction of Ready to eat food products

Kaviya R, 2023 point out that the Indian cooking and the way of life have experienced huge changes over the last 15 years as India becomes the centre of many multinationals and there's a quick alter in individual's ways of life. Nowadays, due to lifestyle changes and busy schedule, people prefer an easy, fast way of cooking food rather than spending too much time in cooking.

Ready to eat food products are very desirable and mostly popular food commodity for consumer due to their ease of preparation, storage, consumption. Various snacks products are come under RTE food including biscuits, sandwiches, roll, chips, fruits and vegetables salad, breakfast cereals, dairy products, etc. Fruits, vegetables and cereals are most important commodity for development of ready to eat food was defined by Malik T, 2018.

Although these types of food are tasty, convenient and easily accessible yet excess intake can be harmful to our health. It can lead to high blood pressure, obesity, diabetes & heart disease. Also there is a higher chance of upper respiratory infection (Running nose, Sore throat, Cold and Cough) caused by most relevant groups of respiratory viruses include rhinovirus, influenza, etc. Various natural plants said to have medicinal values that helps in treatment of such UR symptoms. Those medicinal plants are of great benefit if taken regular in our diet.

2.2. Medicinal leaves and its importance

For hundreds of years, medicinal plant life was used to deal with human diseases. The value of medicinal plants and traditional health systems in resolving global health issues is becoming more widely recognised. As a result of this renewed enthusiasm, research on medicinal plants is exploding on a global scale. Humans have been using medicinal plants as a source of medicine for thousands of years reported by Akinyemi O., 2018.

Medicinal vegetation is appeared as wealthy assets of conventional drug treatments and from that vegetation a number of the current drug treatments are produced. For lots of year's medicinal plant life were used to deal with fitness disorders, to feature taste and preserve meals and to save you sicknesses epidemics. Medicinal flora was gambling a critical function with inside the improvement of human culture. It has been estimated that more than 400 traditional plants or plant derived products have been used for the management of type 2 diabetes across the world discussed by Smruti, 2019.

Medicinal plants are plants used with the intention health maintenance, to be administered for some specific conditions, or both, whether in traditional medicine or in modern medicine observed by Ahn, 2017.

2.2.1. *Solanum trilobatum*

S. Parasuraman 2018, observed that the *Solanum* is a large and diverse genus of flowering plants of about 1500-2000 different species in the family of Solanaceae. *S. trilobatum* Linn... ("thuthuvalai" in Tamil; climbing brinjal in English) belongs to Solanaceae family and is widely used as a vegetable accompaniment to meals in the regions of Tamil Nadu, India. *Solanum trilobatum* (Fig 2.1) is also called as purple-fruited pea eggplant which is widely distributed in India.

Roots & Leaves are bitter and useful for treatment of cough and respiratory ailments. Berries and flowers are used for treatment of cough, cold and chest pain. The whole plant is useful remedy for all kinds of cough, acute and chronic bronchitis. It is reported that aqueous/alcoholic extract of leaf at 300 mg, two times a day for 3 days may be relief from Bronchial asthma was analyzed by Amudha R.R., 2017.

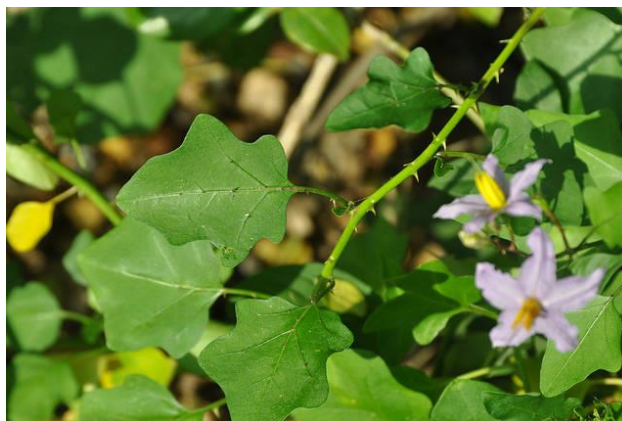


Figure 2.1 Solanum trilobatum leaves

Pharmacological and Microbiological Activity

Anti-diabetic activity

Nartunai Govindarajan 2022 in his research reported that the water extract from the leaves of *S. trilobatum* causes a notable decrease in glucose levels in diabetic rats induced by alloxan.

Anti-cancer Activity

The chloroform extract of *S. trilobatum* leaf was reported to possess anti-cancer activity against MCF-7 and Hep G2 cell lines and useful for the treatment of breast cancer was defined by Amudha, 2017. The methanolic extract of plant material reported to have antitumor effect against diethyl nitrosamine induced hepatocarcinogenesis in Wister rats was studied by Jahan, 2018. The saponin fraction isolated from *S. trilobatum* is reported to inhibit the proliferation of Hep-2 cells through cytotoxicity and apoptosis induction in the human larynx carcinoma was proposed by Kanchana, 2017.

Anti-hyperlipidaemic activity

Ganesan, 2019 his study shows that the aqueous extract of dried leaves of *S. Trilobatum* in doses of 200mg/kg frame weight suggests large antihyperlipidaemic impact in streptozocin precipitated diabetic rats.

Anti-bacterial Activity

The antibacterial activity of aerial parts of *S. trilobatum* was assayed under in vitro conditions by agar disc diffusion and well diffusion method against four bacterial species. All the solvents, ethanol, acetone, ethyl acetate used for the extraction of

leaves, flower and culmination have been proven large antibacterial hobby besides acetone and ethyl acetate extracts of flower against *K. pneumonia*. Gram–positive bacteria *S. aureus* was more susceptible to inhibition when compared to the Gram-negative bacterial species test was proposed by Krishna k., 2018.

2.2.2. *Cardiospermum halicacabum* and its importance

Manju Shree, 2019 observed that in India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in trend for the reason that historic instances and *C. halicacabum* L (Fig 2.2). Belonging to own circle of relatives Sapindaceae is one such plant extensively utilized by conventional practitioners to treatment diverse ailments. *Cardiospermum* is the aggregate of the Latin phrases cardio, which means coronary heart and sperma, which means seed and refers back to the white coronary heart-fashioned sample at the seed. It is a small delicate, smooth, climber and the whole plant has been used for several centuries in the treatment of many diseased conditions.



Figure 2.2 *Cardiospermum halicacabum* leaves

Antibacterial activity

In recent years, the rate of infection and resistant against antibiotics has been increased. The presence of antibacterial materials with inside the plant display

correct antibacterial interest without generating any aspect effects. Antibacterial activity of *C. halicacabum* Linn was investigated by well diffusion technique (SAVITHA G, 2017) against the selected human pathogens.

Antidiabetic activity

Diabetes mellitus is a metabolic disorder which is characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion or insulin action or both. Management of diabetes without any side effect is still challenge to the medical community was reported by Surapaneni KrishnaMohan 2017.

2.2.3. *Mukia maderaspatana* and its importance

M. maderaspatana (Fig 2.3) Linn... Belongs to the own circle of relatives call is Cucurbitaceae. It is a prostrate herb or tendril climber found throughout India. *M. maderaspatana* is a medicinal plant. It occurs in wild areas as well as cultivated in Kitchen gardens. It is traditionally used as a leafy vegetable and to cure several ailments in South India. It is used in vertigo and biliousness reported by S. Kiruthika, 2018.



Figure 2.3 *Mukia maderaspatana* leaves

Anti-diabetic activity

Antihyperglycemic interest turned into proved with the aid of using the ethanolic and aqueous extract of complete plant of *M. maderaspatana*. Ergosterol is an active compound extracted from the methanolic extraction of whole plant of *M. maderaspatana* exhibits antidiabetic activity was point out by Jamuna S, 2015.

Anti-inflammatory activity

Sujeethasai, 2022 observed that the inflammation is caused by the denaturation of protein. The *Mukia maderaspatana* extract shows positive effect on inhibition of protein denaturation and proteinase activity. Proteinase hobby is inhibited through extract of *M.maderaspatana*. Leucocyte proteinase plays a vital role in development of tissue damage in course of inflammatory reaction. Study at the methanol extract of *M, maderaspatana* proved immoderate proteinase inhibitory activity. *Coccinia grangis* species of Cucurbitaceae own circle of relatives own comparable impact as *M.maderaspatana*.

Antioxidant activity

According to reports published by Agata Rolnik., 2020, Oxidative stress is an imbalance between pro-oxidants and antioxidants. Oxidative stress leads the body to diseases such as cancer and obesity. Compounds present in curcubits such as cucurbitacins B and E, belonging to family of tannins exhibits antioxidant activity and also possess free radical scavenging tendency.

Considering the above points this study aims to develop and investigate the nutritional health benefits of incorporating medicinal leaves into the idly powder.

CHAPTER 3

AIM AND OBJECTIVES

AIM

This study aims to develop and investigate the nutritional health benefits of incorporating medicinal leaves into idly podi.

OBJECTIVES

- To examine the presence of bioactive phytochemicals in *S. Trilobatum*, *C. Halicacabum* and *M. Maderaspatana*.
- To develop the instant idly podi using *S.trilobatum*, *C.halicacabum* and *M.maderaspatana*
- To analyze the micro-nutrient composition of idly podi and its potential health benefits.
- To assess the macronutrient composition of the idly podi.
- To evaluate the specific sensory attributes of the idly podi.

CHAPTER 4

MATERIALS AND METHODS

4.1 Locale of the study

The major part of the present study has been carried out in the Department of Clinical Nutrition and Dietetics at the Sathyabama Institute of Science and Technology. The nutritional analysis was performed in the nearest lab.

4.2 Procurement of raw materials

Medicinal leaves (*S.trilobatum*, *C.halicacabum* and *M.maderaspatana*) was purchased from the local areas of villages and the other ingredients for the preparation of the idly podi were purchased from the local markets of Chennai.

4.3 Examine the presence of bioactive phytochemicals in *S. trilobatum*, *C. halicacabum* and *M. maderaspatana* leaves

4.3.1 Alkaloids

One gram of each leaf powder sample was dissolved in 10 ml of methanol and filtered through Whatmann No.1 paper. After that 3 ml of filtrate was mixed with 10 ml of 1% aqueous HCl. Mayer's reagent was then added with the reaction mixture. Appearance of buff-colored precipitate indicates the presence of alkaloids.

4.3.2 Flavonoids

One gram of each leaf powder sample was dissolved in 10 ml of 50% methanol and slightly warmed. After that the solution was filtered and added with metal magnesium and followed by few drops of conc. HCl. Formation of red to purple color indicates the presence of flavonoids.

4.3.3 Saponins

About 0.5gram of each leaf powder sample was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously.

4.3.4 Tannins

Five gram of each leaf powder sample was mixed with 100 ml of sterile double distilled water and then heated at 60° C for 10 minutes. The aqueous extract was

then filtered with Whatmann No.1 filter paper. 1% ferric chloride solution was added to 5 ml of extract in a test tube. Formation of a blue-black or blue-green precipitate in the reaction mixture indicates the presence of tannins.

4.4 Development of instant idly podi using *S. trilobatum*, *C.halicacabum* and *M.maderaspatana*

Medicinal leaves (*S.trilobatum*, *C.halicacabum* and *M.maderaspatana*) were cleaned and washed under running tap water. Then, they were Sun-dried until completely dehydrated. 75g each of black urad dal and Bengal gram, 8g of *Cardiospermum halicacabum*, 12g each of *Mukia maderaspatana* and *Solanum trilobatum*, 8g of curry leaves and red chili were roasted. Subsequently, all roasted ingredients were cooled at room temperature for 10 minutes. Garlic (optional) was added according to taste. Finally, all ingredients were ground together. Following grinding asafoetida and salt were added to taste and mixed thoroughly.

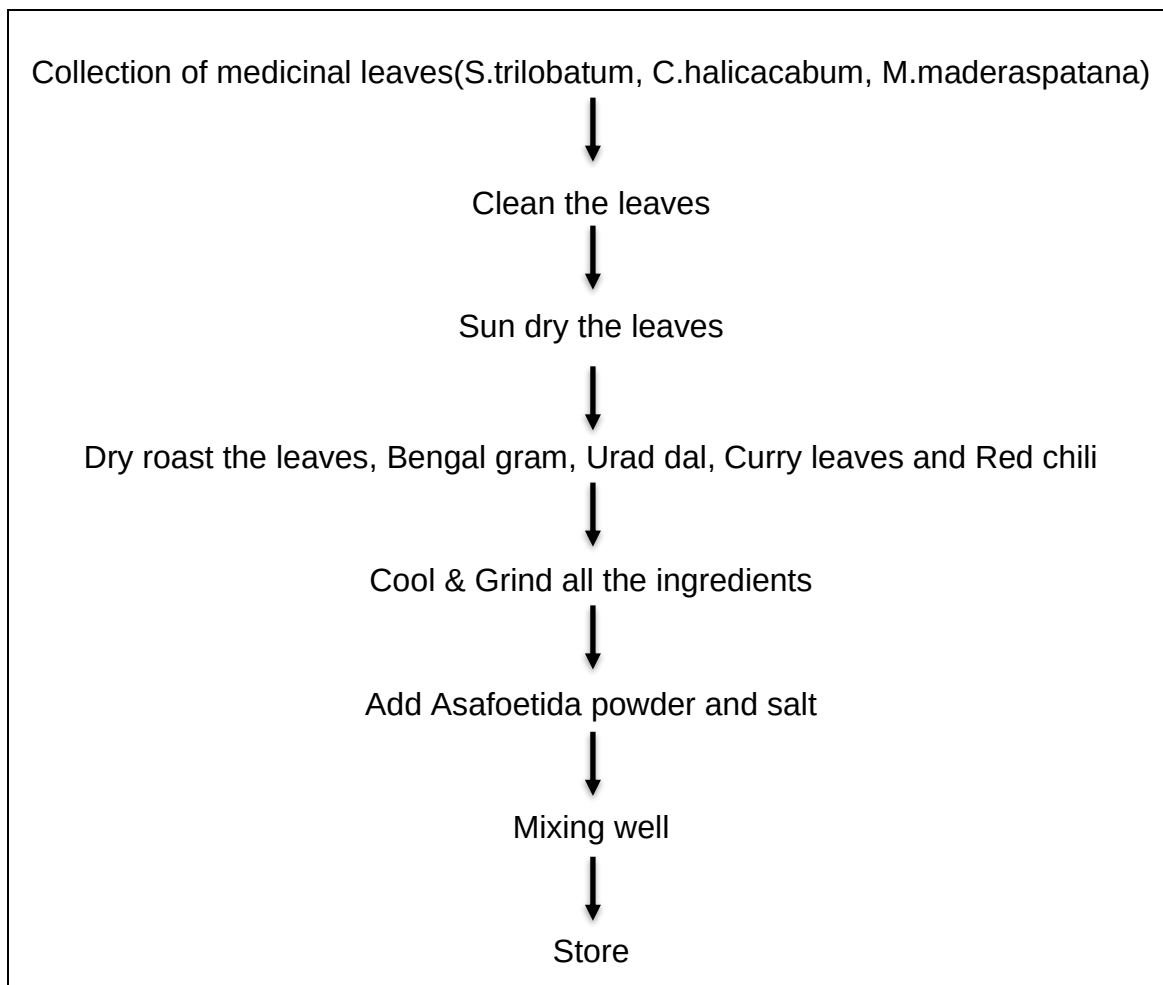


Figure 4.1 Steps in preparation of Instant Idly podi

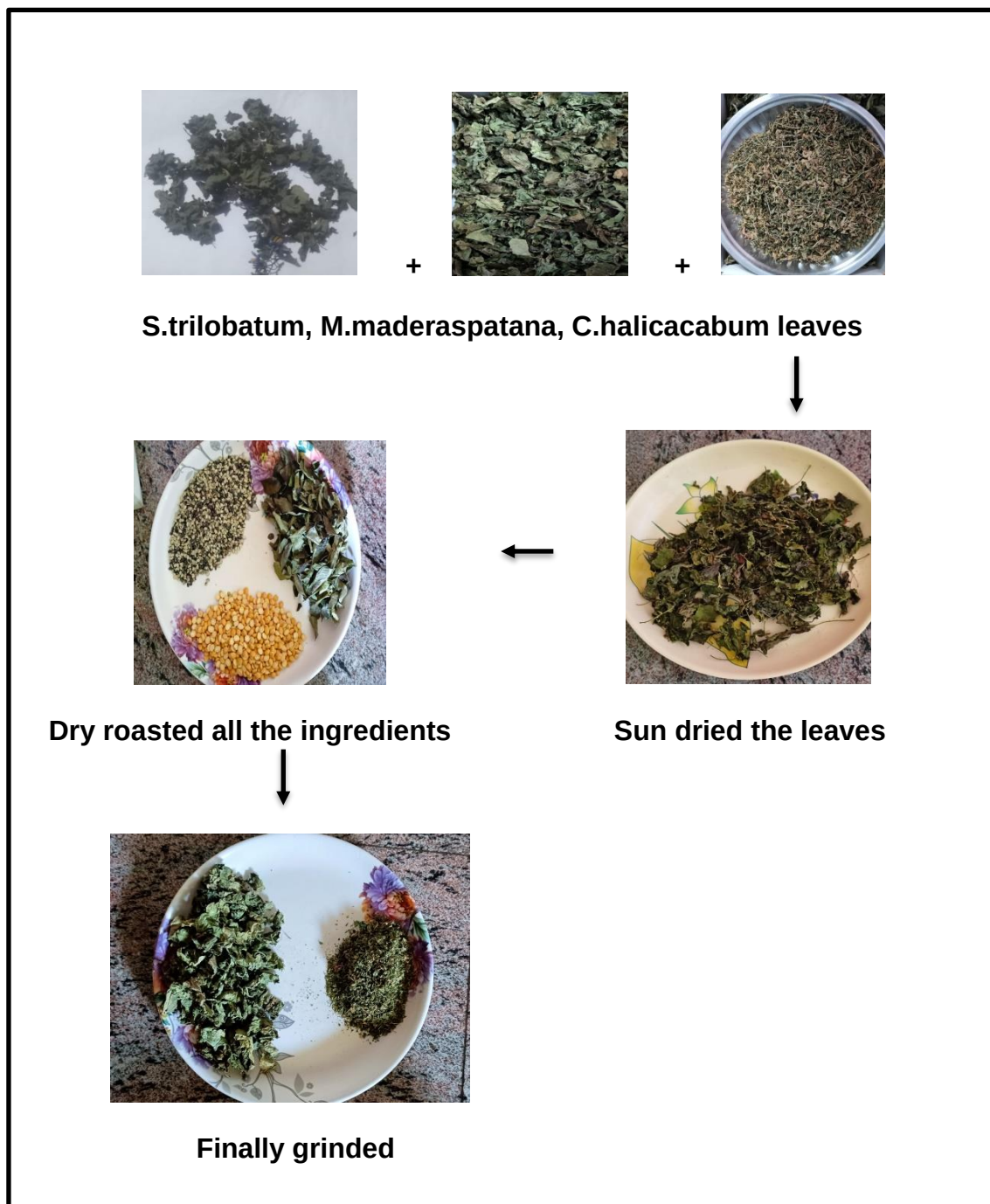


Figure 4.2 Flow chart of pictorial representation in preparation of instant idly podi

4.5 Analysis the micro-nutrient composition of idly podi

4.5.1 Dietary fibre

The dietary fibre was determined by the method of AOAC 20th Edn.2016, 985.29

Procedure:

Weigh duplicate 1g test portion, accurate to 0.1mg into 400ml tall-form beakers. Add 0.1ml tetramethyl solution. Cover the beaker with Al foil and place in boiling water bath for 15mins. Add 5mg protease. Incubate 30 mins at 60°C with continuous agitation and cool down. Add 10ml 0.325M HCl solution. Measure pH and dropwise and add acid if necessary. Add 280ml 95% ethyl alcohol preheated to 60°C. Wash residue successfully with three 20ml, portions of 78%ethyl alcohol and two 10ml portions of acetone. Time for filtration and washing will vary from 0.1h to 6h. Dry crucible containing residue overnight in 70°C vacuum oven. Cool in desiccator and weigh to nearest 0.1mg.

Calculations:

Determination of blank:

$B = \text{blank, mg} = \text{weight residue} - P_B - A_B$

Where,

Weight residue = average of residue weights (mg) for duplicate blank determination; and

$P_B - A_B = \text{weights (mg) of protein and ash}$

Calculate TDF as follows:

$\text{TDF (\%)} = [(\text{weight residue} - P - A - B) / \text{weight test portion}] * 100$

Where,

Weight residue = average of weights (mg) for duplicate blank determination

P and A = weights (mg) of protein and ash;

Weight test portion = average of two test portion weights (mg) takes.

4.5.2 Sodium

The sodium were determined by the method of AOAC 20th Edn.2016, 969.23

Procedure:

Weigh 4g test portion into the crucible, and char on electric hot plate or over low flame. Place on cold furnace and bring to 525°C and 2h to white ash. Add 15ml dil. HNO₃ (1-4) to the crucible, breaking up ash with a stirring rod if necessary. Filter through acid-washed quantitative paper into a 100ml volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume.

For flame emission- Read directly

For flame absorption- Dilute for direct readout as follows: Place 1ml aliquot in 100ml volumetric flask and dilute to volume with H₂O for Na; place 1ml aliquot in 25ml volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2ml HNO₃ to 100ml with H₂O.

Calculation:

For flame emission photometers:

$$Na (100g) = 100 \times F$$

Where,

$$E = (\text{percent T of unknown}) - (\text{percent T of blank})$$

$$E = (\text{percent T of standard lower concentration than test solution}) - (\text{percent T of blank})$$

$$E = (\text{percent T of standard of higher concentration than test solution}) - (\text{percent T of blank})$$

$$C = \text{mg in Na/ml in standard of lower concentration than test solution}$$

$$C = \text{mg in Na/ml in standard of higher concentration than test solution}$$

$$F = \text{dilution factor}$$

For flame absorption photometers: Convert percent absorption to absorbance.

$$\text{Mg in Na/100g} = (\text{concentration unknown} \times F \times 100) / \text{g test portion.}$$

4.5.3 Calcium

Calcium was determined by titration method (IS 5949:1990 (RA.2010))

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 per cent sodium hydroxide solution (4.6) using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass = $V_1 \times 0.04008/M$

Where V_1 = Volume in ml of EDTA solution used in titration; and

M = Mass in g of the sample in the solution for the test

4.5.4 Iron

Iron was determined by the method of AOAC 20th Edn.2016, 999.11

Procedure:

Homogenize the product if necessary, using non-contaminating equipment. In a crucible, weigh 10-20g test portion to nearest 0.1g. Dry in a drying oven or water bath at 100°C. Place dish in the furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C /h to 450°C. Let dish stand for at least 8 hours or overnight. Wet ash with 1-3ml water and evaporate on water bath. Put crucible in furnace at no more than 200°C and raise temperature (50°C -100°C /h) to 450°C. Proceed with ashing at 450°C for 1-2 hours or longer. Repeat the

procedure until the product is completely ashed. Add 5ml 6M HCl to crucible ensuring that all ash comes into contact with the acid. Evaporate acid on water bath and dissolve residue in 10-30ml, to the nearest 0.1ml of 0.1M HNO₃. Cover with watch glass and lid for 1-2 hours. Then, it is determined by atomic absorption spectrometry (AAS).

Calculations:

Calculate the detection limit (DL) for Fe:

DL= 3 * standard deviation of the mean of the blank determination (greater than or equal to 20)

Calculate the concentration, c, of the metal in the test sample according to the formula:

$$c = (a-b) * V / m$$

Where,

c = concentration in the test sample (mg/kg)

a = concentration in the test solutions (mg/L)

b = mean concentration in the blank solution (mg/L)

V = Volume of the test solution (mL)

m = weight of the test portion (g)

If (a-b) is lower than the DL, then (a-b) is substituted with DL for calculation of the limit of detection in the test portion.

4.6 Assess the macronutrient composition of idly podi

4.6.1 Energy

Energy is calculated by the FAO method.

It is calculated by the following formula:

$$\text{Energy (kcal/100g)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

4.6.2 Carbohydrates

The total carbohydrate content in the porridge powder was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fibre, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat(g)} + \text{Protein(g)} + \text{Fibre(g)}]$$

4.6.3 Total fat

The total fat was determined by the method of AOAC 20th Edn.2016, 920.39

Procedure:

Weigh accurately about 2g of sample in a fat extraction tube; add 2.0ml of alcohol to prevent lumping on addition of acid, and shake to moisten all particles. Add 10ml HCl (25+11), mix well and set tube 30 to 40 mins in water bath at 70°C and 80°C, shaking frequently. Cool to room temperature and add alcohol. Add 25ml diethyl ether to the flask and shake for 1.0 mins. Then add 25ml petroleum ether to the flask and shake 1min. Decant ether layer into the pre-weighed dry flask through anhydrous sodium sulphate. Then, Decant ether layer into a pre-weighed dry flask through anhydrous sodium sulphate. Then add 25ml of 1:1 diethyl ether, and petroleum ether to the flask and shake for 1.0min. Ether layer can be evaporated under a water bath. Keep the flask into the oven for further evaporation of ether residue. Remove the flask from the oven and place in desiccators to cool to room temperature. Record the weight of the flask plus fat.

Calculation:

% of Fat = (Weight of flask + fat) – Empty weight of the flask ×100 / Sample weight

4.6.4 Protein

The protein (N×6.25) was determined by the method of AOAC 20th Edn.2016, 986.25

Procedure:

Weigh a suitable test portion of the sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g CuSO₄ and 25mL or more, if necessary, of Conc.H₂SO₄ to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H₂O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

$\%N (w/w) = (TV_s - TV_b) * \text{Normality of standard HCl} \times 1.4007 / \text{Sample weight}$

Where,

TV_b = Titer value of blank; TV_s = Titer value of sample

4.7 Evaluate the specific sensory attributes of idly podi

Instant Idly Podi using *S.trilobatum*, *C.halicacabum* and *M.maderaspatana* was served only podi and food with podi to evaluate for sensory attributes (Appearance, consistency, aroma, mouthfeel, flavour and overall acceptability) through a panel of 10 subjects using a 9-point hedonic scale of scores from 1-dislike extremely to 9-like extremely.

CHAPTER 5

RESULT AND DISCUSSION

5.1 Phytochemical analysis

Table 5.1 phytochemical analysis of instant idly podi

S.NO	TESTS	SOLANUM TRILOBATUM	CARDIOSPERMUM HALICACABUM	MUKIA MADERASPATANA
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Saponins	+	+	+
4	Tannins	+	+	+

Phytochemical analyses for alkaloids, Flavonoids, Saponins, Tannins were screened in water and methanolic extracts of the selected medicinal plants of *S.trilobatum*, *C.halicacabum* and *M.maderaspatana*. The screening of the extracts indicated the presence of Alkaloids, Flavonoids, Saponins, and Tannins in the methanolic extract (shown in table 5.1.)

5.2 Micro-nutrient composition

Table 5.2 Micro nutrient composition of Instant Idly podi

S.NO	PARAMETERS	RESULTS
1	Dietary Fibre	11.7g
2	Sodium	763mg
3	Calcium	664mg
4	Iron	7.12mg

Table 5.2 represents the micro minerals like Dietary Fibre, Sodium, Calcium and Iron content from 100g Instant Idly Podi is contain 11.7g of Dietary Fibre, 763mg of Sodium, 664mg of Calcium and 7.12mg of Iron.

5.3 Macro-nutrient composition

Table 5.3 Macro nutrient composition of Instant Idly podi

S.NO	PARAMETERS	RESULTS
1	Energy	353kcal
2	Carbohydrates	61.8g
3	Total Fat	2.10g
4	Protein	21.6g

From the Table 5.3, it is observed that the macro nutrient composition of 100g of Instant Idly podi using with medicinal leaves (*S. trilobatum*, *C.halicacabum* and *M.maderaspatana*) had 353 kcals of Energy, 61.8g of Carbohydrates, 21.6g of Protein and 2.10g of Total Fat.

5.4 Sensory evaluation of Instant idly podi

Table 5.4 Sensory Evaluation of Instant Idly Podi

ATTRIBUTES	AVERAGE SCORES	
	ONLY PODI	FOOD WITH PODI
Appearance	8	8
Texture	8	8
Aroma	7	8
Mouth feel	7	8
Flavour	7	8
Taste	8	8
Overall acceptability	8	8

It is evident from the Table 5.4 that the Instant Idly Podi was highly acceptable for only podi and also with food by the members. The scores recorded for appearance range between only podi and Food with Podi was 8. Texture range between only podi and Food with Podi was 8. Aroma, Mouth feel and Flavour of only podi range was 7 and Food with Podi was 8. The overall acceptability of only podi and Food with Podi was 8.

CHAPTER 6

SUMMARY AND CONCLUSION

The globalization, commercialization and consumer awareness has led to change in the lifestyle and food purchase pattern of people in developing nations like India. Ready to eat or ready to cook food are also referred as fast food which are easy to prepare and consume. It commonly includes frozen dinner, packed snack food, bread, pasta, breakfast cereals, fruit snacks, sauces, processed meat, protein bars, etc. These food products are processed or ultra-processed which improves its quality or sometimes decreases nutritional quality and microbial quality. While these food items offer palatability, convenience, and accessibility, their excessive consumption has been linked to adverse health outcomes such as hypertension, obesity, diabetes mellitus, and cardiovascular diseases. Additionally, these dietary choices may be associated with an increased susceptibility to upper respiratory tract infections (URTIs) caused by common respiratory viruses like rhinoviruses and influenza viruses. Interestingly, various plants with purported medicinal properties have been shown to alleviate URTI symptoms when incorporated into the regular diet. Considering the above facts this study aimed to develop and analyse the nutritional value of instant idly podi. The product found to be high in its nutritive value with good overall acceptability with idly and rice. Therefore, incorporating underutilized traditional medicinal leaves into daily convenient food in the form of Idly Podi not only prevents higher chance of upper respiratory infection but also proved for its high quality with nutritive value.

Further recommendation of this study would be exploration of these same ingredients in the development of a soup mix and other products. Also investigating potential therapeutic and medicinal benefits of idly podi can be promising.

CHAPTER 7

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**FORMULATION AND STANDARDIZATION OF GUMMIES: INCORPORATING
COCONUT INFLORESCENCE SAP AND SPIRULINA**

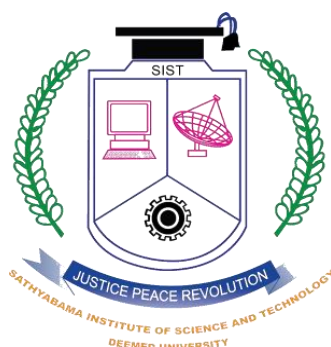
PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science Degree in Clinical Nutrition and Dietetics

By

B. DARSHINI (41736002)

A. GIRIJA (41736004)



DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS

SCHOOL OF ALLIED HEALTH SCIENCES

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BONAFIDECERTIFICATE

This is to certify that this Project Report is the Bonafide work of SATHIYAPRIYA M (41736015) KAVYA SHREE S (41736007) who has the project work entitled **FORUMULATION AND STANDARDIZATION OF NUTRITION BITES USING "DIOSCOREA ALATA"** under my supervision from JUNE 2023 to MAY 2024.

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We A.Girija (41736004) and B.Darshini (41736002) hereby declare that the Project Report entitled **"FORMULATION AND STANDARDIZATION OF GUMMIES: INCORPORATING COCONUT INFLORESCENCE SAP AND SPIRULINA"** done by us under the guidance of Ms. S. Priya (Internal) and Dr.L.Stanley Abraham (External) at the department of Allied Health and sciences and Ocean Research, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science and Degree in Clinical Nutrition and Dietetics.

DATE: 27.04.2024

PLACE: CHENNAI

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SIGNATURE OF THE CANDIDATE

DECLARATION

We B.Darshini (41736002) and A.Girija (41736004) hereby declare that the Project Report entitled **"FORMULATION AND STANDARDIZATION OF GUMMIES: INCORPORATING COCONUT INFLORESCENCE SAP AND SPIRULINA"** done by us under the guidance of Ms. S. Priya (Internal) and Dr.L.Stanley Abraham (External) at the department of Allied Health and sciences and Ocean Research, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science and Degree in Clinical Nutrition and Dietetics.

DATE: 21 - 04 - 2024

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B. Darshini

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ABSTRACT

This study explores the formulation of gummies infused with coconut inflorescence sap and spirulina, presenting a novel approach to creating functional and nutritious confectionery products. coconut inflorescence sap, a traditional drink derived from the sap of coconut palms, is renowned for its unique flavor profile and nutritional richness, offering a natural source of sugars, vitamins, and minerals. Spirulina, a nutrient-dense blue-green microalgae, complements the coconut sap with its essential vitamins, and antioxidant properties. The gummies, formulated with these ingredients, serve as a convenient and enjoyable means to incorporate indispensable nutrients into the diet, especially to special population like sports fraternity . This innovative product combines the cultural significance of coconut sap with the nutritional powerhouse of spirulina, offering consumers a delicious and health-enhancing snack option. The formulation process ensures optimal taste, texture, and nutritional balance, providing a promising avenue for the development of functional food products with broad consumer appeal.

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CHAPTER 1

INTRODUCTION:

Coconut sap, also known as Neera, naturally ferments quickly at room temperature. This fresh sap is initially sweet and has a neutral pH, but within a few hours, it begins to change. The colour turns whitish, the pH becomes acidic, and a noticeable fermented odour develops. Traditional collection methods using mud pots at the top of the palm tree actually encourage this fermentation because the sap sits for extended periods (Hebbar, 2015).

To prevent fermentation and preserve the fresh qualities of the sap, two main approaches are used. Chilling the collection containers keeps the temperature low, slowing down the fermentation process. Alternatively, collecting the sap more frequently (every hour) and storing it in a cold environment maintains its freshness (Arivalagan, 2015).

Neera, known as coconut inflorescence sap is a see-through white sweet juice collected from the spadix of an unopened coconut tree. It has high nutritional value and is a good digestive enzyme. Nevertheless, Neera undergoes spontaneous fermentation, first alcoholic and then acidic because of the presence of natural yeast and bacteria. Nonetheless, Neera continues to be consumed in India, Sri Lanka, Africa, Malaysia, Indonesia, Thailand and Myanmar as a popular nutritious drink. (Femi, 2020)

The coconut sugar, a natural sweetener with low glycemic index compared to cane sugar, is becoming popular in developed countries. Based on (Trinidad 2010) studies, the GI of coconut sap sugar ranges from 35 ± 4 to 42 ± 4 . Additionally, (Solanki 2016) points out that there has been a high demand for palm sugars which include coconut sugar in European Union countries for five years now. This explains why this type of sugar is extracted from fresh saps of all types of palms including coconuts. Neera; which is fresh sap obtained from coconut trees has several benefits.

Neera can also help prevent jaundice, improve digestion promote clear urination and maintain cool body system. When they are already producing steadily which is usually at a young age, coconut trees can be tapped. Tapping takes place on the unopened inflorescence. Once swelling starts at the base as a result of development of female flowers in the spathe (a protective leaf-like structure, about 60 cm long) then tapping is done.



Fig.1.1-Representation of climbing tree

After stroking for 4–5 days, 7–10 cm of the tip of the inflorescence is sliced off. Sap begins oozing immediately after this cut has been made.



Fig .1.2- Representation of spadix

The process starts with preparing the part of the coconut tree called the spadix, just like mentioned before. Once the sap begins to ooze out, tappers apply clay, gummy material, or leaf extract to the cut surface. This isn't to stimulate sap production as believed, but rather to prevent the sap from seeping internally between the peduncles.

They also tie a coconut leaf lamina around the cut area to guide the sap downwards; otherwise, it might just flow along the surface, especially since the coconut spadix is usually upright or at an angle (20° – 30° to the main axis). The sap that trickles from the cut surface is collected in an open earthen pot or bamboo sac, placed at the top of the palm tree for about 8–12 hours. To stop the sap from fermenting, lime is coated on the inside of the collecting pot. This collected sap is oyster white with a strong smell and may contain insects, ants, pollen, and dust particles. The sap gathered without lime is specifically used for making toddy, which is an alcoholic drink.



Fig.1.3 -Representation of sap collection



Fig.1.4-representation of collection process



Fig.1.5-representation of filtration

Biochemical and mineral composition fresh sap is rich in sugars, minerals and proteins. It is also a rich source of phenolics and ascorbic acid and essential elements, viz. N, P, K, Mg and micronutrients Zn, Fe and Cu.

The Central Plantation Crops Research Institute has even developed a special "coco-sap chiller" that uses ice cubes to maintain a cool temperature around the collected sap. This innovative method ensures the sap remains sweet, unfermented, and free of contaminants like insects and dust (Manikantan, 2015). This fresh, high-quality sap is called Kalparasa and is considered a valuable health drink. It can be sold directly under refrigeration or processed into various natural products like sugar, jaggery, or syrup without needing any artificial additives (Mathew, 2015).

Spirulina platensis is a powerful algae with a long history as a superfood. Packed with protein, vitamins, and minerals, it's even more nutritious than some common foods like soybeans (Jung 2019). This led the United Nations to recognize its potential. Beyond its impressive nutritional content, spirulina also boasts antiviral, antibacterial, and antioxidant properties. It's even been linked to benefits like diabetes control, cancer prevention, and reduced inflammation (Krüger 2019). This versatility has earned it the title of "superfood." *Spirulina* thrives in salty or alkaline water, using sunlight to convert carbon dioxide into food and releasing oxygen in the process. This means it can be grown in areas with limited freshwater resources, making it a sustainable and powerful source of nutrition (Waldeck,2019).

Compared to other algae like *Scenedesmus* and *Chlorella*, *Spirulina* is a bit of a diva. It needs a richer environment to thrive. This explains why it's naturally found only in salty lakes. Growing it elsewhere requires a special recipe (Ogawa, 2012). Scientists have tweaked an original formula to create mediums that meet *Spirulina*'s specific demands. These mediums are high in alkalinity and constantly replenish bicarbonate ions, which are essential nutrients for these picky algae (Cifferi,2012).

Spirulina isn't just a powerful food source, it's also a potential medicinal powerhouse. This algae is used in everything from agriculture to medicine because of its unique chemical makeup (Hoseini, 2013). Packed with protein, complex sugars, healthy fats, essential fatty acids, vitamins, and minerals, Spirulina offers a range of health benefits. Studies suggest it can fight viruses, bacteria, and even cancer (Darani, 2013). It may also help protect against heavy metal poisoning, boost the immune system, and combat inflammation due to its antioxidant properties. Researchers are still exploring the full extent of Spirulina's medicinal applications, but the potential is promising (Mozafari,2013).

Agar agar powder, a seaweed extract, is your secret weapon for delicious vegan and vegetarian dishes. This natural gelling agent forms strong gels at cool temperatures, thickening soups, sauces, and desserts without altering their flavour. Unlike gelatin, it's completely plant-based, making it a versatile and cruelty-free addition to your kitchen.

The magic of agar agar powder lies in its ability to transform liquids into gels when heated with water. This makes it a fantastic substitute for gelatin, especially for vegans and vegetarians. Unlike gelatin, which comes from animals, agar agar is plant-based and sets at cooler temperatures, around body temperature. This wider setting range opens it up for use in many more recipes, letting you create delicious dishes with all sorts of textures (Junianto, 2021).

CHAPTER 2

REVIEW OF LITERATURE:

2.1. COCONUT INFLORESCENCE SAP

The aptly named “tree of life,” the coconut palm offers a treasure trove of benefits. Neera, the sap collected from its unopened flowers, is a particularly valuable product. This healthy and therapeutic drink boasts a wealth of nutrients, surpassing even milk in terms of calories. Neera fights various ailments, from diabetes and cancer to electrolyte deficiencies and hair loss (Maria, 2022).

2.1.1 NUTRITIVE VALUE

Packed with minerals, vitamins, and the protein-building block glucamic acid, neera promotes healthy digestion. Its vitamins A and C act as antioxidants, protecting cells from damage (Malhija, 2022). Diabetics can also enjoy this beverage as its low glycemic index and load prevent blood sugar spikes. However, to fully understand its potential, further research is needed to explore the exact chemical makeup of neera, particularly the levels of total sugar, reducing sugar, ethanol, and specific vitamins (Sherin, 2022).

2.1.2 COLLECTION PROCESS

Coconut inflorescence sap is commonly collected using earthen pots hung on the unopened flower clusters of the coconut tree. Although this method is straightforward and popular, it has some drawbacks. The sap can quickly ferment at room temperature, leading to the creation of an alcoholic drink known as “toddy.” Additionally, the open containers allow the sap to come into contact with dust, insects, and other impurities, which can reduce the quality of the product. This exposure can also lower the sap’s Ph level due to fermentation, affecting its overall taste and usability (Hebbar, 2015). Researchers have developed improved methods for collecting coconut inflorescence sap that maintain its freshness. The coco-sap chiller uses a container with ice cubes to keep the temperature low, around 2-3°C, which slows down fermentation and allows for longer collection periods.

2.1.3 SELECTION OF INFLORESCENCE AND PREPARATION:

Tapping Coconut:

When they are already producing steadily which is usually at a young age, coconut trees can be tapped. Tapping takes place on the unopened inflorescence. Once swelling starts at the base as a result of development of female flowers in the spathe (a protective leaf-like structure, about 60 cm long) then tapping is done.

Tapping Stage:

After stroking for 4–5 days, 7–10 cm of the tip of the inflorescence is sliced off. Sap begins oozing immediately after this cut has been made.



Fig.2.1.3.1-Representation of spadix tapping

2.1.4 TRADITIONAL METHOD OF COLLECTING COCONUT SAP:

The process starts with preparing the part of the coconut tree called the spadix, just like mentioned before. Once the sap begins to ooze out, tappers apply clay, gummy material, or leaf extract to the cut surface. This isn't to stimulate sap production as believed, but rather to prevent the sap from seeping internally between the peduncles. They also tie a coconut leaf lamina around the cut area to guide the sap downwards; otherwise, it might just flow along the surface, especially since the coconut spadix is usually upright or at an angle (20°–30° to the main axis). The sap that trickles from the cut surface is collected in an open earthen pot or bamboo sac, placed at the top of the palm tree for about 8–12 hours. To stop the sap from fermenting, lime is coated on the inside of the collecting pot. This collected sap is oyster white with a strong smell and may contain insects, ants, pollen, and dust particles. The sap gathered without lime is specifically used for making toddy, which is an alcoholic drink.

Another method uses elongated cuboidal boxes that limit the sap's exposure to air, further preventing fermentation and preserving the sap's quality (Samsudeen et al, 2020).

2.1.5 POST HARVESTING CHANGES OF SAP

These factors were tested over time, with the sap being analyzed after 4 and 8 hours. Each factor had three different levels, offering a variety of conditions to compare. To keep the temperature of the sap below the surrounding environment, the researchers used ice or a mix of ice and water in coolers, depending on whether the temperature needed to be maintained at 10 °C or below, or between 11–20 °C (Jintana Wiboonsirikul, 2024).

2.1.6 POST FERMENTATION PROCESS

The movement of sap is sluggish and it is also highly susceptible to fermentation, which makes it very difficult for one to collect unfermented sap. Lime is often applied during tapping to prevent fermentation (M. Arivalagan, 2015). However, this sap still turns white and smells awful as if unhealthy when lime is added into it.

The fermented sap is known as toddy, which has an off smell making it inedible despite its nutritional value (K. B. Hebbar, 2015).

2.1.7 FACTORS AFFECTING SAP YIELD

The sap yield varies with the species, seasons, spathes of the same tree, and time of tapping. In one spathe of an inflorescence, sap can be harvested for a month or even more than a month, and in the same period, the second spathe might also be ready for the tapping. The sap can be harvested for up to six months from three spathes exuding sap from the same tree simultaneously (Chayanika Sarma, 2022).

2.1.8 ANTIOXIDANTS IN COCONUT SAP

Coconut inflorescence sap or neera is a type of sweet liquid that comes from the non-opened flower clusters of coconut palms. With the coming of ages, it became famous for a healthy drink due to its abundance of bioactive ingredients usually associated with antioxidants (Hebbar, 2022).

Neera has been ascertained to contain a variety of types of phenolic compounds, which include phenolic acids and flavonoids. These types of antioxidants are known to exert strong antioxidant activity, and such antioxidants can effectively scavenge free radicals in the body and thus reduce oxidative stress and promote health (Sharafudeen, 2022).

The chemical composition of coconut inflorescence sap and ascertained that the sap had a great antioxidant activity. According to the study, the sap contains substances that help to donate hydrogen atoms to free radicals, thereby neutralizing them and reducing their evil effects (Hebbar, 2015).

The results are encouraging, but a lot of research needs to be done on the specific types and intensities of antioxidants in coconut inflorescence sap and its health benefits. It also becomes the need to investigate how different processing and storage methods can affect the antioxidant levels in the sap.

2.1.9 OTHER PRODUCTS FROM UNFERMENTED SAP

Coconut sugar has been widely used in the cuisine of Indonesian and many other Asian countries for centuries and is also very popular as a natural sweetener in Indonesia and now also gaining popularity as a sweetener in some developed countries (Somawiharja, 2018).

The sweet sap i.e **neera** from mature coconut trees has been enjoyed as a natural drink for many years, but selling it on a large scale has been challenging. This is because the sap can ferment quickly, turning it into an alcoholic beverage. This change in the drink's nature affects its marketability and stability, making it hard to offer it as a consistent, non-alcoholic product (Mohankumar, 2019).

Coconut sap honey is a delicious and unique product made from the sap of coconut trees. The sap used to make the honey has a Ph level between 6.0 and 6.5, ensuring a perfect balance of acidity and sweetness.

Making this honey is similar to producing jaggery, but it's important not to heat the sap for too long. Overheating can cause the sap to burn and change color, affecting the flavor and quality of the honey. This delicate process results in a rich, flavorful coconut honey that's perfect for adding a touch of sweetness to your meals (Balasubramanian, 2023).

2.2.SPIRULINA

The transformative impact of Spirulina farms as environmentally sustainable green food machines. Thriving in shallow ponds, this versatile algae exhibits rapid growth, doubling its biomass every 2 to 5 days. This remarkable productivity breakthrough yields an abundance of protein, surpassing traditional agricultural crops by significant margins - over 20 times more than soybeans, 40 times more than corn, and a staggering 400 times more than beef, all within the same land area (Robert, 2009).

2.2.1 NUTRITIVE VALUE

The investigators determined the nutritional value of processed *Spirulina maxima* in vitro in rat skeletal muscle. Dietary-fed rats were provided with six successive days of a diet containing 20% protein levels (Saranraj, 2012). *Spirulina platensis* had a nutritional value that fit somewhere between those of wheat gluten and casein supplemented with methionine.

Casein, along with supplementation from methionine, prepared from *Spirulina platensis* had been considered fairly similar in quality to casein sample with methionine (Usharani, 2012).

2.2.2 CULTIVATION

Spirulina, a commercial organism within the family *Spirodaceae*, is seen as a filamentous type of cyanobacterium. It is among the world's largest microalgal cultivation industries that are focused on growing it and currently generate 10,000 tons in annual production of the algae. Because it is enriched in proteins, phycocyanin, essential amino acids, polysaccharides, carotenoids, minerals, vitamins, and essential fatty acids, spirulina has lately been regarded as an ideal bio-resource and has attained increasing interest in recent years (Kanchana, 2012). Cultivation is generally considered an option for its extraction due to the abundant amounts of this pigment, and easy cultivation. The Cyanobacteria *Spirulina platensis* has a high tolerance towards alkaline Ph, for ease of cultivation; a large size for its cell aggregates for easy harvest; and an easily digested cell wall (Usharani, 2012).

2.2.3 Market prospects

The fluctuating price and the high labor costs made life hard for Indian coconut farmers. Somewhere, along the line, the fresh coconut sap, the Kalparasa, has provided the opportunity for Indian coconut farmers who have a load of price fluctuations and many labor costs (Hebbar, 2015).

Kerala-based company Palakkad Coconut Producers Company Ltd (PCPCL) leads the way in implementing the CPCRI method for sap collection as it becomes the first-to-market entity in the sale of fresh, unfermented coconut sap. The farmers harvest fresh sap, which is then taken to the storage center and then to several kiosks. Here, the fresh sap is sold to the consumers through juice dispensers. Market trends indicate that the Kalparasa is much in demand as a refreshing and an instant, natural drink for thirst quenching (Manikantan, 2015).

With the Kalparasa tapping, not only did the farmers increase their income, but a lot of employment opportunities also found for the youth and the tappers. The change has brought many economic benefits to the local economy and the consumers, who would definitely pave the way to a bright future for the industry (George, 2015).

2.2.4 HEALTH BENEFITS OF SPIRULINA

Research on Spirulina has revealed a wide array of potential health benefits beyond its antioxidant and anti-inflammatory effects. Some of these include: Spirulina, a type of blue-green algae, offers a wide range of potential health benefits. It has been shown to protect the liver and kidneys from damage, improve blood quality, and prevent anaemia. Some studies suggest that Spirulina may also have benefits for diabetes by helping to regulate blood sugar levels (Capelli, 2019). Additionally, Spirulina has been found to lower blood pressure, remove heavy metals from the body, and provide radioprotection against radiation-induced harm. It also shows promise in preventing liver and renal toxicity, boosting the immune system, and relieving allergic reactions. With its strong antioxidant properties, Spirulina is a valuable addition to a healthy lifestyle (Cysewski, 2019).

2.2.5 SPECIES

Various spirulina species are *Spirulina maxima*; *Spirulina platensis*, *Spirulina pacifica* (also known as *Arthrospira platensis*, *Arthrospira maxima*). *Arthrospira platensis* is the predominant species and commercially cultivated worldwide though *Arthrospira maxima* is produced in the South and Central American regions (Thomas, 2010).

2.2.6 INDUSTRIAL CULTIVATION

Microalgae can hardly be harvested from the environment and thus has to be produced in small-scale industrial facilities, or better, large-scale industrial production facilities—recently, offshore production of microalgae has been studied.

Spirulina is not an eukaryotic microalga but a prokaryotic cyanobacteria (Bacteria, Cyanobacteria, Cyanophyceae, Oscillatoriales, Microcoleaceae, *Arthrospira*) (Zurano, 2021). Nevertheless, it has been in a common trend among both the reviewers and other scientists in addressing the extraction of *Spirulina* proteins.

Though *Spirulina* contains high protein content, when used in the production of hydrolysates and bioactive peptides, the extraction of *Spirulina* proteins should first be accomplished to speed up proteolysis and increase the degree of purification of the products (Silvia, 2021).

The need for the disruption step of cell walls is in general mandatory because most microalgal strains contain strong cell walls. Not only with regard to microalgal products in general, but when it comes to food applications, this step will also improve its extractability (Ainoa, 2021).

2.2.7 FUNCTIONAL FOOD

Functional foods are a rather new food category, which promises health benefits after consuming the product that go beyond basic nutrition. The idea for functional foods started in Japan back in 1984 with government-funded research projects that were called “Systematic analyses and development of functional foods.” Since that time, the functional foods market has been growing continuously, and it seems likely that it will continue to grow in the future (Tomas, 2020).

2.2.8 POWDER FORM PIGMENTATION

Arthrospira (*Spirulina*) *platensis* or *A. maxima* most-commonly comprise commercial spirulina powder. Those species, otherwise known as spirulina, are Gram-negative, nontoxic species of cyanobacteria that are, in its broadest sense, utilized either as a dietary supplement or as natural additives. In this aspect, spirulina is just one of the best food and quality drug resource due to its high content of protein, lipids, vitamins, minerals, chlorophyll, β -carotene, and polysaccharides (Park, 2018).

Two distinct natural colors exist, namely carotenoids and C-phycocyanin, which have enabled spirulina use to be in line with consumer awareness regarding the importance of natural color agents for their nutritional, pharmacological, and health-related benefits.

By this measure, the number of applications of natural pigments, especially spirulina as a source of these pigments, is on the increase, especially in the food and cosmetic industries (Kim, 2018).

2.3. AGAR AGAR POWDER

Agar agar powder, a seaweed extract, is your secret weapon for delicious vegan and vegetarian dishes. This natural gelling agent forms strong gels at cool temperatures, thickening soups, sauces, and desserts without altering their flavour. Unlike gelatin, it's completely plant-based, making it a versatile and cruelty-free addition to your kitchen (Najia, 2016).

2.3.1 FOOD GRADE

Agar agar powder is a key ingredient loved for its special way of setting foods. It turns into a gel at about 35°C (95°F) and melts when hotter. This feature is perfect for making jelly, pudding, sweets, and vegan options like fake cheese.

Agar's skill in forming a clear, firm gel is great for making food look good and feel different. Plus, agar is a no-cal choice, doesn't have fat, and fits a vegan diet, making it a hit for those who watch what they eat (Najia, 2016).

2.3.2 INDUSTRIAL USES

In the world beyond cooking, agar agar powder is used in many work areas. In biotech, agar is key in growing tiny life forms and samples because it jells, making a firm space for cells to grow and get checked. Also, agar can soak up water and stay the same for a long time, which helps in making drugs, clothes, and even in teeth care (Rao, 2016).

2.4. FOOD GRADE PRESERVATIVES

2.4.1 CITRIC ACID

Citric acid reigns supreme as the go-to acid in the food world. This molecule, with its three acidic groups, it dissolves easily in water. It's what gives citrus fruits their zing, and food manufacturers love it for that reason. But citric acid is a multi-talented player (Hattori, 2011). Its acidity helps fight off microbes, keeping foods like tomato juice and jams fresher for longer.

It also acts as a behind-the-scenes antioxidant by grabbing metal ions that would otherwise spoil food. So next time you enjoy a refreshing salad dressing or a scoop of sherbet, remember, citric acid might be playing a part in keeping it delicious. (Kirimura, 2011).

2.4.2 POTASSIUM SORBATE

Potassium sorbate, a form of sorbic acid, is a common preservative found in many store-bought foods. Scientists have developed various methods to test for potassium sorbate and other preservatives to ensure food safety and compliance with regulations (Sushanth, 2022). To make food last longer and prevent spoilage from mould, fungus, and other microbes, food manufacturers often add preservatives.

2.4.3 PROPYLENE GLYCOL

Propylene glycol is a synthetic food additive belonging to the same chemical group as alcohol and is a colorless, odorless syrupy liquid that is denser than water and leaves almost no taste in it (Wei, 2018). It is considered to have greater solubility in most substances than water and is moisturizing, hence it is a good food additive. PG is widely used in all kinds of processed foods and beverages for its functionalities (Zhang, 2018).

While having similar melting points, PG and EG are not interchangeable. Whereas EG is toxic to humans and hence considered unfit for use as a food additive, PG is regarded as safe for consumption. The most common uses of PG, with respect to food products, are related to texture modification, flavour, appearance, and extension of shelf life (Wu, 2018).

These additives are carefully regulated, especially in the European Union. Rules specify which foods they can be used in, how much can be added, and their exact chemical makeup (Salwan, 2022).

CHAPTER 3

AIM AND OBJECTIVE

3.1. AIM

The aim of this thesis is to develop and optimize a unique gummy product that combines novel and traditional foods, specifically spirulina and coconut inflorescence sap. This includes the formulation and standardization of the gummies, sensory evaluation to assess consumer appeal, analysis of their nutritive value, and the implementation of innovative packaging and marketing strategies to enhance product quality and marketability.

3.2.OBJECTIVE

1. New and Old Foods (Spirulina and Coconut Inflorescence Sap):

- This objective seeks to study new and old foods like spirulina and coconut inflorescence sap used in the preparation of gummies. Spirulina, for its rich nutrient content, and coconut inflorescence sap, a natural sweetener, could be combined to make a unique product very rich in health but also pleasing to the taste buds.

2. Standardization of Gummies Incorporating Coconut Sap and Spirulina:

- This objective focuses on the standardization of the formulation of gummies incorporating coconut sap and spirulina, including the best ingredient ratio and the appropriate processing method that would result in the desired texture, taste, and nutritional value, but with the same consistency across different batches.

3. Sensory Evaluation :

- Objective 3 focuses on determining the acceptability and liking of the gummies, through the conduct of sensory evaluation among a sample of 100 people. They were asked to rate the taste, texture, and overall liking and hence the product received the desired feedback for further improvement.

4. Nutritional Value Analysis of Gummies and Coconut Inflorescence Sap:

- This objective focuses on the analysis of the nutritional value of the prepared gummies and the coconut inflorescence sap to determine the presence and levels of essential nutrients such as vitamins, minerals, proteins, and sugars. It provides insights into the health benefits of the product and gives support to the nutritional labelling and marketing claims.

5. Innovative Packaging and Marketing of the Gummies:

Objective This level aims at developing effective packaging and marketing strategies for the gummies. Such measures may bring into play innovative packaging to enhance the shelf life and appeal of the product; marketing strategies can be focused on the health benefits and unique ingredients to attract consumers interested in natural and nutritious snacks.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

The methodology section introduces the formulation and standardization procedures employed for the production of the gummies.

4.1.1 MATERIALS USED:

Agar Agar, Xanthum Gum, Water, Sugar, Propylene glycol, Coconut inflorescence sap, Spirulina, Lemon juice, Sorbitol, Citric acid and Potassium sorbate.

4.2 METHODOLOGY

4.2.1 PRE-PREPARATION:

Three solutions were prepared:

Solution 1, containing Agar Agar, xanthan gum, and Lemon juice

Solution 2, composed of water, sugar, and propylene glycol and

Solution 3, a mixture of coconut inflorescence sap, spirulina, sorbitol, citric acid, and potassium sorbate.

4.2.2. PREPARATION:

First, Solution 1 is prepared by combining agar agar powder, grams of sugar, and lemon juice that has been diluted. Additionally, propylene glycol is mixed into Solution 1.

Meanwhile, Solution 2 is created with a mixture of coconut inflorescence sap, spirulina, citric acid, and potassium sorbate.

To prepare the gummies, Solution 1 is heated for 3 minutes. Just before turning off the heat, Solution 2 is introduced and thoroughly mixed into Solution 1. This ensures that all ingredients are evenly distributed throughout the mixture.

Once the solutions are combined, the resulting mixture is poured into gummy molds and left to set for approximately 1 hour at room temperature. After this initial setting period, the molds are transferred to a refrigerator and left to chill for 24 hours.

Following the refrigeration process, the gummies are fully prepared and ready to be enjoyed. This method ensures that the gummies have the proper consistency and flavor, making them a delicious and satisfying treat.

The addition of Lemon flavor further enhances the taste profile, providing a refreshing and citrusy element to the coconut inflorescence sap and spirulina-infused health gummies. This combination not only offers potential health benefits but also a delightful sensory experience for consumers.

4.3 FORMULATION

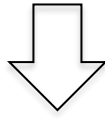
Materials used	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Coconut Inflorescence sap	30 ml	30 ml	30 ml	30 ml
Spirulina	1.5g	1g	0.75g	0.5 g
Lemon Juice	20ml	20ml	20ml	20ml
Sorbitol	10ml	10ml	10ml	10ml
Agar Agar	3g	3g	3g	3g
Sugar	180g	150g	120g	90g

Table 4.3.1-Representation of formulations

Propylene glycol is added as binder and citric acid, potassium sorbate, are added as preservatives. The fourth formulation, which utilizes 30ml of Coconut nectar, 0.5g of Spirulina, 0.5g of Citric Acid, 0.5ml of Propylene Glycol, 20ml of lemon juice, 0.5g potassium sorbate, 10ml of sorbitol, 3g of 3g of Agar Agar, and 90g of Sugar, was selected for preparation due to the optimal consistency of the resulting gummies.

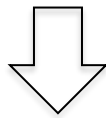
FLOW CHART OF GUMMIES PREPARATION

Solution 1 is heated

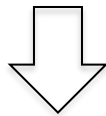


Prepare Solution 2

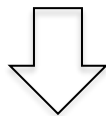
- Mix coconut nectar, spirulina, citric acid, and potassium sorbate



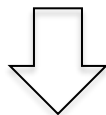
Combine Solution 1,2 and mix thoroughly



Pour the mixed solution into Molds



Allow the mixture to set for 1 hour at room temperature



Refrigerate the filled Molds for 24 hours

Table 4.3.2-Representation of gummies preparation

4.4 QUALITY ANALYSIS:

This study delves into consumer perception of gummy candies through sensory evaluation with a diverse group of 100 participants. By analysing their feedback on factors like appearance, texture flavour, aroma and mouthfeel, this research aims to identify the ideal gummy characteristics across different age demographics.

CATEGORIES	MEAN $\pm$ STANDARD DEVIATION
APPERANCE	7.68 $\pm$ 1.32
TEXTURE	7.41 $\pm$ 1.55
FLAVOUR	7.42 $\pm$ 1.51
AROMA	6.86 $\pm$ 1.67
MOUTHFEEL	7.28 $\pm$ 1.60

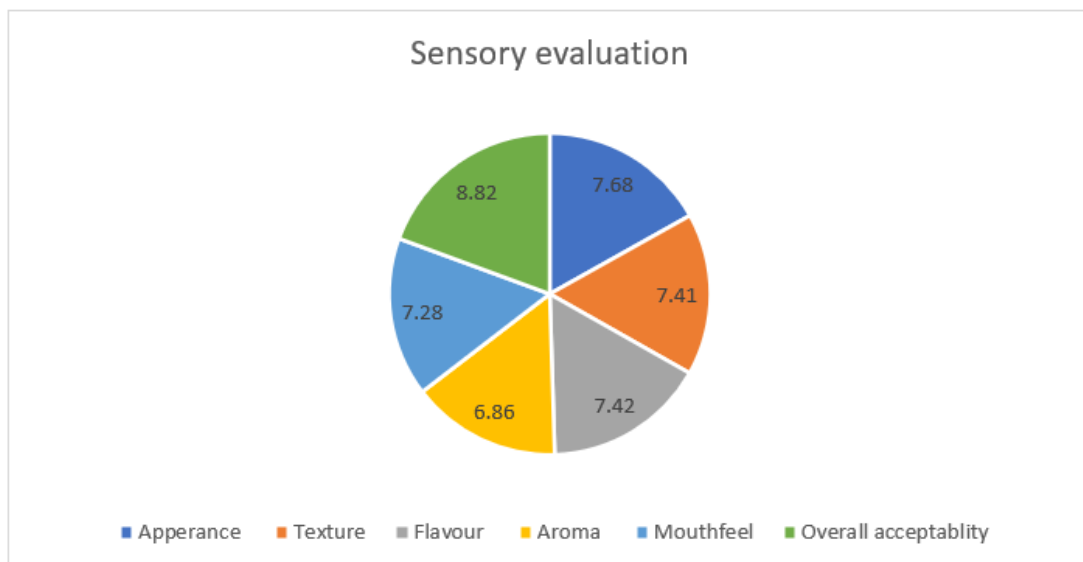


Fig.4.4.1 - representation of sensory evaluation

4.5 QUESTIONNAIRE

1. Appearance

RATING RANGE (1-10)	NO. OF RESPONSES
1-5	2
6-10	98

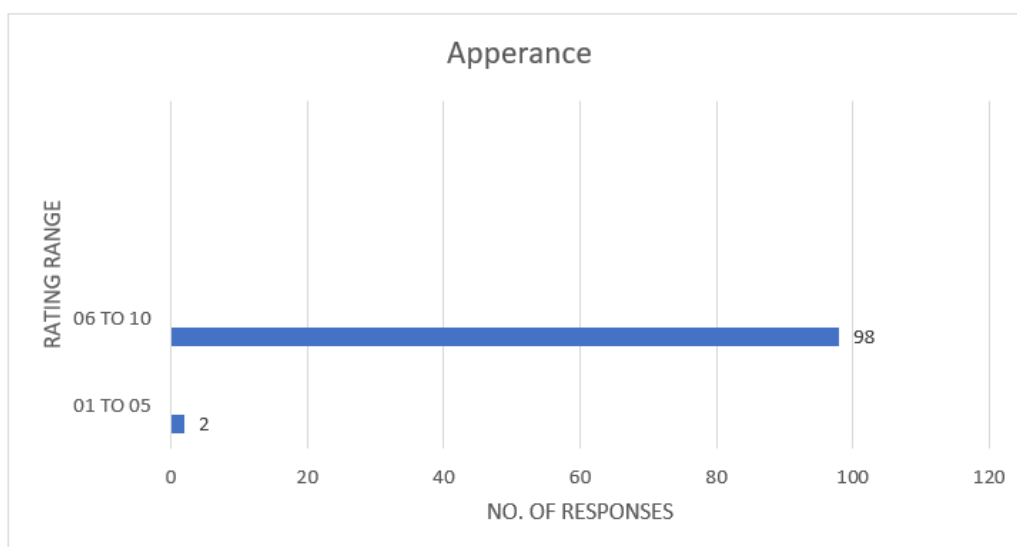


Fig. 4.4.2- representation of appearance

2.Texture

RANGE OF RATING (1-10)	NO. OF RESPONSES
1-5	0
6-10	100

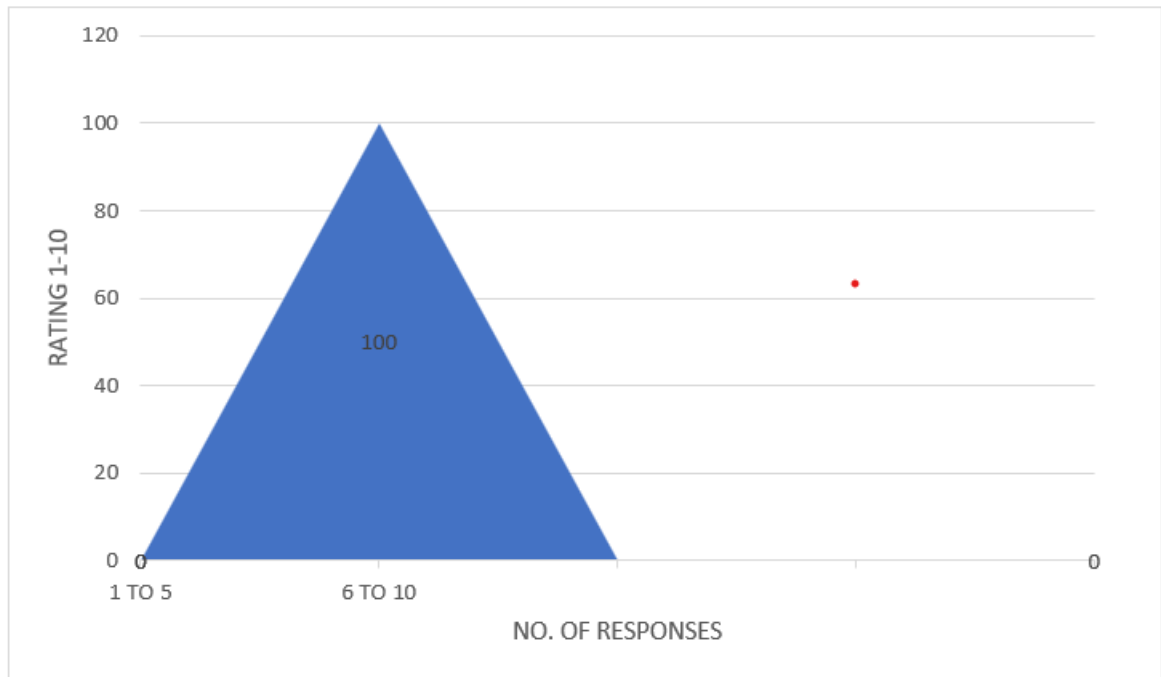


Fig. 4.4.3 - representation of texture

3. Flavour

RANGE OF RATING (1-10)	NO. OF RESPONSES
1-5	5
6-10	95

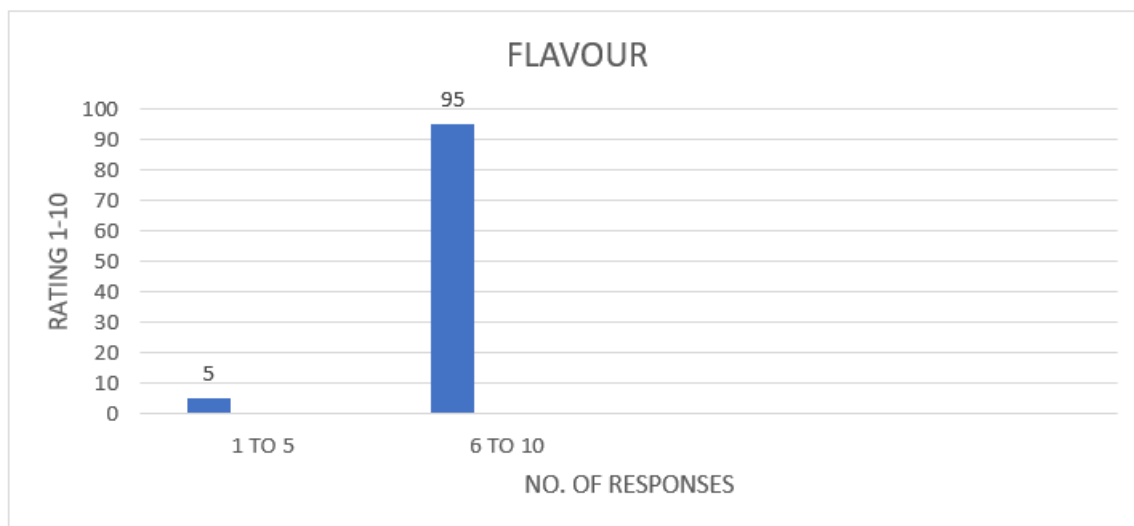


Fig.4.4.4- representation of flavour

4. Aroma

RANGE OF RATING (1-10)	NO. OF RESPONSES
1-5	15
6-10	85

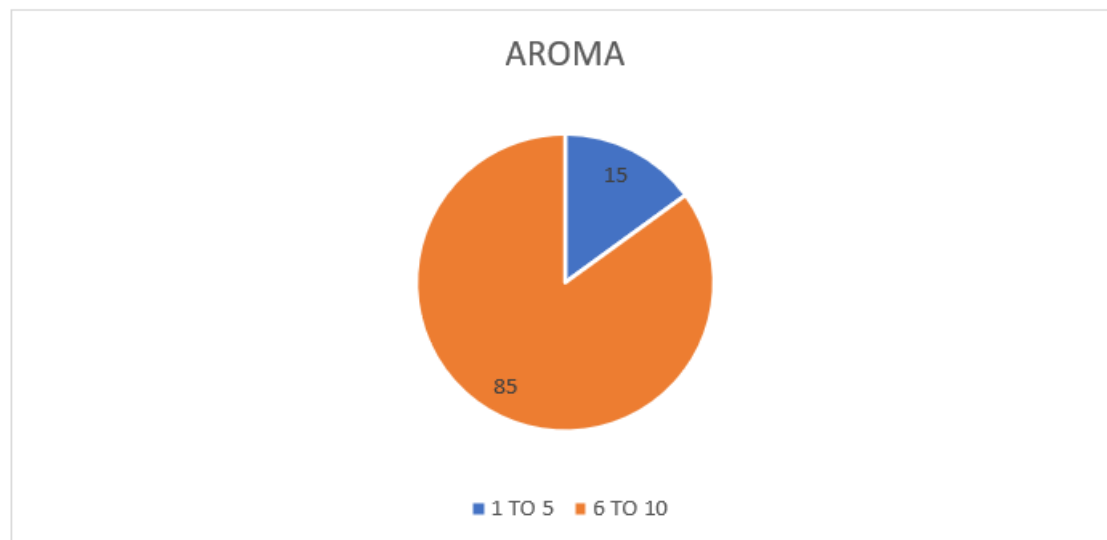


Fig. 4.4.5- representation of aroma

5. Mouthfeel

RANGE OF RATING (1-10)	NO. OF RESPONSES
1-5	2
6-10	98

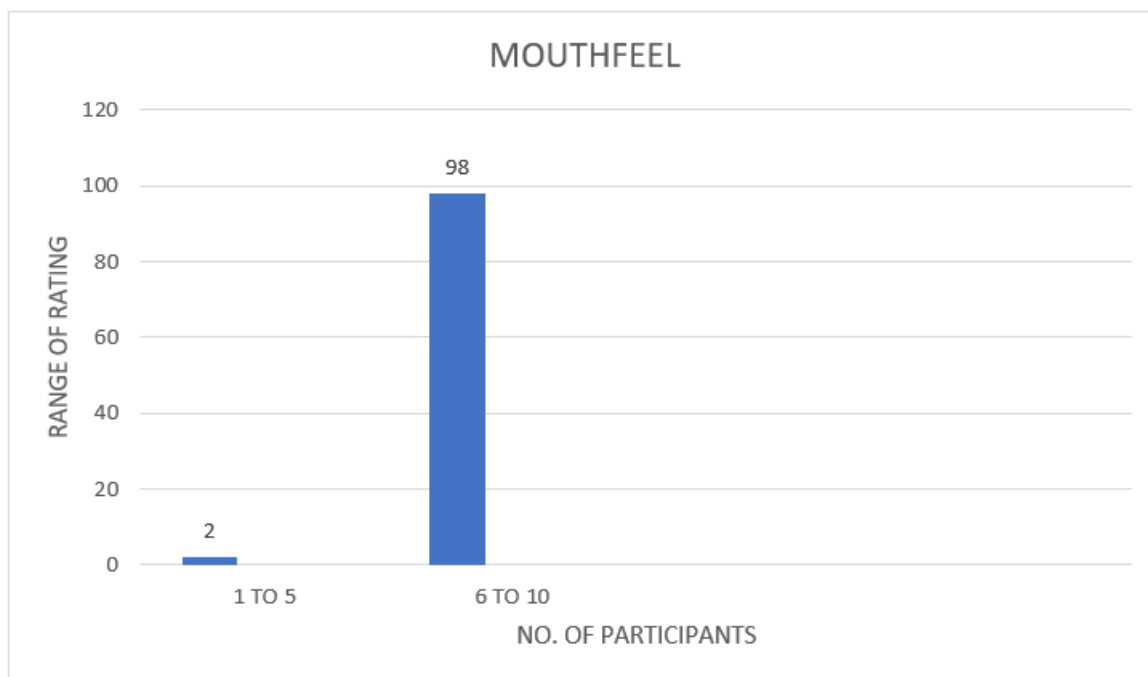


Fig . 4.4.6 - representation of mouthfeel

6. Overall acceptability

RANGE OF RATING (1-10)	NO. OF RESPONSES
1-5	0
6-10	100

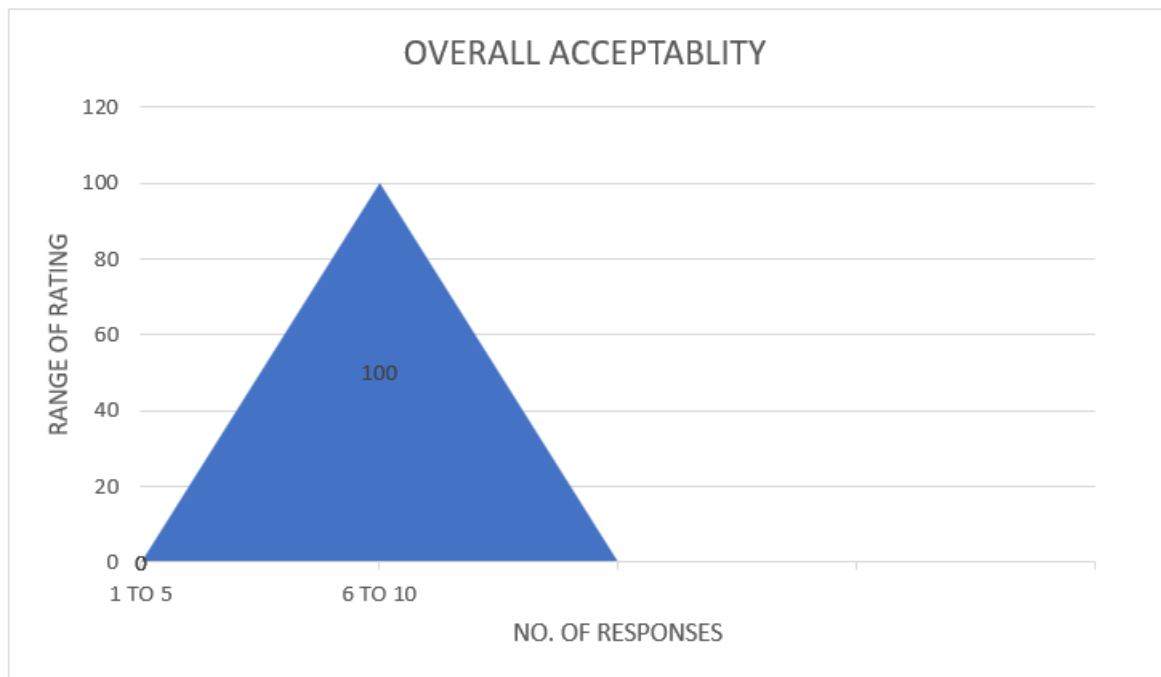


Fig. 4.4.7- representation of overall acceptability

CHAPTER 5

RESULTS AND DISCUSSION

5.1 FORMULATION

The formulated spirulina and coconut-infused gummies have been successfully prepared using the described methodology and ingredients.

5.2 Taste and Texture

The gummies exhibit a delightful combination of flavours, with the refreshing citrusy note from the lemon juice complementing the sweetness of the coconut inflorescence sap. The agar agar contribute to the gummies' firm yet chewy texture, providing a satisfying mouthfeel.

5.3 Nutritional Benefits

These gummies are not just tasty treats; they are also packed with nutritional benefits. The coconut inflorescence sap provides essential minerals, vitamins, and glucamic acid, which promote healthy digestion. Spirulina, known for its superfood status, offers a wealth of nutrients, including protein, vitamins, essential fatty acids, and antioxidants. This combination makes the gummies a nutritious snack option.

5.4 Preservation

The addition of citric acid and potassium sorbate serves dual purposes. Citric acid not only enhances the flavour profile but also acts as an antioxidant, helping to preserve the gummies by preventing oxidation. Potassium sorbate is a common food preservative that inhibits the growth of molds and yeast, extending the shelf life of the gummies.

5.5 Consistency and Flavour

The selected formulation, includes Coconut Inflorescence sap, Spirulina, Citric Acid, Propylene Glycol, lemon juice, potassium sorbate, sorbitol, Agar Agar, and Sugar, has resulted in gummies with optimal consistency and flavor. This formulation strikes a balance between the sweetness of the sugar, the tanginess of the lemon juice, and the earthy notes of spirulina.

5.6 Consumer Appeal

With the rising demand for functional foods and plant-based snacks, these spirulina and coconut-infused gummies cater to health-conscious consumers. The natural ingredients, vegan-friendly formulation, and potential health benefits make them an attractive option for those seeking nutritious and delicious snacks.

Sensory Experience

Beyond the nutritional aspects, these gummies offer a delightful sensory experience. The combination of flavours and textures, from the chewiness of the gummies to the burst of lemony freshness, provides a satisfying treat for consumers of all ages.



Fig.5.1.1-Representation of final gummy product

Summary and Conclusion

The formulated spirulina and coconut-infused gummies represent an innovative and healthy snack option that merges old and new foods, such as spirulina and coconut inflorescence sap. This unique combination of flavors and textures appeals to health-conscious consumers, offering a vegan-friendly and plant-based formulation that includes natural ingredients like coconut inflorescence sap, spirulina, lemon juice, and agar agar. These ingredients provide essential nutrients and health benefits, making the gummies a nutritious and appealing snack. The preparation methodology results in gummies with a refreshing citrus flavor from lemon juice, sweetness from coconut inflorescence sap, and an earthy undertone from spirulina. The use of agar agar ensures the gummies have a firm yet chewy texture, enhancing the overall sensory experience for consumers.

The addition of citric acid and potassium sorbate acts as a preservative, extending the shelf life of the gummies and preventing oxidation. The gummies strike a balance between taste and nutrition, offering essential minerals, vitamins, and glucamic acid from coconut inflorescence sap, as well as protein, vitamins, essential fatty acids, and antioxidants from spirulina. This combination makes the gummies a nutritious snack option and a convenient way to incorporate superfoods like spirulina into one's diet. Sensory evaluation among 100 participants rated the gummies positively for taste, texture, and overall satisfaction, providing valuable feedback for further product improvements. These gummies cater to the growing demand for functional foods and plant-based snacks, making them a valuable addition to the functional foods market. Nutritional value analysis of the gummies and coconut inflorescence sap revealed essential nutrients such as vitamins, minerals, and sugars, supporting the nutritional labeling and marketing claims.

Effective packaging and marketing strategies, focused on the health benefits and unique ingredients, are essential to enhance the shelf life and appeal of the product. This approach can attract consumers interested in natural and nutritious snacks. In conclusion, the spirulina and coconut-infused gummies successfully fuse health and taste, offering an enjoyable sensory experience with their chewy texture and delightful flavor profile. They provide essential nutrients and antioxidants, making them a nutritious and convenient snack option for health-conscious consumers. The standardization of the formulation ensures consistent quality across different batches, and the inclusion of natural preservatives helps maintain product freshness and safety.

Sensory evaluation indicates that the gummies are well-received by consumers, offering valuable insights for further enhancements. Overall, the spirulina and coconut-infused gummies offer a win-win solution for consumers seeking a guilt-free indulgence that contributes to overall well-being. The combination of innovative formulation, standardization, and consumer appeal positions these gummies as a valuable addition to the functional foods market. With the right packaging and marketing strategies, the product has the potential to achieve significant success and widespread adoption.

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EXPLORING THE UNTAPPED POTENTIAL: A COMPREHENSIVE STUDY ON THE PROPERTIES OF JACKFRUIT LEAVES TEA POWDER

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Clinical Nutrition & Dietetics

by

PAVITHRA K (41736011)

GAYATHIRI S (41736003)



**DEPARTMENT OF CLINICAL NUTRITION & DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of GAYATHIRI S (41736003) and PAVITHRA K (41736011) who carried out the project entitled "EXPLORING THE UNTAPPED POTENTIAL: A COMPREHENSIVE STUDY ON THE PROPERTIES OF JACKFRUIT LEAVES TEA POWDER" under my supervision from JUNE 2023 to APRIL 2024.

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Submitted for Viva – voce Examination held on 26.04.24

Internal Examiner

External Examiner

DECLARATION

We Gayathiri S and Pavithra k hereby declare that the project entitled "Exploring The Untapped Potential: A Comprehensive Study On the Properties Of Jackfruit Leaves Tea Powder" done by me under the guidance of Mrs. Kanya S is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

PLACE: CHENNAI

Gayathiri.S

SIGNATURE OF THE CANDIDATES

DECLARATION

We Pavithra k and Gayathiri S hereby declare that the project entitled "Exploring The Untapped Potential: A Comprehensive Study On the Properties Of Jackfruit Leaves Tea Powder" done by me under the guidance of Mrs. Kanya S is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

PLACE: CHENNAI

K-Pavithra
SIGNATURE OF THE CANDIDATES

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ABSTRACT

Artocarpus heterophyllus, commonly known as jackfruit, is a tropical tree with leaves that have been historically utilized in traditional medicine and culinary practices. This study aimed to explore the phytochemical composition, nutritional content, antibiotic sensitivity, and sensory attributes of *A. heterophyllus* leaves. Phytochemical screening was conducted to identify various secondary metabolites present in the leaves using standard qualitative methods. Nutritive analysis involved determining the proximate composition, mineral content, and vitamin levels of the leaves. Antibiotic sensitivity testing was performed using well-established methods to evaluate the susceptibility of potential microbial contaminants to commonly used antibiotics. Additionally, sensory evaluation was carried out to assess the organoleptic properties, including taste, aroma, texture, and overall acceptability of *A. heterophyllus* leaf preparations. This involved a panel of trained assessors using standardized sensory evaluation techniques. The results revealed the presence of diverse phytochemicals such as alkaloids, flavonoids, phenols, tannins, and terpenoids in the leaves. Nutritive analysis indicated that the leaves are rich in essential nutrients, including proteins, carbohydrates, vitamins, and minerals, making them a potential source of dietary supplements. Antibiotic sensitivity testing demonstrated varying degrees of susceptibility among microbial strains tested, suggesting potential antimicrobial properties of *A. heterophyllus* leaves. Furthermore, sensory evaluation highlighted the palatability and culinary versatility of *A. heterophyllus* leaves, with positive responses regarding taste, aroma, and overall sensory appeal. In conclusion, this comprehensive study provides valuable insights into the phytochemical composition, nutritional quality, antibiotic sensitivity, and sensory characteristics of *A. heterophyllus* leaves, emphasizing their potential as functional foods, dietary supplements, and sources of natural antimicrobial agents. Further research is warranted to explore their therapeutic and culinary applications in greater depth.

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LIST OF ABBREVIATIONS

AI	:	Adequate Intake
DRI	:	Dietary Reference Intake
TDEE	:	Total Daily Energy Expenditure
DHA	:	Docosahexaenoic Acid
EPA	:	Eicosapentaenoic Acid
HDL	:	High-Density Lipoprotein
LDL	:	Low-Density Lipoprotein
TG	:	Triglycerides
BG	:	Blood Glucose
HbA1c	:	Hemoglobin A1c
GI	:	Glycemic Index
GERD	:	Gastroesophageal Reflux Disease
PERT	:	Pancreatic Enzyme Replacement Therapy
FODMAPs	:	Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols
FOD	:	Frequency of Distribution
PRAL	:	Potential Renal Acid Load
DGA	:	Dietary Guidelines for Americans
ADA	:	American Diabetes Association

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1. INTRODUCTION

The *Artocarpus heterophyllus* is a species of tree which belongs to mulberry, fig and breadfruit family (Moraceae) is known by other names jackfruit (Eng), Kathal, Panas (Hindi), Kanthal (Beng), Palua (Tamil), Pharmas (Guj & Mar) & Chakka (Malayalam). *Artocarpus heterophyllus*, commonly called as jackfruit. It is a tropical tree species belonging to the Moraceae family (Om Prakash, 2010).



FIG 1.1 : JACKFRUIT TREE WAS FOUND IN ALATHUR, KERALA

Jackfruit is originated in areas such as India, Indonesia, Central Africa, Florida, Brazil, Australia, and the Pacific Islands, jackfruit is a plant with several therapeutic uses for its roots, stems, leaves, and fruit. Notably, β -sitosterol, tannin, cycloartenone, cycloartenol, and sapogenins are abundant in jackfruit leaves. (A Utari, 2020) Furthermore, because jackfruit contains a high concentration of phenolic compounds, particularly flavonoids, which are very poisonous, it shows promise as a cytotoxic agent (L Warly, 2020). Many agricultural wastes are currently being investigated as possible feed ingredients, and their effectiveness is being assessed. In tropical regions, feed availability varies greatly with the seasons, frequently leading to poor quality and irregular supplies. Feed shortages

occur frequently during the dry season; on the other hand, during the rainy season, feed becomes plentiful. Using substitute feed ingredients, such as those made from forages like jackfruit, is one way to address this problem((Rajesh Kumar, 2010). Members of this family usually have small, petalless male or female blooms paired with alternating or opposite leaves and a milky latex. This family has many species that fuse the fruits of different flowers to form numerous fruits. This family of flowering plants has more than 1180 species and about 38 genera. (Vijaya poojitha, April 2017)Southeast Asia's jackfruit has been cultivated for a long time—between 3000 and 6000 years, according to archeological finds in India. This large evergreen tree can grow to a height of 10 to 15 meters. It is indigenous to evergreen woods that are found between 450 and 1,200 meters above sea level. Widely grown in India's warmer regions, it requires soil that is both moist and well-drained, with a pH range of 4.3 to 8.0 and moderate fertility. (Devarakonda Ramadevi, April 2017). These plant-based compounds have been used for many years to treat a wide range of illnesses and infections, such as cancer, toxicity from paracetamol, stroke, diabetes, and many more. (A. A. Uwakwe, June 2018). Humans consume minerals as vital nutrients, which are naturally occurring chemical components found in soil and plants. They are an essential part of diet and are required for animals' bodies to function properly. Iron promotes blood flow, calcium keeps teeth and bones strong, while potassium helps muscles and skeleton contract. (F. C. Anacletus, June 2018). It is commonly known that the use of herbal treatments is becoming more and more popular. (L F Mustanti, 2020).The leaf of *A. heterophyllus*, commonly known as jackfruit, is used for treating a variety of conditions, such as asthma, wound healing, ringworm infestation, gallstones, abscesses, antisyphilitic properties, anthelmintic effects, lactagogue properties, relief from earaches, antiulcer properties, dental cavity prevention, toxin absorption, anemia, dermatitis, cough, diarrhea, fever, sedation, and promoting healthy digestion. (Periyanayagam K, May 2013).

1.1 DESCRIPTION OF ARTOCARPUS HETEROPHYLLUS LEAVES

The leaves of *Artocarpus Heterophyllus*, more commonly recognized as jackfruit, stand out not only for their visual appeal but also for their considerable importance across different spheres of human existence. (Jonathan Smith, 2024)

heterophyllus tree are also very important, even if the fruit of the jackfruit tree is well-known for its distinct flavor and adaptability in cooking. (Benjamin Nguyen, 2024). The qualities, therapeutic possibilities, and culinary significance of Artocarpus Heterophyllus leaves will all be thoroughly examined in this essay. By examining the size, shape, color, traditional medicinal uses, and culinary versatility of these leaves, a more comprehensive understanding of their complex nature can be gained. (Sarah Johnson, 2024).



FIG 1.2 PHYSICAL CHARACTERISTICS ARTOCARPUS HETEROPHYLLUS

Artocarpus Heterophyllus leaves are distinguished in part by their unusual physical characteristics, which include their huge size, oblong form, and vivid green color. With up to 9 inches of length, these leaves rank among the largest in the plant kingdom. Their smooth surfaces with pronounced veins add visual appeal, and their robust but flexible nature allows for a variety of uses, both in the culinary and medical arts. Because of these qualities, Artocarpus Heterophyllus leaves are quite versatile and easy to identify in a variety of settings. (Olivia Garcia, 2024).

TAB 1 : PHYSICAL CHARACTERISTICS OF THE LEAVES

SIZE	The leaves are typically large and can reach up to 9 inches in length and 4 inches in width.
SHAPE	They are generally elliptical or ovate in shape with pointed tip.
TEXTURE	The leaves have a smooth texture on the upper surface and a slightly hairy texture on the underside.
COLOR	The color of the leaves can vary from bright green to dark green , and they often have a glossy appearance.
VEINS	The leaves have prominent veins running through them, which contribute to their overall appearance.
DISTINCTIVE FEATURES	Prominent veining , with deeply impressed veins on the upper surface and raised veins on the lower surface. The leaves are arranged alternately on the stems.

1.2 LEAF ARRANGEMENT

The leaves of the *Artocarpus heterophyllus* (jackfruit tree) are alternately and spirally arranged on the branches. The petiole is 1 to 3 cm long, and the leaf blade is elliptic to obovate, measuring 7 to 15 cm in length and 3 to 7 cm in width. On young trees, the leaves are lobed, while on mature trees, the leaves are entire with a leathery texture. The leaves have a pale green underside with scattered resin cells and a dark green, shiny, and glabrous upper surface. The base of the leaf is cuneate, the margin of mature leaves is entire, and the apex is blunt to acuminate. The midvein is prominently visible on the underside, and there are 6 to 8 secondary veins on each side of the midvein.

Phyllotaxis: The leaves are arranged alternately and spirally on the branches of the tree. This means that the leaves emerge from the stem in a helical pattern, with each leaf positioned at an angle to the previous one.

Petiole: The leaves are attached to the stem via petioles, which are generally 1 to 3 cm long. The petioles serve to support the leaf and position it for maximum exposure to sunlight.

Leaf Blade: The leaf blade is elliptic to obovate in shape, measuring approximately 7 to 15 cm in length and 3 to 7 cm in width. These dimensions can vary slightly depending on the age of the tree and the specific growing conditions.

Leaf Texture: On young trees, the leaves may be lobed, while on mature trees, the leaves are entire, meaning they are undivided and have a smooth, leathery texture.

Leaf Color and Surface: The underside of the leaves is pale green and contains scattered resin cells. In contrast, the upper surface of the leaves is dark green, shiny, and smooth (glabrous).

Leaf Base, Margin, and Apex: The base of the leaf is cuneate (wedge-shaped), while the margin of mature leaves is entire, meaning it is smooth and unbroken. The apex of the leaves can range from blunt to acuminate, depending on the specific leaf.

Veins: The midvein of the leaf is prominently visible on the underside, and there are typically 6 to 8 secondary veins on each side of the midvein.

The jackfruit tree efficiently captures sunlight for photosynthesis and contributes to its overall growth and development.

1.3 LEAF BASE:

- The leaf base refers to the lower part of the leaf where it is attached to the stem or petiole.
- In the case of *Artocarpus heterophyllus* leaves, the leaf base is cuneate, meaning it is wedge-shaped, tapering to a narrow point at the attachment to the petiole or stem.

- The base of the leaf provides structural support and serves as the point of attachment for the leaf, allowing it to remain connected to the rest of the plant and facilitating the transport of water, nutrients, and sugars. (Smith,J.A, 2019)

1.4 LEAF APEX:

- The leaf apex, or tip, is the uppermost part of the leaf.
- In the context of Artocarpus heterophyllus leaves, the apex can vary from blunt to acuminate, with some leaves having a more rounded or blunt tip, while others have a sharper, pointed tip.
- The apex of the leaf is involved in regulating transpiration and gas exchange. It also plays a role in protecting the leaf meristem, the growing tip of the leaf, and may aid in deterring herbivores. (Garcia, 2020)

1.5 UNIQUE FEATURES OF THE LEAVES:

Glossy Texture: One of the most notable features of the leaf surface is its glossy texture. The upper surface of the leaves has a smooth and shiny appearance, which gives them a distinct visual appeal. This feature sets them apart from many other types of leaves and adds to their aesthetic value. (Patel, 2017)

Waxy Coating: The leaf surface may possess a natural waxy coating, which contributes to the glossy appearance and helps reduce water loss through transpiration. This waxy layer also serves as a protective barrier against pests and pathogens, enhancing the leaf's resilience. (Wang, 2018)

Microscopic Structures: Under a microscope, the leaf surface reveals intricate microscopic structures, such as stomata and trichomes. Stomata are small pores that facilitate gas exchange, while trichomes are hair-like outgrowths that can have various functions, including deterring herbivores and reducing water loss. (y, 2015)

Distinct Vein Patterns: The leaf surface showcases distinct vein patterns that contribute to its overall appearance. The network of veins, including the midrib

and secondary veins, creates a visually appealing and intricate design on the leaf surface. (H, 2017)

1.6 MEDICINAL AND TRADITIONAL USE OF LEAVES

Apart from their aesthetic value, *Artocarpus Heterophyllus* leaves have gained recognition for their wide range of medicinal and therapeutic properties, which have been valued for ages by a variety of cultures. These leaves have been used historically to treat a wide range of ailments, including fever, diarrhea, and skin infections. They are highly valued for their medicinal properties. Their abundance of vitamins, minerals, and antioxidants is what makes them so effective. For those who wish to enhance their health and well-being with natural therapies, *Artocarpus Heterophyllus* leaves can be added to teas or applied topically. These leaves have long been used in traditional medicine, which emphasizes their value and efficacy in complementary therapies. (Lily Chen, 2024)

In addition to their therapeutic uses, *Artocarpus Heterophyllus* leaves are important in culinary and cultural traditions, especially in Asian nations where they are frequently used in traditional dishes. When used in several cooking techniques, like steaming, frying, or as organic wrappers, these leaves provide food a mildly flavorful but unique taste that improves the overall flavor profile. Especially, they give savory foods a hint of sweetness, which enhances their distinct flavor profile. In addition, *Artocarpus Heterophyllus* leaves are a great complement to a balanced diet because they are a great source of fiber, antioxidants, and vitamins A and C. These leaves have a crucial function in fostering holistic nutrition and enhancing culinary traditions due to their culinary variety and cultural significance. (David Kim, 2024)the leaves of *Artocarpus Heterophyllus* are distinguished by their wide variety of health advantages in addition to their striking appearance. With their eye-catching look, culinary applications, and therapeutic qualities, these leaves present a plethora of opportunities to improve human well-being. We can continue to appreciate the diverse nature of *Artocarpus Heterophyllus* leaves and gain a deeper grasp of their value by exploring their exquisite intricacies in a variety of contexts. (Maria Rodriguez, 2024)

CHAPTER – 2

AIM AND OBJECTIVES

Nutritional Analysis: The nutritional composition of *Artocarpus heterophyllus* to determine health benefits and culinary applications.

Phytochemical Analysis: Analyzing the phytochemical compounds present in *Artocarpus heterophyllus* to explore its potential medicinal properties.

Value-Added Products: Exploring innovative ways to utilize *Artocarpus heterophyllus* in human diet, by developing value-added products such as tea powder, soup mix and chappathi flour.

- The main aim is to evaluate the phytochemical components, antimicrobial reaction and antioxidant activity present in *Artocarpus hirsutus*.
- It serves multiple purposes which include culinary and medicinal properties with potential health benefits.
- It is also used as a biodegradable plate.

CHAPTER 3

REVIEW OF LITERATURE

The *Artocarpus heterophyllus* is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands. It is a large, evergreen tree, 10-15m in height, indigenous to the evergreen forests at altitude of 450-1,200m and cultivated throughout the hotter parts of India. Stem of this plant is straight rough whereas bark is green or black, 1.25cm thick, exuding milky latex, leaves broad obovate, elliptic, decurrent, glabrous, entire inflorescence solitary axillaries, cauliflorous and ramiflours on short leafy shoots. Male head is sessile or on short peduncles receptacles, sometimes born on the ultimate twing, Female head are oblong ovoid receptacle, syncarpus, cylindrics. Seeds are separated horny endocarpus enclosed by sub-gelatinous exocarpus (1mm thick) oblong ellipsoid in nature. The sweet yellow sheaths around the seeds are about 3–5 mm thick and have a taste similar to that of pineapple, but milder and less juicy Jackfruit (*Artocarpus heterophyllus* Lam) produces heavier yield than any other tree species, and bear the largest known edible fruit (up to 35 kg). The jackfruit tree has several uses. Flakes of ripe fruits are high in nutritive value; every 100 g of ripe flakes contains 287-323 mg potassium, 30.0-73.2 mg calcium and 11-19 g carbohydrates. In Bangladesh, it is commonly referred to as “poor man’s food” as it is cheap and plentiful during the season. The nutritious seeds are boiled or roasted and eaten like chestnuts, added to flour for baking, or cooked in dishes. The tree is also known for its durable timber, which ages to an orange or reddish brown color, with anti-termite properties. The leaves and fruit waste provide valuable fodder for cattle, pigs and goats. Jackfruit wood chips yield a dye, which is used to give the famous orange-red color to the robes of Buddhist priests. In addition, many parts of the plant, including the bark, roots, leaves and fruits have medicinal properties. Leaves are dark green, alternate, entire, simple, glossy, eathery, stiff, large (up to 16 cm [6 in] in length), and elliptic to oval in form. Leaves are often deeply lobed when juvenile and on young shoots. The leaves are useful in fever, boils, wounds and skin diseases. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic, and

constipating. The wood is nervine, antidiabetic, sedative and is useful in convulsions. The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent .

The ash of Jackfruit leaves is used in case of ulcers. The dried latex yields artostenone, convertible to artosterone, and a compound with marked androgenic action. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings . The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhoea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property and its pith is said to be abortifacient. Latex is used as an anti-inflammatory agent (Om prakash., 2010).

Jackfruits comprises a wide range of nutrients, including minerals, carbohydrates, volatile compounds, proteins, and vitamins. The fruit, bark, leaves, and roots are endowed with therapeutic attributes and are utilized in the many traditional medicinal systems for the management of various ailments. Fruit and seeds are commonly used to prepare various food items, including sauce, ice creams, jams, jellies, and marmalades. Due to unique texture, jackfruit is becoming a popular meat substitute. Based on preclinical studies, jackfruit exhibits antimicrobial, antioxidant, anti-melanin, antidiabetic, anti-inflammatory, immunomodulatory, antiviral, anthelmintic, wound-healing, and antineoplastic activities. Clinical studies reveal that the leaves possess antidiabetic action in healthy and insulin-independent diabetic individuals. (Rajiv Gupta et., 2022)

The maceration process is carried out, the Jackfruit leaves to be used are cleaned first and then dried at room temperature without sunlight . This is because exposure to direct sunlight at high temperatures can damage and cause degradation of the compounds contained in the sample. After drying, the jackfruit leaves and then mashed using a blender. Phytochemical Screening After obtaining the thick extract, the extract was then tested for the chemical compounds contained in it using a phytochemical screening test .At this stage, five types of examinations are carried out, namely the examination of alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids.

The phytochemical screening test result was done for presence in jackfruit leaves alkaloid, Flavonoid, Fenolic/tannin. Steroid and Triterpenoid and absent for jackfruit leaves in saponin. This is supported by the color change that occurs due to the supply reagent for extracting jackfruit leaf . (Mishra, 20210) .Phytochemical test results show that jackfruit leaves contain various types of secondary metabolites .

The taller plants have good flavonoids in the vegetative section, especially in the flower. Flavonoids as flower pigments play an important role. Other functions of flavonoids are able to absorb ultraviolet light to direct insects, regulate plants, regulate photosynthesis, work in anti-microbial and anti-virus work on insects. The effects of flavonoids on many organisms are numerous. flavonoids are widely used in traditional medicine. In addition content of flavonoids work as a strong respiratory inhibitor, inhibiting enzyme and non-enzyme oxidation reactions The highest chemical extracts of jackfruit leaves are obtained from tritertenoid and steroid substances .flavonoids are found in low content. (warly, 2021)

The Preliminary phytochemical screening of EAAH leaves .The presence of flavonoids, sterols, carbohydrates, proteins, tannins, phenolic compounds and absence of alkaloids, volatile and fixed oils, glycosides anthroquinone, cardiac, cyanogenetic and isothiocyanate. (PERIYANAYAGAM, 2013) The total phenolic content of EAAH was found to be 376.5mg/g.The trace element content of XRF analysis showed the presence of Calcium (39.4%), Potassium (29.6%), Magnesium (2.06%), Iron (0.99%), Sulphur (1.83%), Zinc (0.083%), Strontium (0.23%), Manganese (0.13%) and Aluminium (0.005%). The HPTLC analysis of EAAH contain 134mg/g of ursolic acid . (V, 2013)

The ethanolic leaf extract Showed in the phytochemical screening in flavanoids, phenols, terpenoids, saponins, steroids,and aqueous extracts showed the presence of flavonoids , phenols, saponins,and steroids .The analgesic activity is presented in hot plate method , writhing method and Tail flick method . The antioxidant activity is presented in nitric oxide radical scavenging property of aqueous extarct and ethanolic extract with ascorbic acid , DPPH free radical scavenging property of aqueous extarct and ethanolic extract with ascorbic acid . (widyawati, 2020)

The preliminary phytochemical test for methanolic extract in leaves alkaloids, amino acid, carbohydrate, phenols, tannin, terpenoids, saponins, flavonoids, protein, steroids, fixed oil and fat. The ash content is (27.50%), water soluble content is (24.92%), Acid soluble content is (14.06%), Alcohol soluble content is (13.05%). Methanolic extract of *Artocarpus heterophyllus* was found to exhibit maximum antimicrobial activity against *E. coli*. With respect to the zone of inhibition. Zone of inhibition was exhibited by leaf extract with the high concentration. The Gram-positive bacterial strains were found to be more susceptible to the extracts of various plant parts tested as compared to Gram-negative bacteria (POOJITHA, 2017)

The phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrate, tannins, steroid, terpenoids, cardiac glycoside, saponins, carboxylic acid, all phytochemical screening content is Positive in *Artocarpus heterophyllus* leaves. The macro and micro mineral content in jack fruit leaves (7.150)Mg, (34.945)K, (85.108) Ca, (47.05) Na, (5.410) P. The mineral content is (8.579) Zn, (0.640) Cu, (1.296)Mn, (3.949) Cr, (40.740) Fe. (Onuah, 2018)

Aqueous methanol exhibited highest yield percentage in 10.98% . Methanol solvent least percentage in 0.22% . Ethyl acetate solvent yield 4.68% . Methanol extract presence in coumarin , steroids . Ethyl acetate presence in coumarin , terpenoid. Aqueous methanol presence in coumarin, tannin, saponin. Coumarin was present in all three extract blood thinning , antifungicidal, and antitumor activity . Terpenoid presence in aromatic properties and antibacterial , anti cancer properties (Uwakwe, 2018).

This plant reduce of cholesterol problem presence of steroid . Saponin present in aqueous methanol extract lowering of blood cholesterol and inhibition of cancer cell growth . Saponin used are traditional medicines, especially asia and intensively used in food, veterinary and medicinal industries. Tannin present in aqueous methanol extract naturally occurring polyphenols binding and precipitating proteins (Anacleto, 2018).

Phytochemical screening from the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids, and diterpenes . The antioxidant activity of tannin result from their free radical and reactive oxygen species- scavenging

properties, as well the chelation of transition metal ions that modify the oxidation process. Among three solvents used for the methanol extract the best antioxidant activity. So, the leaf extract of the plant has some anti-cancer activity (Nita thapa ET AL., 2016).

The proximate composition of jack fruit leaves. The moisture content range from (85.33%). The crude fiber content range from (4.91%). Ash content range (2.53%). Protein content range (1.19%). Fat content range (0.73%). Carbohydrate content range (5.33%). Minerals and vitamin content for jack fruit leaves. Calcium content range (0.52g/100g) Potassium content range (0.21g/100g) Manganese content range (12.75g/100g) Iron content range (59.50g/100g) Zinc content range (5.73g/100g) Vitamin c content range (0.99g/100g) Phytochemical composition of jack fruit leaves. Phytic acid content range (7.5 /mg/100g) Oxalate content range (0.42 /mg/100g) Alkaloid content range (12.43/mg/100g) Tannin content range (0.07/mg/100g) Flavonoid content range (3.91/mg/100g) (bhandari, 2016).

The calcium content for jackfruit leaves is high. Ash content is higher in jackfruit leaves. Potassium consumption improves blood pressure. The iron content is highest in jack fruit leaves. Vitamin c content is very low for jack fruit leaves. Phytic acid content is low. The tannin content is very low compared to oxalate and alkaloids. The presence of flavonoids indicates medicinal benefits. Antioxidant properties and increased activity of enzymes detoxify carcinogens (Amadi, 2018).

CHAPTER 4

MATERIALS AND METHODS

4.1 COLLECTION OF LEAVES

Fresh leaves of jackfruit were collected in November 2023 from a village in Alathur of Kerala. They were identified by Assistant professor S. Kanya Department of clinical nutrition and dietetics, Sathyabama institute of science and technology, Chennai. The leaves were washed slightly in distilled water, dried in sunlight for 1-2 weeks and used immediately.



FIG 4.1 DRIED JACK FRUIT LEAVES

4.2 EXTRACTION OF LEAVES MATERIAL

The extraction methods described by Professor James Department of Medical Laboratory Technology were adopted. Leaves were grinded into fine powder. 2gm of leaf powder is mixed with 50ml of ethanol and kept in a beaker. Water bath of leaf extract using ethanol at 40°C for a day. Leaf extract using ethanol was continued by the process of filtration using filter paper. Filtration has been done twice and preserved in deep freezer. The extract was again kept in water bath. 50% of ethanol extract has been reduced to 25% after 3 hours of water bath.



FIG 4.2 : WATERBATH LEAVES



FIG 4.3 : FILTRATION PROCESS

ETHANOL EXTRACT

4.3 PHYTOCHEMICAL SCREENING

The method of jackfruit leaves ethanol extract were used to identify the following phytochemicals in the extract :

Flavonoids, tannins, alkaloids, steroids, phenols, amino acid, carbohydrate, anthraquinone, terpenoids.

DETECTION OF FLAVONIODS:

Two to three drops of sodium hydroxide were added to 2ml of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

DETECTION OF TANNINS:

About 2.5mg of each plant extract was boiled in 5ml of water in a test tube and then filtered through WHATMAN'S no. 1 filter paper. Two to three drops of 0.1% ferric chloride added and read for brownish green or a blue –black precipitate indicating a positive result.

DETECTION OF ALKALOIDS:

By adding 1ml of Dragendorff's reagent to 2ml of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

DETECTION OF STEROIDS:

Leaf extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing leaf extract with 2ml of chloroform. Then 2ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

DETECTION OF PHENOLS :

Appearance of red color indicates the presence of phenols. Lead acetate test. Three drops of Pb(OAc)₂ was added to 5ml of extract and mixed well. Formation of yellow precipitate indicates the presence of phenols.

DETECTION OF AMINO ACID :

Take 2ml of given sample compound in a test tube. A few drops of concentrated sulphuric acid and heat. If there is formation of yellow precipitate then the presence of amino acid .

DETECTION OF CARBOHYDRATE:

One ml of the analyte sample must be mixed with 2ml of Benedict's reagent and heated in a bath of boiling water for 3 to 5 minutes. The development of a brick red coloured precipitate of cuprous oxide confirms the presence of reducing sugars in the analyte. The test for carbohydrate is negative.

DETECTION OF ANTHRAQUINONE:

To 2ml of extract 1ml of concentrated H₂SO₄ and 0.5ml diluted ammonia was added. Appearance of rose pink colour indicates the presence of anthraquinone. The test for anthraquinone is negative.

DETECTION OF TERPENOID:

Salkowski test was used to detect terpenoids . Extract (5ml) was mixed with chloroform(2ml), and concentrated sulphuric acid (3ml) was carefully added to

form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids. The test for terpenoids is positive.

TAB 2 : PHYTOCHEMICAL SCREENING

PHYTOCHEMICALS	NEGATIVE	POSITIVE
FLAVANOIDS		+
TANNIS		+
ALKALOIDS		+
STEROIDS		+
PHENOLS		+
AMINO ACIDS		+
CARBOHYDRATE	-	
ANTHRAQUINONE	-	
TERPENOIDS		+

4.4 NUTRITIVE ANALYSIS

CARBOHYDRATE

Procedure:

Total carbohydrates are calculated as follows after determining the percentage of moisture, total protein, fat and total ash

$$\text{Total Carbohydrate} = 100 - (A + B + C + D)$$

ENERGY

Procedure:

Food energy values in both kilocalories (kcal) and kilojoules use the same factors.

$$\text{Energy (kcal/100g)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

A = P by mass of moisture

B = Percent by mass of total ash,

C = Percent by mass of fat

D = Percent by mass

FAT

AOAC Official Method 954.02, 20th editions, 2016

Principle: Not applicable

Apparatus:

- Hot air oven.
- Aluminum dish.
- Desiccators
- Soxhlet apparatus
- Thimble

Chemicals and Reagent :

- Hexane - Food grade
- Ethanol
- Petroleum ether
- Diethyl ether
- Hydrochloric acid

Procedure:**For more than 10% of fat contained samples:**

Weigh accurately about 2 g of sample in a pre-weighed aluminum dish with a cover.

Shake the dish until the contents are evenly distributed. With cover removed place the dish and cover in hot air oven maintained at 100°C and dry for at least 2 hours. Place cover on dish, cool in a desiccators and weigh. Weigh accurately about 2.5 g of moisture free sample in thimble. Extract with Hexane in a Soxhlet apparatus for 4 hours. Dry the extract on a hot air oven for overnight, cool in desiccators and weigh.

For Less than 10% of fat contained samples:

Applicable to fruit juices, cereal based products also.

Weigh accurately about 2 g of sample in a fat extraction tube; add 2.0 ml of alcohol to prevent lumping on addition of acid, and shake to moisten all particles. Add 10 ml HCL (25+11), mix well and set tube 30 to 40 mins in water bath at 70°C to 80°C, shaking frequently. Cool to room temperature and add alcohol. Add 25 ml diethyl ether to flask and shake 1.0 mins. Then add 25 ml petroleum ether to flask and shake 1 mins. Decant ether layer in to pre weighed dry flask through anhydrous sodium sulphate. Then Decant ether layer in to pre weighed dry flask through anhydrous sodium sulphate. Then add 25 ml of 1:1 diethyl ether, petroleum ether to flask and shake 1.0 min. Ether layer can be evaporated under water bath. Keep the flask in to oven for further evaporation of ether residue.

Remove flask from oven and place in desiccators to cool to room temperature. Record the weight of flask plus fat.

Calculation:

$$\% \text{ of Fat} = \frac{(\text{Weight of flask +fat}) - \text{Empty weight of the flask} \times 100}{\text{Sample Weight}}$$

PROTEIN

Procedure:

Weigh 0.7 to 2.2 g sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4 g CuSO₄ and 25 mL. or more, if necessary of Con H₂SO₄ to the flask. Heat the flask for 90 min for complete digestion. Cool, cautiously add 250 ml. H₂O, and cool to room temperature. Add 100 ml of 4% boric acid to the beaker, that condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150 mL. distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

$$\% \text{ N (w/w)} = (\text{TV}_s - \text{TV}_b) \times \text{Normality of standard HCl} \times 1.4007 \text{ Sample Weight}$$

Where

TV_B Titer value of Blank.

TV_s Titer value of sample

Calculate Crude protein from Nitrogen

TAB 3 : NUTRITIVE ANALYSIS

Animal feed (General factor)	NX 6.25
Milk and Dairy products	NX 6.38
Wheat flour	NX 5.7
Gelatine	NX 5.55
Egg	NX 6.68
Soya flour	NX 5.70
Ground Nuts	NX 5.46
Rice	NX5.95
Rye/Barley/Oats	NX 5.83
Wheat meal	NX 5.83

SODIUM

This standard operating procedure describes a method for the determination of sodium and potassium all type of ready to eat food products.

REFERENCE

AOAC20th edition 2016

PRINCIPLE

Flame emission is directly proportional to the concentration of the solution.

APPARATUS

Silica crucible

Muffle furnace

Standard measuring flasks-100ml

Flame photometer

CHEMICALS AND REAGENTS

Conc. Nitric acid

Sodium chloride High purity

Potassium chloride High purity

Sodium standard solution

Stock solution - 1mg na/ml. Dry reagent grade Nacl 2 h at 110o c ; cool in deciccators. Weight 2.5421gram in to 1L Volumetric flask and dilute to volume with H2O.

Working standard solution

5,10,25,50, 100mg 1 prepare from 1000 ppm of stock solution .

Potassium standard solution

1,9068 g into 1L volumetric flask and dilute to volume with H2O (or) Use potassium 1000 mg/1 if available.

Working standard solution

5,10,25,50, 100mg/l prepare from 1000 ppm of stock solution.

Procedure :

Dry ash method:

Weigh 4 g test sample into crucible, and char on electric hot plate or over low flame. Place in cold furnace and bring to 525°C. Ash 2 h to white ash. Cool, and weigh if total ash is desired. Add 15 ml dilute HNO₃ (1+4) to crucible, breaking up ash with stirring rod if necessary. Filter through acid-washed quantitative paper into 100 mL. volumetric flask. Wash residue and paper 3 times with H₂O. Dilute to volume and read directly. Prepare blank solution by diluting 2 mL HNO₃ to 100 ml with H₂O. If sample goes to out of calibration make suitable dilution and read once again.

Calculation:

$$\text{Sodium or potassium (mg/kg)} = \frac{\text{Concentration} \times \text{Made up volume} \times \text{Dilution}}{\text{Weight of the sample}}$$

$$\text{Sodium or Potassium (mg/100g)} = \frac{\text{Sodium or Potassium (mg/kg)}}{10}$$

CALCIUM**Reference:**

IS : 5949:1990 RA 2020 volumetric determination of calcium and magnesium using EDTA.

Principle: Not applicable.

Apparatus:

- Silica crucible
- Muffle furnace
- volumetric flasks
- Gooch filter
- Pipettes
- What man filter paper No.40

Chemicals and Reagents:

- P & R indicator
- EBT indicator
- Sodium hydroxide diluted -20%
- Standard EDTA Solution - 0.01M
- Triethanolamine solution
- Buffer solution
- Hydroxylamine Hydrochloride

Procedure:

Weigh 5 g of sample in Silica crucible and ignite at 550 deg * C plus/minus 25 deg * C in furnace to C- free ash. Boil residue with 40 mL dil HCl and few drops concentrated $\text{HN}^*\text{O}_{\{3\}}$ and Cool Transfer to 250 mL volumetric flask, dilute to volume, and mix thoroughly.

For Calcium:

Take a suitable aliquot of the sample solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust ph to 12.5 to 13.0 with 20% sodium hydroxide solution using a ph meter. Add P & R indicator. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. The end point the colour turns a violet which on further drop wise addition gives the pure blue end point. Where a slight excess of EDTA has been added. Note the volume as V1.

For Magnesium:

Take a same as above aliquot of the solution. Expected to contain approximately 10 to 30 mg of calcium and magnesium combined oxides in a 500 ml conical flask. Add 10 ml of hydroxylamine hydrochloride solution, 2 ml of potassium cyanide solution and 25 ml of triethanolamine solution. Dilute to 150 to 200 ml and add sufficient quantity of buffer solution to bring the pH to 9.5 to 10. Add 3 to 4 drops of EBT indicator solution and titrate with 0.01 M EDTA solution till the red colour changes to pure blue end point free from violet tinge. It is easier to note the end point by comparing the colour with that of previously titrated solution having a pure blue end point, where a slight excess of EDTA Solution has been added. Note the volume as V2.

Note: Make necessary dilution and take necessary aliquots according to the Calcium and magnesium concentration.

Calculation:

$$\% \text{ of calcium (mg/100g)} = \frac{V1 \times 40.08 \times \text{Molarity} \times \text{Dilution} \times 100}{\text{Weight of the sample}}$$

4.5 ANTIBIOTICS SENSITIVITY TEST

The same procedure is followed as we have done for Phytochemical testing. The broth has been inserted in the test tube and it has been closed tightly using cotton.

The extract has been kept in autoclave for 45minutes. There 8 different bacteria inserted Into the test tube. The test tube is kept in incubator for incubator to view the bacterial growth.



FIG 4.4 : BROTH WAS COLLECTED



FIG 4.5: MATERIALS USED



FIG 4.6 : 8 SPECIFIC BACTERIA

4.6 DATA ANALYSIS AND INTERPRETATION

GENDER :

TAB 4 : NUTRITIVE ANAIYSIS GENDER

Male	13
Female	37

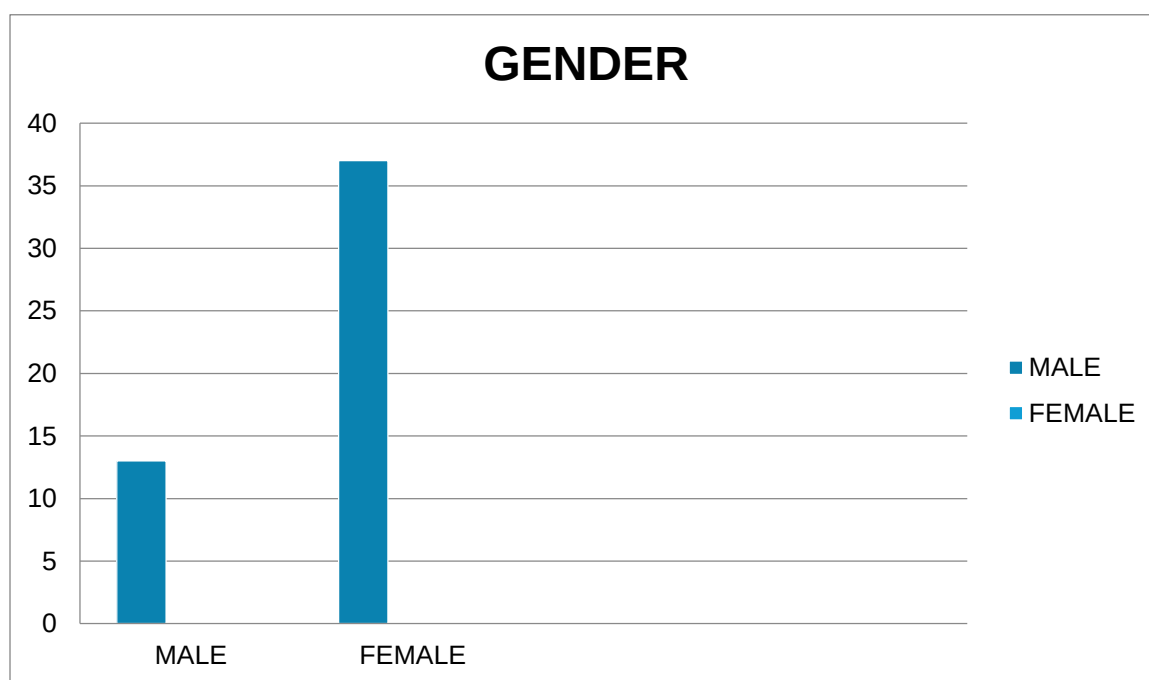


FIG 4.7 : SENSORY ANALYSIS BASED ON GRAPH

Calculation :

$$\text{Male} = 13 \times 50 \div 100 = 6.5$$

$$\text{Female} = 37 \times 50 \div 100 = 18.5$$

INTERPRETATION :

In this data was collected in both male and female people. 13 male people and 37 female people were collected.

OCCUPATION :

TAB 5 : NUTRITIVE ANALYSIS OCCUPATION

RANGE OF OCCUPATION	NO.OF RESPONSE
Bio chemistry	35
Bio technology	10
B.com	5

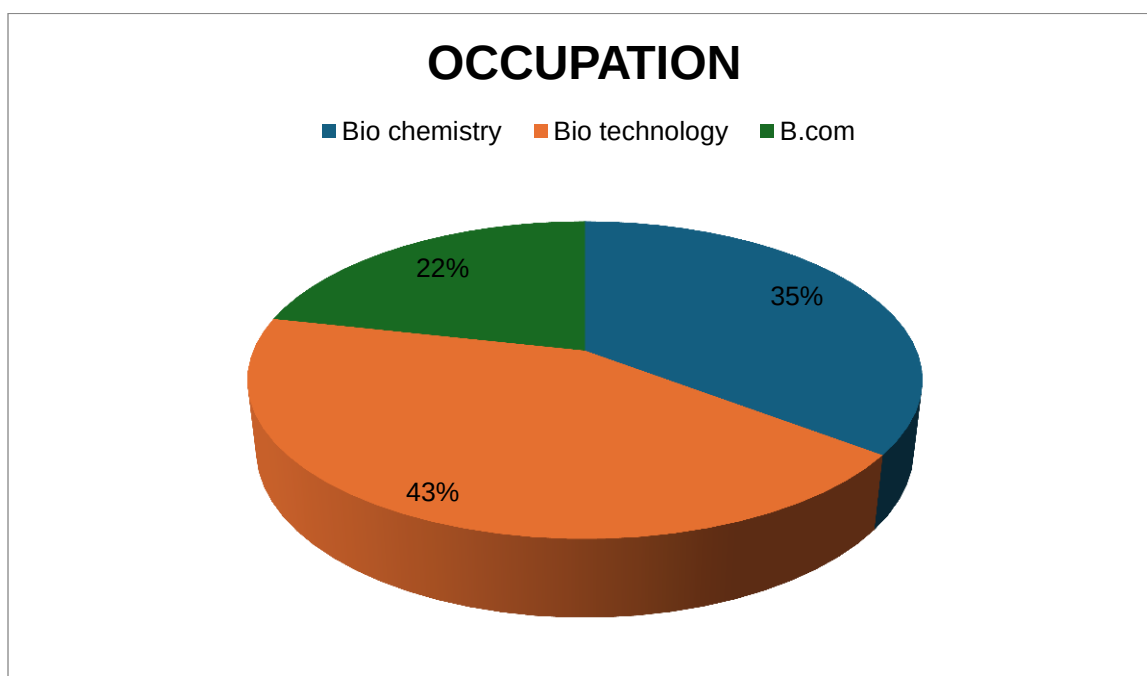


FIG 4.8 : SENSORY ANALYSIS BASED ON PIECHART

Calculation :

$$\text{Biochemistry} = 35 \times 50 \div 100 = 17.5$$

$$\text{Biotechnology} = 10 \times 50 \div 100 = 5$$

$$\text{B.com} = 5 \times 50 \div 100 = 2.5$$

INTERPRETATION :

It was found that people lifestyle. bio chemistry student is 35. Biotechnology student is 10 people. B.com student is 5 people.

APPEARANCE :

TAB 6 : NUTRITIVE ANALYSIS APPEARANCE

Range of Appearance	No. of Response
Like	28
Like extremely	10
Like very much	12

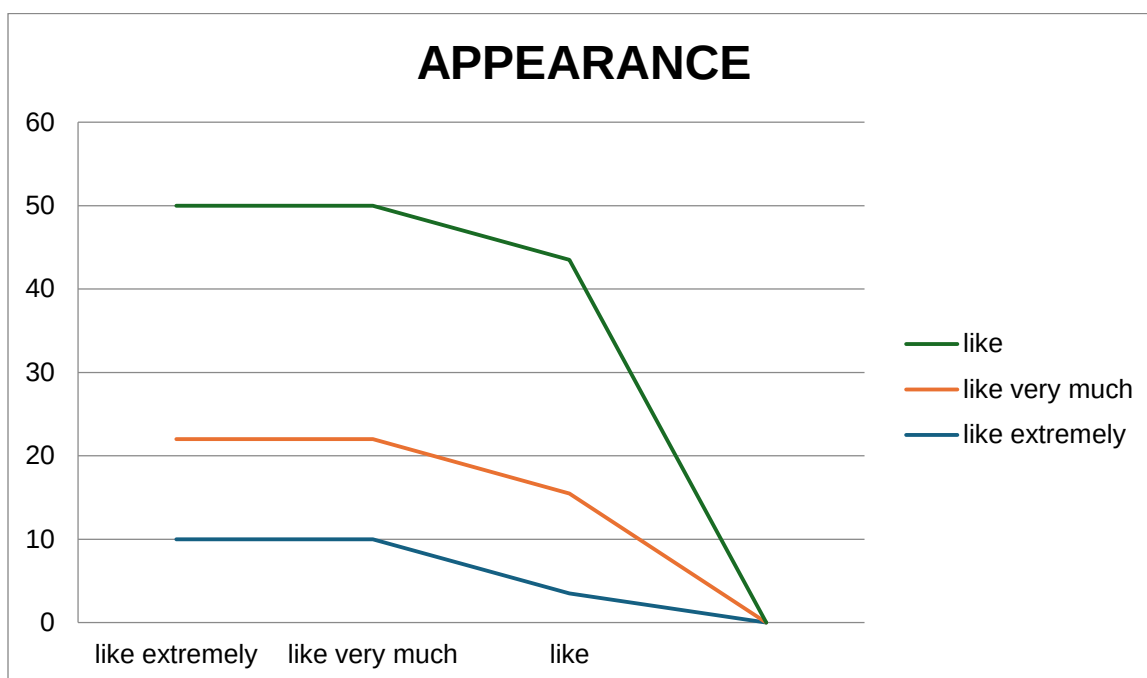


FIG 4.9 : SENSORY ANALYSIS BASED ON LINECHART

Calculation :

$$\text{Like} = 28 \times 50 \div 100 = 14$$

$$\text{Like extremely} = 10 \times 50 \div 100 = 5$$

$$\text{Like very much} = 12 \times 50 \div 100 = 6$$

INTERPRETATION :

The data was collected in appearance in tea powder for people. Like extremely for 27, like very much for 8, like 15 people.

TEXTURE :

TAB 7 : NUTRITIVE ANAIYSIS TEXTURE

Range of texture	No. of Response
Like extremely	20
Like slightly	17
Neither like or dislike	8
Like	5

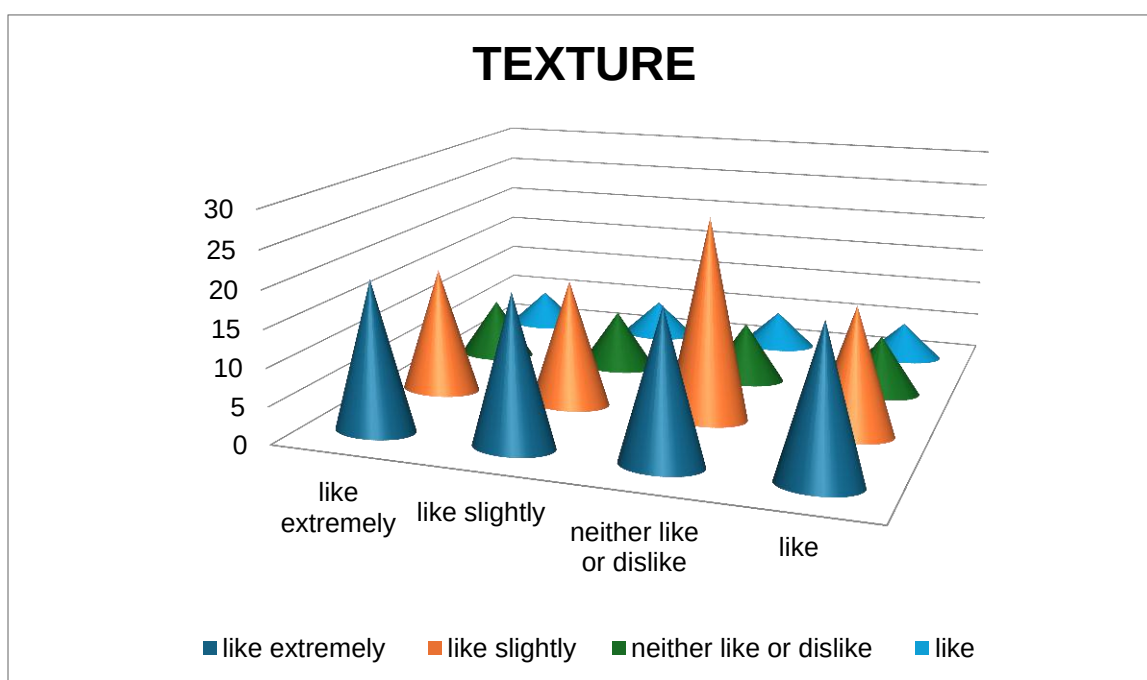


FIG 4.10 : SENSORY ANALYSIS BASED ON BARCHART.

Calculation :

Like	=	$5 \times 50 \div 100 = 2.5$
Like extremely	=	$20 \times 50 \div 100 = 10$
Like slightly	=	$17 \times 50 \div 100 = 8.5$
Neither like or dislike	=	$8 \times 50 \div 100 = 4$

INTERPRETATION :

The data was collected in appearance in tea powder for people. Like extremely for 27, like very much for 8, like 15 people.

FLAVOUR / TASTE :

TAB 8 : NUTRITIVE ANAIYSIS FLAVOUR/TASTE

Range of flavor/ taste	No. of Response
Like extremely	18
Like very much	10
Neither like or dislike	7
Like	15

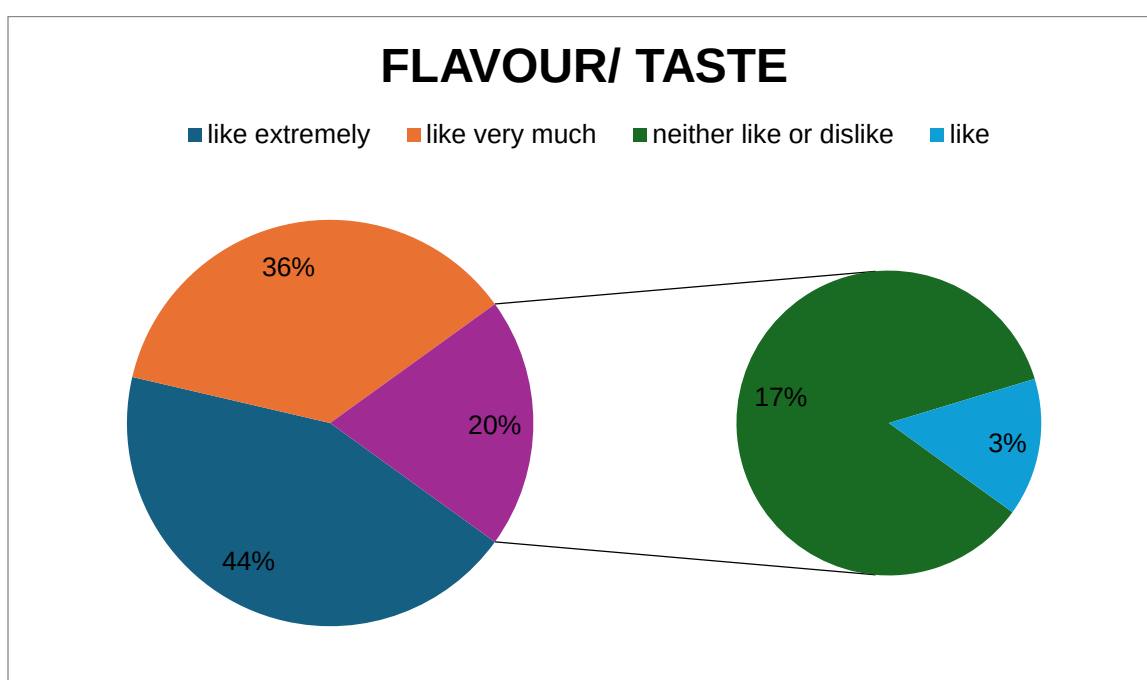


FIG 4.11 : SENSORY ANALYSIS BASED ON PIECHART.

Calculation :

Like	=	$15 \times 50 \div 100 = 7.5$
Like extremely	=	$18 \times 50 \div 100 = 9$
Like very much	=	$10 \times 50 \div 100 = 5$
Neither like or dislike	=	$7 \times 50 \div 100 = 3.5$

INTERPRETATION :

The data was collected in flavor/ taste tea powder for people. Like extremely for 18, like very much for 10, like 15, neither like or dislike 7 people.

AROMA :

TAB 9 : NUTRITIVE ANALYSIS AROMA

Range of aroma	No. of Response
Like extremely	28
Like	12
Neither like or dislike	4
Like very much	6

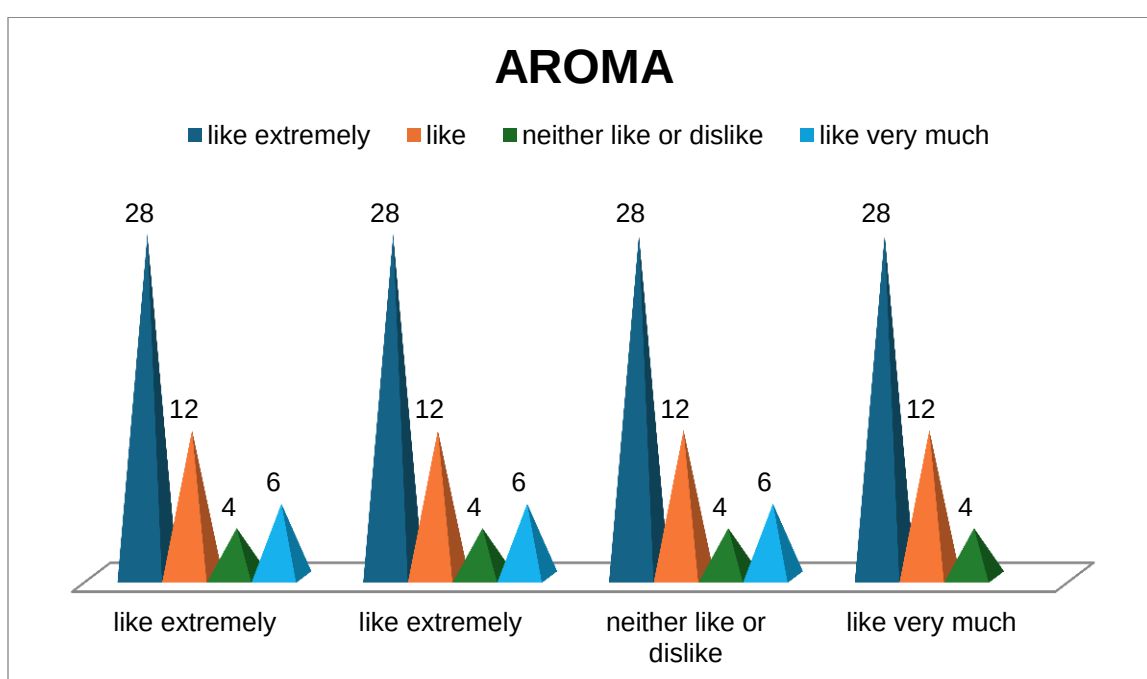


FIG 4.12 : SENSORY ANALYSIS BASED ON BARCHART

Calculation :

$$\begin{aligned}\text{Like} &= 12 \times 50 \div 100 = 6 \\ \text{Like extremely} &= 28 \times 50 \div 100 = 14 \\ \text{Like very much} &= 6 \times 50 \div 100 = 3 \\ \text{Neither like or dislike} &= 4 \times 50 \div 100 = 2\end{aligned}$$

INTERPRETATION :

The data was collected in aroma for tea powder for people. Like extremely for 28, like very much for 6, like 12, neither like or dislike 4 people.

OVER ALL ACCEPTABILITY

TAB 10: NUTRITIVE ANALYSIS OVER ALL ACCEPTABILITY

Range of over all acceptability	No. of Response
Like extremely	27
Like	13
Neither like or dislike	3
Like very much	7

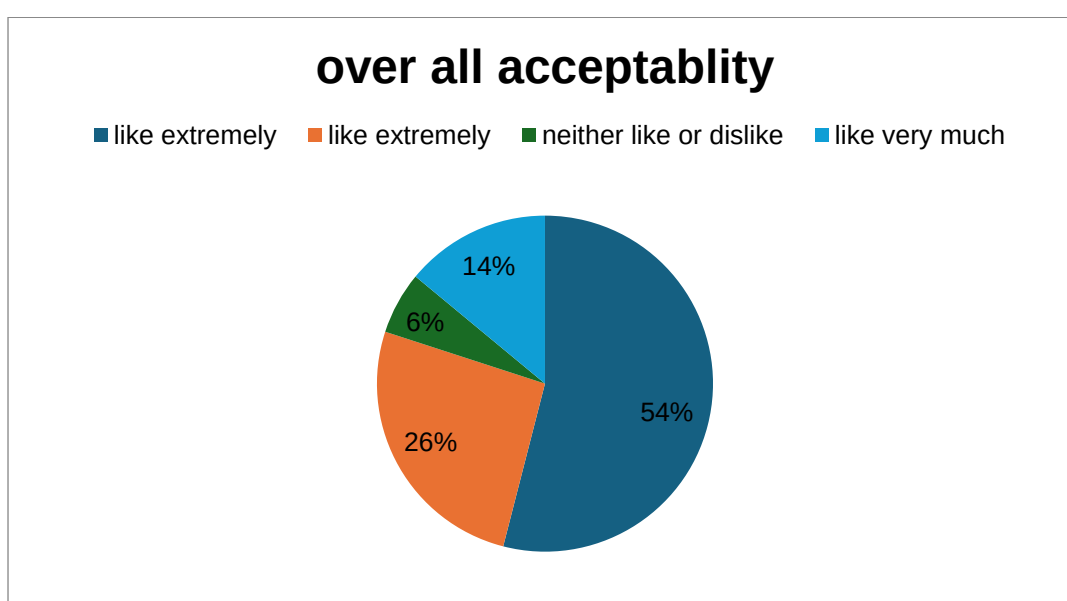


FIG 4.13 : SENSORY ANALYSIS BASED ON PIECHART.

Calculation :

Like	=	$13 \times 50 \div 100 = 6.5$
Like extremely	=	$27 \times 50 \div 100 = 13.5$
Like very much	=	$7 \times 50 \div 100 = 3.5$
Neither like or dislike	=	$3 \times 50 \div 100 = 1.5$

INTERPRETATION :

The data was collected in over all acceptability tea powder for people. Like extremely for 27, like very much for 7, like 13, neither like or dislike 3 people.

CHAPTER – 5

RESULTS AND DISCUSSION

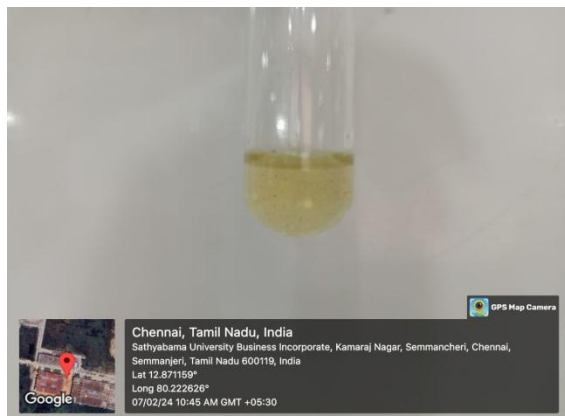


FIG 5.1 : FLAVONOIDS



FIG 5.2 :TANNINS



FIG 5.3 :ALKALOIDS



FIG 5.4 :STEROIDS

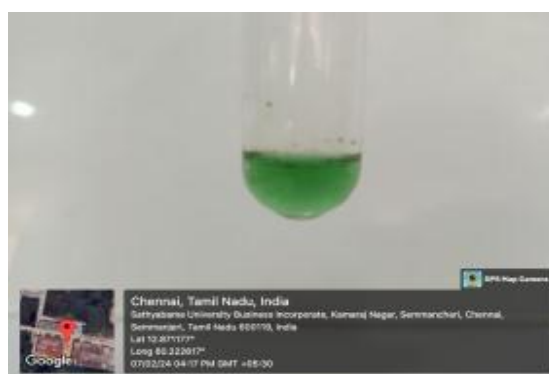


FIG 5.5 :CARBOHYDRATE



FIG 5.6 : AMINO ACIDS

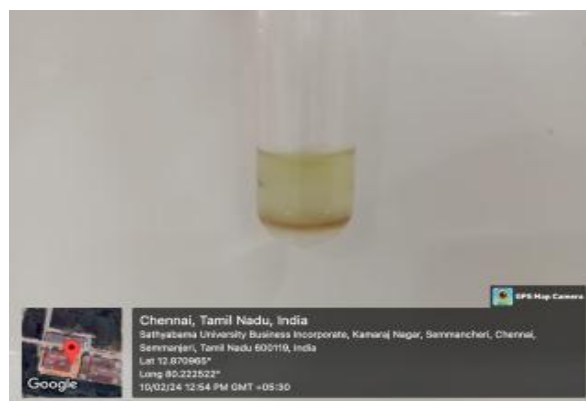


FIG 5.7 : TERPENOIDS



FIG 5.8 : PHENOLS



FIG 5.9 : ANTHRAQUIONE

Phytochemical screening of *Artocarpus heterophyllus* leaves revealed the presence of various secondary metabolites, indicating their potential medicinal and

nutritional value. The qualitative analysis detected alkaloids, flavonoids, phenols, tannins, and terpenoids in the leaf extracts.

Alkaloids are nitrogen-containing compounds known for their diverse pharmacological activities, including analgesic, anti-inflammatory, and antimicrobial properties. Their presence in *A. heterophyllum* leaves suggests potential therapeutic applications in traditional medicine.

Flavonoids are polyphenolic compounds with antioxidant, anti-inflammatory, and anti-cancer properties. The presence of flavonoids in the leaves indicates their potential role in scavenging free radicals and reducing oxidative stress, thus contributing to overall health benefits.

Phenols are another class of compounds with antioxidant properties that can protect against oxidative damage and reduce the risk of chronic diseases such as cardiovascular disorders and cancer. The presence of phenols in *A. heterophyllum* leaves underscores their potential as functional foods with health-promoting properties.

Tannins are polyphenolic compounds known for their astringent properties and ability to precipitate proteins. They have been traditionally used for their antimicrobial and anti-diarrheal effects. The presence of tannins in *A. heterophyllum* leaves suggests their potential role in gastrointestinal health and microbial defense.

Terpenoids are widely distributed in plants and possess diverse biological activities, including antimicrobial, anti-inflammatory, and anti-cancer properties. Their presence in *A. heterophyllum* leaves indicates their potential as natural therapeutic agents.

Overall, the phytochemical screening results suggest that *A. heterophyllum* leaves contain a rich array of bioactive compounds, which may contribute to their traditional medicinal uses and potential as functional foods. Further studies are warranted to isolate and characterize specific phytochemicals and elucidate their pharmacological effects and mechanisms of action.

NUTRITIVE ANALYSIS

S.NO	PARAMETERS	METHODS	UNITS	RESULTS
1	Energy (By calculation)	FAO Method	Kcal/100g	344
2	Carbohydrate(By difference)	CTL/SOP/FOOD/262-2014	g/100g	62.9
3	Total fat	AOAC 20 TH Edn2016,952.02	g/100g	2.14
4	Protein (Nx 6.25)	AOAC 20 TH Edn2016,952.25	g/100g	18.4
5	Total sugars	FSSAI Manual 2015-Beverages Sugar and Confectionaries	g/100g	BDL(DL:2.0)
6	Dietary fibre	AOAC 20 TH Edn2016,952.29	g/100g	19.6
7	Sodium as Na	AOAC 20 TH Edn2016,952.23	mg/100g	79.4
8	Calcium as Ca	IS 5949:1990(RA 2010)	mg/100g	1082
9	Iron as Fe	AOAC 20 TH Edn2016,999.11	mg/100g	7.12

The nutritive analysis of *Artocarpus heterophyllus* leaves revealed valuable insights into their composition and potential nutritional benefits. The proximate composition analysis showed that the leaves are rich in essential nutrients, including proteins, carbohydrates, fats, fiber, moisture, ash, and energy content.

Proteins are crucial for building and repairing tissues, enzymes, and hormones in the body. The presence of proteins in *A. heterophyllus* leaves suggests their potential role as a plant-based protein source, especially for individuals following vegetarian or vegan diets.

Carbohydrates are the primary source of energy for the body and play a vital role in maintaining blood sugar levels. The carbohydrate content of *A. heterophyllus* leaves contributes to their energy-giving properties and may support overall metabolic function.

Fats are essential for various physiological processes, including hormone production, cell membrane structure, and nutrient absorption. While the fat content of *A. heterophyllum* leaves may be relatively low, the presence of healthy unsaturated fats can still provide valuable nutritional benefits.

Dietary fiber is crucial for digestive health, promoting regular bowel movements, and preventing constipation. The fiber content of *A. heterophyllum* leaves indicates their potential role in supporting gastrointestinal function and overall digestive wellness..

The energy content of *A. heterophyllum* leaves provides an estimate of the caloric value of the leaves, which can contribute to overall dietary intake and energy balance.

Their consumption could contribute to meeting daily iron requirements and preventing iron deficiency anemia, especially in populations with limited access to animal-based iron sources.

Calcium is essential for bone health, muscle function, nerve transmission, and blood clotting. The presence of calcium in *A. heterophyllum* leaves suggests their potential role in promoting bone strength and overall skeletal integrity, particularly in individuals with lactose intolerance or those following dairy-free diets.

Sodium is an electrolyte involved in fluid balance, nerve function, and muscle contraction. While excessive sodium intake is associated with adverse health effects such as hypertension and cardiovascular disease, moderate consumption is necessary for maintaining physiological balance. The sodium content of *A. heterophyllum* leaves provides insight into their overall mineral composition and potential impact on sodium intake in the diet.

The presence of significant levels of iron and calcium in *A. heterophyllum* leaves highlights their potential as nutrient-rich foods that can contribute to meeting daily mineral requirements and supporting overall health and well-being. Additionally, the moderate sodium content suggests that their consumption can be part of a balanced diet without significantly impacting sodium intake levels.

In conclusion, the nutritive analysis results demonstrate that *Artocarpus heterophyllus* leaves are a valuable source of essential nutrients, including proteins, carbohydrates, fats, fiber, sodium, iron, calcium, and energy. Incorporating these nutrient-rich leaves into the diet can contribute to overall health and well-being. Further research is warranted to explore their potential nutritional benefits and culinary applications in greater depth.

ANTI BIOTICS SENSITIVITY

The antibiotic sensitivity testing of *Artocarpus heterophyllus* leaves aimed to assess their potential antimicrobial activity against common bacterial strains. The results of the sensitivity testing revealed varying degrees of susceptibility among the tested bacterial strains to the leaf extracts.

The leaf extracts exhibited notable antimicrobial activity against a range of bacterial pathogens, including Gram-positive and Gram-negative bacteria. The observed inhibition zones indicated the ability of the leaf extracts to inhibit the growth of bacterial colonies, suggesting their potential as natural antimicrobial agents.

The sensitivity testing also provided insights into the spectrum of antimicrobial activity of *A. heterophyllus* leaves, with some bacterial strains showing greater susceptibility compared to others. This variability in sensitivity may be attributed to differences in bacterial cell wall structure, membrane permeability, and susceptibility to the bioactive compounds present in the leaf extracts.

The antimicrobial activity of *A. heterophyllus* leaves can be attributed to their rich phytochemical composition, including alkaloids, flavonoids, phenols, tannins, and terpenoids, which have been reported to possess antimicrobial properties in previous studies.

The findings of this study suggest that *A. heterophyllus* leaves have the potential to be used as natural alternatives to conventional antibiotics in the treatment of bacterial infections. Their broad-spectrum antimicrobial activity and relatively low toxicity make them promising candidates for further research and development of novel antimicrobial agents.

Furthermore, the use of plant-based antimicrobials can help mitigate the growing problem of antibiotic resistance, offering sustainable and eco-friendly solutions to combat infectious diseases.

In conclusion, the antibiotic sensitivity testing results demonstrate the potential antimicrobial activity of *Artocarpus heterophyllus* leaves against common bacterial pathogens. Further research is warranted to elucidate the mechanisms of action of their bioactive compounds and to explore their therapeutic applications in the treatment of bacterial infections.

CHAPTER – 6

SUMMARY AND CONCLUSION

The study involved the collection and extraction of fresh jackfruit leaves from Alathur, Kerala, in November 2023. The identification of the leaves was performed by Assistant Professor S. Kanya from the Department of Clinical Nutrition and Dietetics at Sathyabama Institute of Science and Technology, Chennai. The leaves were washed, dried, and subjected to ethanol extraction following the method described by Professor James from the Department of Medical Laboratory Technology.

Phytochemical screening of the ethanol extract revealed the presence of flavonoids, tannins, alkaloids, steroids, phenols, and amino acids, while tests for carbohydrates, anthraquinone, and terpenoids showed negative results.

Detection of phytochemicals was carried out using specific tests:

- Flavonoids: Sodium hydroxide test
- Tannins: Ferric chloride test
- Alkaloids: Dragendorff's reagent test
- Steroids: Chloroform and concentrated sulfuric acid tests
- Phenols: Lead acetate test
- Amino acids: Concentrated sulfuric acid test
- Carbohydrates: Benedict's test
- Anthraquinone: Concentrated sulfuric acid and diluted ammonia test
- Terpenoids: Salkowski test

The nutritive analysis included the determination of carbohydrate, protein, fat, sodium, and calcium content in the jackfruit leaf extract using standard methods. The carbohydrate content was calculated, and energy value was determined based on the carbohydrate, protein, and fat content.

For fat determination, the AOAC Official Method 954.02 was followed, while protein content was determined using the Kjeldahl method. Sodium and calcium

were analyzed using flame photometry and volumetric determination methods, respectively.

The study concludes that jackfruit leaves contain a variety of phytochemicals and nutrients, including flavonoids, tannins, alkaloids, phenols, amino acids, carbohydrates, and terpenoids. Additionally, the leaves were found to be rich in carbohydrates, proteins, fats, sodium, and calcium. These findings suggest that jackfruit leaves may possess potential health benefits and can be further explored for their medicinal and nutritional properties.

In the antibacterial sensitivity testing, a procedure similar to that of phytochemical testing was followed. Broth was added to test tubes, which were then sealed with cotton and autoclaved for 45 minutes to ensure sterility. Eight different strains of bacteria were introduced into the test tubes, and the tubes were subsequently placed in an incubator to observe bacterial growth.

The purpose of this testing was likely to evaluate the antibacterial properties of the jackfruit leaf extract against a range of bacterial strains. By exposing the bacteria to the extract under controlled conditions, researchers aimed to determine if the extract could inhibit or reduce bacterial growth, indicating potential antibacterial activity.

A conclusion drawn from the results of this testing would depend on the observed bacterial growth patterns. If bacterial growth is significantly inhibited or reduced in the presence of the jackfruit leaf extract compared to control samples, it suggests that the extract possesses antibacterial properties. Conversely, if bacterial growth is similar to or greater than control samples, it may indicate a lack of antibacterial activity in the extract.

Overall, the antibacterial sensitivity testing provides valuable insights into the potential therapeutic applications of jackfruit leaf extract in combating bacterial infections. If positive results are obtained, further studies could be conducted to elucidate the mechanisms of action and identify the specific compounds responsible for the antibacterial activity.

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**PREPARATION AND NUTRIENT ANALYSIS OF BOTTLEGUARD JAM
(Enriched with Prebiotic)
PROJECT WORK**

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Clinical Nutrition and Dietetics

By

**JILLELA BHARATHI (41736005)
KARRU LIKHITHA REDDY (41736006)**



DEPARTMENT OF ALLIED HEALTH SCIENCES

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(DEEMED TO BE UNIVERSITY)

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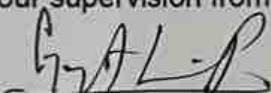
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of, **JILLELA BHARATHI (41736005)**, **KARRU LIKHITHA REDDY (41736006)** who carried out the project entitled **PREPARATION AND NUTRIENT ANALYSIS OF BOTTLEGUARD JAM (Enriched with Prebiotic)** under our supervision from January 2024 to April 2024.

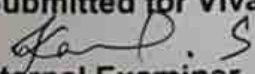

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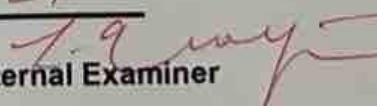
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Submitted for Viva-voce Examination held on

27/4/2024


Internal Examiner


External Examiner

DECLARATION

We KARRU LIKHITHA REDDY (41736006), JILLELA BHARATHI (41736005) hereby declare that the project Report entitled "**PREPARATION AND NUTRIENT ANALYSIS OF BOTTLE GUARD JAM (Enriched with Prebiotic)**" done by us under the guidance of Dr. Gayathri.P Assistant professor Internal guide, Department of Medical Laboratory Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition and Dietetics.

DATE: 27-04-2024

PLACE: CHENNAI

K. Likhitha Reddy
SIGNATURE OF THE CANDIDATE

DECLARATION

JILLELA BHARATHI (41736005) hereby declare that the project Report entitled **"PREPARATION AND NUTRIENT ANALYSIS OF BOTTLE GUARD JAM (Enriched with Prebiotic)"** done by me under the guidance of Dr.Gayathri.P Assistant professor Internal guide, Department of Medical Laboratory Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition and Dietetics.

DATE: 27-04-2024

PLACE: CHENNAI

J. Bharathi

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Bottle gourd and banana jam is an innovative fruit preserve that combines the nutritional benefits of both ingredients. This study investigates the nutritional composition and microbial content of bottle gourd and banana jam, while also providing detailed insights into the jam preparation process. Nutritional analysis reveals the rich presence of essential vitamins, minerals, and dietary fiber in both bottle gourd and banana, highlighting their potential health benefits. Microbial analysis ensures the safety and quality of the final product by evaluating the microbial load and identifying any potential contaminants. The jam preparation process involves selecting fresh bottle gourd and ripe bananas, followed by thorough cleaning and sanitization. The fruits are then chopped and cooked with sugar and lemon juice to enhance flavor and aid in preservation. The mixture is carefully monitored to ensure proper consistency and texture. The resulting jam exhibits a harmonious blend of sweetness from bananas and the subtle earthiness of bottle gourd, enhanced by natural pectin for a perfect texture. Nutrient analysis revealed significant levels of vitamins, minerals, and dietary fiber, making it a wholesome alternative to traditional jams. Overall, this innovative jam offers a delightful culinary experience while promoting health and sustainability.

Keywords: bottle gourd, banana, nutrient analysis, microbial analysis, traditional jams.

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LIST OF ABBREVIATIONS

ACS	American Chemical Society
ASTM	American society for testing materials
Ca	Calcium
CFU	Colony forming unit.
CNS	Central nervous system
CuSO ₄	Copper sulphate
DV	Daily Value
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediaminetetraacetic acid
g	Grams
GERD	Gastroesophageal reflux disease
HCL	Hydrogen chloride
H ₂ O	Hydrogen chloride
Kcal	Kilocalorie
KOH	Potassium hydroxide
LDL	Low-density lipoprotein
M EDTA	Metal ethylene diamine tetra acetic aid
Mg	milligrams
N	Nitrogen
NaOH	Sodium hydroxide
pH	Potential of hydrogen
spp	Species
TPC	Total plate count
TSS	Total soluble solids

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CHAPTER-1

INTRODUCTION

Lagenaria siceraria, known as bottle gourd in English and Lauki in Hindi, is a common vegetable in India. Bottle gourd has been used traditionally to help with many health conditions like fever, cough, pain, and asthma. It has been used since ancient times for its benefits. It is also considered a good source of vitamin B, C, and other nutrients. It is known for its shape, a bottle, dumbbell, or oval shape. (Kumar A *et.al.*, 2012)

Bottle gourd indeed holds a significant place in traditional Indian medicine, known as Ayurveda, for its various health benefits (Singh, 2017). Its rich nutrient profile, including vitamins B and C, makes it a valuable addition to the diet (Goyal, 2016). Its unique shape also adds to its charm and versatility in culinary preparations. It's important to approach any potential treatment or supplement with caution, especially when it comes to liver health. While animal studies suggest that bottle gourd (Singh *et.al.*, 2013) might have properties beneficial for the liver (Singh R. e., 2019), it's crucial to consult with a healthcare provider before incorporating it into the regimen, particularly if have any liver-related concerns or conditions.

It's important to note that while bottle gourd may have potential benefits for memory and CNS activity, consulting with a healthcare provider before using it is crucial to ensure it's safe and appropriate for individual health conditions and potential interactions with medications or existing treatments. (Singh K & Sharma *et.al.*, 2018)

It's important to approach herbal remedies with caution and consult with a healthcare professional, especially when considering their potential as cancer treatments. While studies suggest that bottle gourd extract may have anticancer properties, further research is needed to understand its effectiveness and safety in humans. Always prioritize medical advice and informed decision-making when it comes to your health. (Gupta.s *et.al.*, 2019)

Incorporating bottle gourd into your diet can be beneficial for weight management due to its low calorie and high-water content, along with its rich dietary fibre. However, it's essential to consult with your healthcare provider before using any herbal remedy for weight loss to ensure it's safe and appropriate for your individual health needs. (Amanda *et.al.*, 2023)

It's true that bottle gourd has been traditionally used to aid in managing diabetes due to its low fat and high Fiber content. Animal trials have shown that bottle gourd extract can effectively lower blood glucose levels in diabetic animals. However, more research is needed to confirm these potential anti-diabetic benefits in humans. (Johnson *et.al.*, 2022)

It's important to acknowledge the potential benefits of bottle gourd for skin health, particularly its vitamin C and zinc content. However, further research is needed to fully understand and establish its effectiveness. (Smith *et.al.*, 2020)

1.1 BOTTLE GOURD AND ITS BENEFITS

Bottle gourd is a good source of vitamin C and zinc that may offer several skin benefits. Vitamin C is an essential vitamin for overall skin health. It may protect the skin from the damage caused by ultraviolet radiation. It may also help to prevent signs of skin ageing, such as skin sagging. It may also help strengthen the skin barrier by enhancing the production of skin barrier lipids. (Smith *et.al.*, 2021)



Fig 1.1: Benefits of bottle gourd

Though there are studies that show the benefits of bottle gourds in various conditions, these are insufficient, and there is a need for further studies to establish the true extent of the benefits of bottle gourds on human health. (Johnson *et.al.*, The Insufficiency of Studies on the Health Benefits of Bottle Gourd, 2022)

1.2 HOW TO USE BOTTLE GUARD

Using bottle gourd, also known as calabash or lauki, in various ways can be a beneficial addition to your culinary repertoire and daily routine. From its versatility in cooking to its potential health benefits, bottle gourd offers a wide range of uses that can enhance your diet and lifestyle.

Firstly, bottle gourd can be utilized in cooking in numerous ways. Its mild flavour and soft texture make it suitable for various dishes, including soups, stews, curries, stir-fries, and even desserts. In Indian cuisine, it is commonly used in dishes like lauki ki sabzi (bottle gourd curry), dal (lentil stew) with bottle gourd, and lauki kheer (bottle gourd pudding). Its ability to absorb flavors makes it a versatile ingredient that can be incorporated into both savoury and sweet recipes.

When preparing bottle gourd, it is essential to peel off the tough outer skin and remove any seeds before cooking. The flesh of the vegetable can then be diced, sliced, grated, or cubed, depending on the recipe requirements. Bottle gourd cooks relatively quickly, making it a convenient option for busy individuals or families looking for nutritious meal options.

In addition to its culinary uses, bottle gourd is also valued for its potential health benefits. It is low in calories and rich in essential nutrients, including vitamins C and K, potassium, and dietary Fiber. Consuming bottle gourd regularly may help support digestion, promote hydration, and aid in weight management due to its high-water content and Fiber. Moreover, it is believed to have cooled properties, making it a popular choice in hot climates or during the summer months.

To incorporate bottle gourd into your diet, consider adding it to your weekly meal plan in various ways. You can experiment with different recipes and cooking methods to discover your favourite way to enjoy this versatile vegetable. Whether prefer it in Savory dishes like soups and curries or in sweet treats like puddings and smoothies, there are plenty of options to explore.

Furthermore, can also preserve bottle gourd by pickling or drying it for later use. Pickled bottle gourd, also known as lauki ka achar, is a tangy and flavourful condiment

that pairs well with rice, bread, or as a side dish. Dried bottle gourd slices can be rehydrated and added to soups or stews for an extra burst of texture and nutrition.

In conclusion, bottle gourd is a versatile vegetable that can be used in various culinary applications to enhance flavour, texture, and nutritional value. By incorporating it into your diet regularly, you can enjoy its potential health benefits while exploring new and exciting recipes. Whether you're a seasoned cook or a beginner in the kitchen, bottle gourd offers endless possibilities for delicious and nutritious meals. (Patel *et.al.*, 2023)

1.3 NUTRITIONAL COMPOSITION OF BOTTLE GUARD

Bottle gourd is very health and easily available vegetable. It is composed of 96.1% moisture, 0.1% fat, 0.5% minerals, 0.6% Fiber, 2.5% carbohydrates, 12 kcal energy, 0.7 mg/100g iron, 0.03 mg/100g thiamine, 0.2mg/100g niacin and 0.01mg/100g riboflavin. It is a good source of antioxidant and phenols. It also contains 10.10 mg/100g ascorbic acid, 5.80g/100g total sugar, 5.22g/100g reducing sugar and 1.31 g/100g starch (Srivastiva *et.al.*, 2022), (Milind *et.al.*, 2011)

Table:1.1: Parameters of bottle gourd

PARAMETERS	BOTTLE GOURD (per 100g)
Moisture	96.1 %
Total sugar	5.80g
Reducing sugar	5.22g
Carbohydrates	2.5%
Fiber	0.6%
Fat	0.1%
Energy	12 kcal
Minerals	0.5%

The scientific name for banana is *Musa*, from the Musaceae family of flowering tropical plants, which distinctively showcases the banana fruit clustered at the top of the plant. The mild-tasting and disease-resistant Cavendish type is the main variety sold in the U.S. and Europe. Despite some negative attention, bananas are nutritious and may even carry the title of the first “superfood,” endorsed by the American Medical Association in the early 20th century as a health food for children and a treatment for celiac disease. (Smith *et.al.*, The Nutritional and Historical Significance of Bananas., 2021)

Bananas are one of the most widely consumed fruits globally, prized for their sweet taste, convenience, and nutritional value. This project aims to provide a thorough understanding of bananas, covering their origins, cultivation, nutritional composition, health benefits, economic significance, and environmental impact. (Smith *et.al.*, Understanding Bananas: Origins, Cultivation, Nutrition, Health Benefits, Economics, and Environmental Impact., 2023)

Bananas are also considered an easy-to-digest food that's part of a bland diet prescription for people experiencing gastrointestinal issues like gas, diarrhoea, or vomiting. A bland diet may be useful for people with gastrointestinal conditions such as gastrointestinal reflux disease or GERD, traveller's diarrhoea, diverticulosis, and for individuals recovering from gastrointestinal-related surgeries.

It's recommended that people consume between 22 to 34 grams (g) of Fiber per day, depending on their age and gender. Unfortunately, many Americans only consume half of this amount. Fiber, also known as roughage, is found in plant foods like fruits, vegetables, and whole grains, and is an important nutrient that helps support digestive and heart health. It may also play a role in reducing the risk of type 2 diabetes and certain types of cancer, such as colon cancer.

Adding more soluble Fiber to the diet, found in fruits, can lower LDL or 'bad' cholesterol, reducing the risk of heart disease and stroke. The way that soluble Fiber does this is by binding to cholesterol molecules in the small intestine and preventing their absorption.

In addition to Fiber, bananas contain potassium, an essential mineral that helps manage blood pressure according to the American Heart Association. A medium-sized

banana delivers 422 milligrams (mg) of potassium, meeting 9% of the recommended intake, or daily value (DV).

Bananas provide and support energy levels in a few ways. Firstly, they are a source of carbohydrates, the body's primary fuel source. The carbohydrate content in bananas is primarily starch when they're unripe and as they ripen, the starch content drops, and the sucrose (a type of simple sugar) content increases. Simple sugars are readily absorbed into the bloodstream, providing a quick burst of energy. (Smithet.al. .. , 2022)

Potassium intake increases urinary excretion of sodium. It also helps ease the tension in blood vessel walls, aiding in lowering blood pressure.⁷ This is important to note because excess sodium in the bloodstream pulls water into the blood vessels, increasing the blood flow and blood pressure. High blood pressure is a major risk factor for heart disease.

Banana (*Musa* sp.) is the second most important fruit crop in India after mango. Its year-round availability, affordability, varietal range, taste, nutritive and medicinal value make it the favourite fruit among all classes of people. Bananas and plantain are very important fruit crops in the tropical world. Banana constitutes a major staple food for millions of people and provides a valued source of income through local and international trade. Banana is a principal source of food, employment, and income in its major production areas.

1.4 NUTRITIONAL COMPOSITION OF BANANA

Banana is an excellent source of nourishment and a well-balanced diet to people of all ages ((Jyothirmayi, 2015). It is a cheap and energy-rich delicacy relished by both rich and poor alike. It forms the staple food for man villagers and tribal of eastern and southern India ((Shruthi., 2019). Bananas are considered as a rich source of vitamin A, vitamin B complex, vitamin C, manganese, potassium, and digestible food Fibers are present in the fruits in sizeable levels.

Table.1.2 Nutritional composition of banana

Constituents	Units	Banana
Energy	Kcal	89
Water	g	74
Protein	g	1.1
Total lipid	g	0.3
Carbohydrate	g	21.8
Dietary Fiber	g	2.0
Na	mg	1.0
K	mg	385.0
Ca	mg	8.0
Mg	mg	30
P	mg	22
Fe	mg	0.42
Cu	mg	0.11
Zn	mg	0.18
Mn	mg	0.2
Equivalent β carotene	μ g	68.0
Tocopherol (Vitamin e)	mg	0.29
Ascorbic acid (Vitamin c)	mg	11.7
Thiamine(Vitamin B1)	mg	0.04
Riboflavin(Vitamin B2)	mg	0.07
Niacin(Vitamin B3)	mg	0.61

Pantothenic acid(Vitamin B5)	mg	0.28
Pyridoxine(Vitamin b6)	mg	0.47
Total folate	µg	23.0
Serotonin	mg	3.3

Fruits are of great importance in human nutrition. However, due to their perishable nature and seasonal availability, they are generally processed into more stable forms such as jams, jellies, juices, pickles and many more products. Jam is a processed fruit product having an intermediate moisture content which is prepared by boiling homogenised fruit pulp with sugar, acid, and pectin. It may have some other ingredients like preservatives, flavouring or colouring compounds. It should have a proper consistency so that it could easily spread and should be firm enough so that it does not flow like a fluid. Jam should contain at least 68.5% total soluble solids (TSS), and the fruit should contribute at least 45% of the total weight of jam. (Gargettet.al., 2004)

CHAPTER-2

LITERATURE SURVEY

2.1 BOTTLEGOURD HISTORY

Lagenaria siceraria, the bottle gourd is a member of the Cucurbitaceae family. It goes by the name calabash, white flowered gourd, and lauki throughout the region. One of the first species that humans are said to have named is the bottle gourd. The bottle gourd is an annual herbaceous plant that grows in a prostrate manner. *Lagenaria siceraria*, the bottle gourd, is a member of the Cucurbitaceae family. It goes by the name calabash, white flowered gourd, and kado throughout the region. One of the first species that humans are said to have tamed is the bottle gourd. The bottle gourd is an annual herbaceous plant that grows in a prostrate manner. (Etthadul ummah *et.al.*, 2012) There are 118 general and 825 species Harika. (Harika *et.al.*, 2012).

LaGuardia breviflora, *Lagenaria rufa*, *Lagenaria sphaerica*, *Lagenaria Abyssinia*, and *Lagenaria guineensis* are the other five wild species that make up *Lagenaria*. wherein *Lagenariasiceraria* spp. are primarily grown. There are identified species of bottle gourd. *Lagenaria siceraria* species, including ssp. *siceraria* and spp. *asiatica*. The crop was first cultivated between 9 and 10,000 BP (before present) in America (the New World), between 6 and 10,000 BP in East Asia, and between 4 and 5000 BP in Africa. *LaGuardia breviflora*, *Lagenaria rufa*, *Lagenaria sphaerica*, *Lagenaria Abyssinia*, and *Lagenaria guineensis* are the other five wild species that make up *Lagenaria*. wherein *Lagenariasiceraria* spp. are primarily grown. There are identified species of bottle gourd. *Lagenaria siceraria* species, including ssp. *siceraria* and spp. *asiatica*. The crop was first cultivated between 9 and 10,000 BP (before present) in America (the New World), 6 and 10,000 BP in (Jamal uddin *et.al.*, 2014).

2.2 FERTILIZATION OF BOTTLE GOURD

The leaves of *L. siceraria* are asymmetrical and varied, with tendrils virtually always present. The blooms are monoecious, with the male and female flowers located on separate plant axes within the same plant. The leaves of *L. siceraria* are asymmetrical and varied, with tendrils virtually always present. The blooms are monoecious, with the male and female flowers located on separate plant axes within the same plant. (Etthadul ummah *et.al.*, 2012)

Cross-pollination is therefore very beneficial. There are wild or non-cultivated varieties of Bering hermaphrodite flowers that have both andromonoecious and dioecious sex. Bottle gourd sex ratio is extremely high. Cross-pollination is therefore very beneficial. There are wild or non-cultivated varieties of Bering hermaphrodite flowers that have both andromonoecious and dioecious sex. Bottle gourd sex ratio is extremely high. (Harika *et.al.*, 2012).

It has been demonstrated that the ratios of male to female flowers have a major impact on output. It has been demonstrated that the ratios of male to female flowers have a major impact on output. (Jamal uddin *et.al.*, 2014)

This ratio can be changed using a variety of growth regulators, such as auxin, ethylene, gibberellic acid, and others, as well as environmental factors like temperature, precipitation, and light intensity. The shape and size of bottle gourd fruits vary greatly, both within and between cultivars. *Lagenaria siceraria*, for example, shows the greatest variation in fruit shape; it can be long, cylindrical, necked, oblong flat or round, conical pyriform to club-shaped, and its skin can be smooth or warded. This ratio can be changed using a variety of growth regulators, such as auxin, ethylene, gibberellic acid, and others, as well as environmental factors like temperature, precipitation, and light intensity. The shape and size of bottle gourd fruits vary greatly, both within and between cultivars. *Lagenaria siceraria* is the cultivar with the greatest fruit shape variations; they might be long, cylindrical, necked (Etthadul ummah *et.al.*, 2012)

2.3 PROPOGATION OF BOTTLEGOURD

The desire of any farmer is to see the germination and growth of all seeds planted in a field. This way the farmer is assured to obtain reasonable yield if all growing conditions are optimal (Etthadul ummah *et.al.*, 2012)

Vegetables are short-duration crops that can be cultivated in constrained areas. Vegetables are short-duration crops that can be cultivated in constrained areas. (Harika *et.al.*, 2012).

The impoverished in both urban and rural areas will be able to afford vegetable costs as long as vegetable yield increases. Gourds are a crop that are grown extensively in

Pakistan and exported outside. The impoverished in both urban and rural areas will be able to afford vegetable costs as long as vegetable yield increases. Gourds are a crop that are grown extensively in Pakistan and exported outside. (Jamal uddin *et.al.*, 2014).

2.4 COMPOSITION OF BOTTLEGOURD

Bottle gourds are rich in calcium and vitamins C and K, have a cooling effect on the body, and even work well as a laxative. Because of their high-water content, they prevent dehydration in humans. To Treat patients with cardiac issues, urological conditions, and sleeplessness. It is also recommended due to its aphrodisiac and anti-diabetic qualities.

The pulp from bottle gourds helps treat constipation, cough, and night blindness. It also functions as an antidote for several poisons. For those who are lactose intolerant, its pulp makes a good alternative.

Beneficial in the treatment of numerous illnesses, such as hepatic and cardiac conditions. Because of its high potassium content, hypertension individuals' blood pressure is controlled. Because it has a high dietary fiber content and is low in fat and cholesterol, it can aid in rapid weight loss. A high concentration of choline found in bottle gourds helps to repair mental health conditions. (gupta *et.al.*, 2022).

When compared to other vegetables grown in India, the bottle gourd is the most reasonably priced and nutrient-dense vegetable option for a diet. Bottle gourds are bitter and eaten. (Pandravada&Sivaraj *et.al.*, 2022).

Additionally, this vegetable is very useful for maintaining heart health. Drinking lauki juice twice or three times a week will help to keep the heart healthy and control blood pressure. (Ganesh &G. Dhakad *et.al.*, 2022)

The various products that can be developed from bottle gourd fruit are pickles, chutney, juice, and sweets (Walters *et.al.*, 2022).

Bottle gourd candy is prepared by dipping bottle gourd cubes in sugar syrup (Ahmad *et.al.*, 2021).

2.5 NUTRITIVE COMPOSITION OF BANANA

All parts of the banana, including the skin, leaves, pulp, inflorescence, and banana seeds, are used to make food or non-food substances with nutraceutical qualities. Natural fiber is a source of micro- and macronutrients, physiologically active substances, and biofertilizers. (Padam & Abdullah *et.al.*, 2021).

A banana offers wholesome nutrients and instant energy. (Maraschin&Pereira *et.al.*, 2015).

This fruit has a high calorie content and is a good source of dietary fiber, minerals like manganese, potassium, and copper, and micronutrients like vitamin B6, vitamin C, and biotin. (Bhaskar & Salimath *et.al.*, 2011).

Bananas' antiallergic, antibacterial, and antinuclear qualities assist to heal dysentery, prevent ulcers in the intestines, and speed up the healing of allergic skin lesions. (Jyothirmayi &Zafar *et.al.*, 2011).

It contains significant levels of linoleic acid, sugar derivatives, fatty acids, sterols, and esters. which include long chain fatty alcohols, aromatic compounds, sterylglucosides, and diacylglycerols as the main replacements for lipophilic chemicals found in banana tissues. (Al-Mqbali & Hossain *et.al.*, 2019).

2.6 NUTRITIVE COMPOSITION OF BEETROOT

This vegetable is also extremely beneficial for keeping one's heart healthy. Consuming lauki juice twice or thrice in a week will support in maintaining a healthy heart and will also regulate blood pressure. (Shivani chauhan&karthik chamoli *et.al.*, 2020)

The Beetroot is the taproot portion of the plant. It is an excellent food which is important for development and growth of human body. It is a rich source of antioxidant and minerals. It also acts as fruits as well as vegetables. Fresh form of beetroot generally consumed as a salad. It contains beta lacin essential for cardiovascular health. It plays another role as a natural color in the textile industries and as a medicinal plant to cure the various illnesses. (Shilpa sharma*et.al.*, 2020)

Beetroot pigment is used as a food dye. It changes color when heated so can only be used in ice-cream and other confectionary, but it is both cheap and has no known

allergic side effect. Beetroot juice is very potent, a beautiful rich ruby red color it is known to help purify the blood.

The use of seasoned remedies severally or together with customary medicines has been utilized in varied medical treatises for the cure of various malady, ailment or disease. Beetroot is one amongst the well-known edible plants and has substantial healthful properties because of the presence of distinctive natural edible substances. It contains antioxidant, vitamins and minerals varied vital antidepressant, antimicrobial and anti-carcinogenic. (Shivani chauhan *et.al.*, 2020)

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM:

To prepare bottle guard jam by adding prebiotics (banana) and to analyse its nutrient content.

3.2 OBJECTIVES:

- ❖ Preparation of bottle gourd for jam preparation.
- ❖ Preparing and adding banana to increase fibre content, quality and content of jam.
- ❖ Microbiological analysis of bottle gourd jam (Total plate count, Yeast and Mould, E. coli, Coliforms & Salmonella)
- ❖ Nutrient analysis of bottle gourd jam {energy, fat. total sugars, fat, protein}

CHAPTER-4

MATERIALS AND METHODS

4.1 COLLECTION OF RAW MATERIAL:

Bottle gourd, banana and beetroot was purchased from a local market in Chennai city. The general store ingredients such as sugar, butter, vanilla essence.

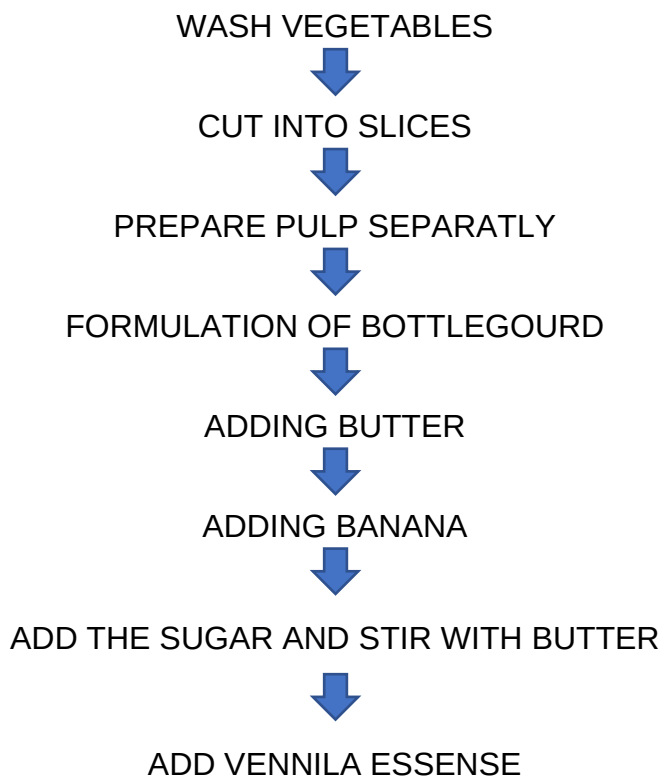
4.2 PREPARATION OF JAM:

The vegetables were sliced, and then the jam was made. Banana and beetroot extract, two varieties of vegetable and fruit bottle gourd, are added to the regular formulation after that.

In order to cook the bottle gourd pulp, 5 grammes of unsalted butter must be added at first, stirring continuously. After 5 minutes, the banana pulp must be added, stirring continuously, along with 5 grammes of unsalted butter, sugar, and two drops of vanilla essence and agar agar.

Beetroot extract will be added as a last step and left aside for a while.

4.3 STEPS FOR PREPARATION OF JAM



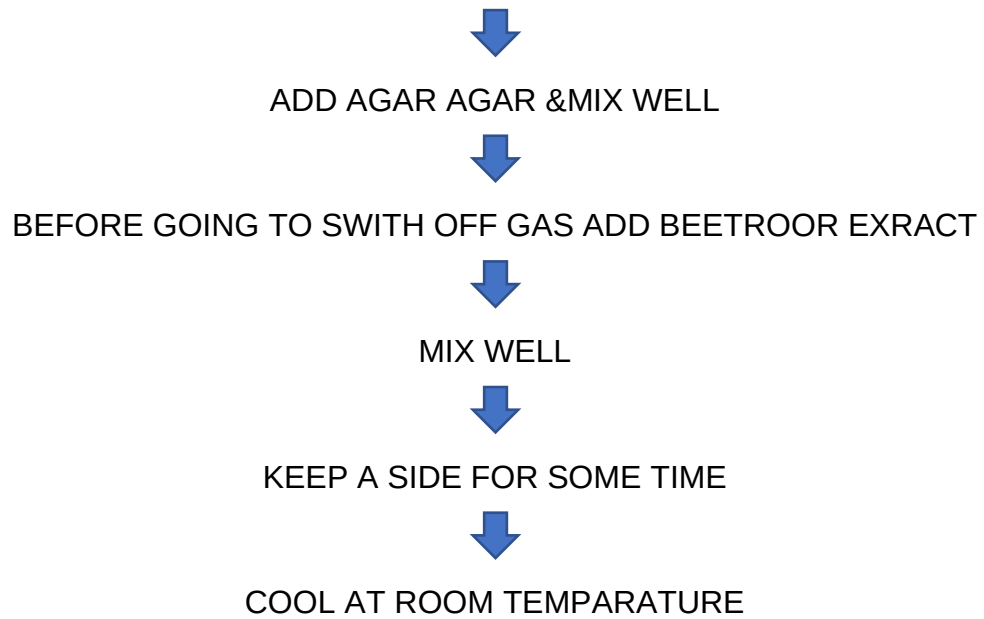
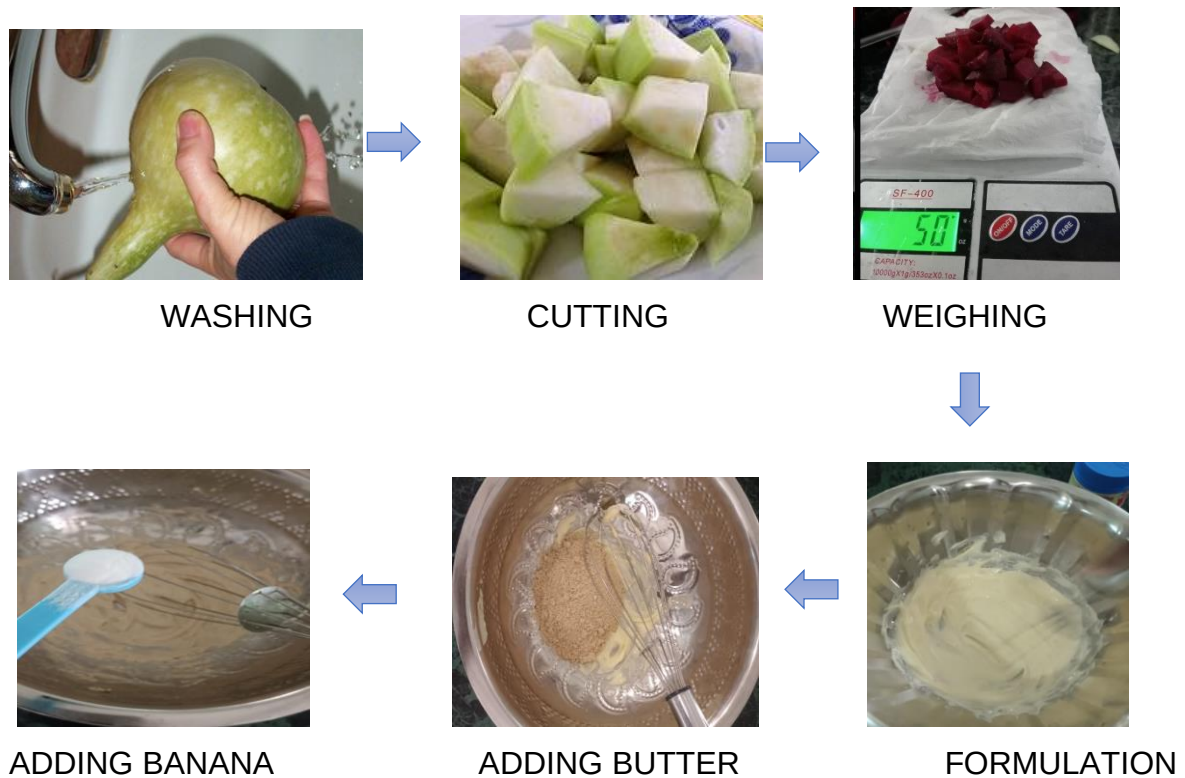


Fig 4.1: Flow diagram for preparation of bottle gourd jam





ADDING SUAGR



ADDING SUGAR &
VENNILA ESSENCE



ADDING BEETROOT
EXTRACT & KEEP
A SIDE



JAM

4.4 STANDARD FORMULATION OF BOTTLE GOURD:

Table 4.1: Standard formulation of bottle gourd jam

INGREDIENT	WEIGHT IN GM
Bottle gourd	200
Banana	100
Beetroot	50
Butter	5
Agar agar	5
Vanilla essence	0.1
Potassium sorbitol	0.1

4.5 NUTRITIONAL EVALUATION ANALYSIS:

4.5.1 ENERGY

The caloric value was calculated in kilo-calories per 100g (kcal/100g) by multiplying the crude fat, protein and carbohydrate values by at waterll factors of 37, 17 and 17 respectively. This method is represented by the following formula.

$$\text{Energy (KCAL/100g)} = (\text{Carbohydrate} \times 4) + (\text{protein} \times 4) + (\text{fat} \times 9)$$

4.5.2 TOTAL CARBOHYDRATES

The total carbohydrate content in the quinoa millet biscuit was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fiber, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat (g)} + \text{Protein(g)} + \text{Fiber(g)}]$$

FAT

Use method A or C for feed ingredients and mixed feeds other than baked or expended, dried milk products, containing urea or mixed feeds that have at least 20% of crude fat derived from baked and expanded or dried milk products.

- A. Indirect methods: determine moisture as in 934.01 or 920.36 then extract dried substance as in C And dried again. Report loss in weight as ether extract.

Direct methods:

- B. Reagents: Anhydrous ether: wash commercial ether with two or three portions H₂O, and solid NaOH or KOH, and let stand until most of H₂O is abstracted from the ether. Decant into Dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until H₂ evolution ceases. Keep ether, thus dehydrated, over metallic Na in lossely stoppered bottles.
- C. Determination:(Large amounts H₂O – soluble components such as carbohydrates, urea, latucacid, glycerol and others may interfere with extraction of fat if present, extract 2 g test portions on small paper in funnel with five 20 mL portion H₂O prior to drying for ether extraction).
- D. Extract ca 2 g test portions dried as in 934.01 or 920.36 with anhydrous ether. Extraction period may vary from 4 h at condensation rate of 5 – 6 drops /s to 16 h at 2-3 drops/ s. Dry extract 30 min at 100°C, cool and weigh.

4.5.3 PROTEIN

Weight suitable test portion of sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g CuSO₄ and 25mL or more, if necessary, of Conc.H₂SO₄ to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H₂O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

$$\%N (w/w) = (TVs-TVb) \times \text{Normality of standard HCl} \times 1.4007 / \text{Sample weight}$$

where, TVb = Titer value of blank; TVs = Titer value of sample

4.5.4 FIBER

Duplicate test portion of dried foods, fat extracted if containing > 10% fat, are gelatinized with termally (heat stable α - amylase), and then enzymatically digested with protease amyloglucosidase to remove protein and starch. (When analyzing mixed diets, always extract fat prior to determining total dietary fiber.) Four volumes of ethyl alcohol are added to precipitate soluble dietary fiber. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying residue is weighed. One duplicate is analyzed for protein, and other is Incinerated at 525°C and ash in determined.

CALCULATION

$$\text{Total dietary fiber} = \text{weight residue} - \text{weight (protein + ash)}$$

Apparatus

a) Fritted crucible- Porosity No. 2 (Pyrex No. 32940, coarse, ASTM 40-60 μ m; or Corning number and soak 529°C 36060 Buchner, fritted disk, Pyrex 60ML ASTM 40-60 μ m) Clean thoroughly heat 1 h at 525 °C and soak and then rinse in H₂O. Add

ca 0.5 g Celite to air-dried crucibles and dry at 130°C to constant weight ($\geq 1\text{h}$). Cool and store in desiccator until used.

b) Vacuum source- Vacuum pump or aspirator equipped with in-line double vacuum flask to prevent contamination in case of H_2O

c) Vacuum oven- 70°C . Alternatively, 105°C air oven can be used.

d) Desiccator

e) Muffle furnace

(f) Water baths – (1) Boiling (2) Constant temperature- Adjustable to 60°C , with either multi station shaker or multi station magnetic stirrer to provide constant agitation of digestion flasks during enzymatic hydrolysis.

(g) Beakers. Tall-form, 400 or 600 ml

(h) Balance. Analytical, readability to 0.1 mg.

(i) pH meter Standardized with pH 7 and pH 4 buffers.

Reagents

(a) 95% Ethanol-v/v. technical grade.

(b) 78% Ethanol - Place 207 mL H_2O into 1 L volumetric flask. Dilute to volume with 95% ethyl alcohol. Mix and dilute to volume again with 95% ethyl alcohol if necessary. Mix One volume HO mixed with four volumes 95% ethyl alcohol will also give 78% ethyl alcohol final concentration.

(c) Acetone.

(d) Phosphate buffer. -0.08 M. pH 6.0. Dissolve 1.400 g sodium phosphate dibasic, anhydrous (Na_2HPO_4) (or 1.753 g dihydrate) and 9.68 g sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) (or 10.94 g dihydrate) in ca 700 mL H_2O . Dilute to 1L with H_2O . Check pH with pH meter.

(e) α -amylase (heat stable). Termamyl. (1) Store in refrigerator. Based on Nelson/Somogyi reducing sugar with soluble starch as substrate-10 000+1000 units/mL. (1 unit is defined as the amount of enzyme required to release 1 μmole reducing sugar equivalents/ min at pH 6.5 and 40°C). (2) Based on Ceralpha method using p-nitrophenyl-maltosaccharide as substrate in the presence of α thermostable α -glucosidase- 3000 + 300 Ceralpha units/ml. (1 unit of enzyme is required to release 1 μmole p-nitrophenyl/min at pH 6.5 and 40°C)

(f) Protease-Keep refrigerated. (1) Casein assay- 300-400 Units/mL. [1 protease unit is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μ mole tyrosine equivalents/min from soluble casein at pH 8.0 and 40°C]; 7-15 units/mg (1 unit will hydrolyze casein to produce color equivalent to 1.0 μ mole tyrosine/min at pH 7.5 and 37°C). Color by Folin-Ciocalteu reagent (2) Azo-casein assay-300-400 Units/mL. 1 unit endo-peptidase activity defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μ mole tyrosine from soluble casein at pH 8.0 and 40°C]

(g) Amyloglucosidase-Keep refrigerated. (1) Strach / glucose oxidase -peroxidase 2000-3300 Units /mL (1 unit enzyme activity is defined as the amount of enzyme required to release 1 μ mole glucose/ min at Ph 4.5 and 40°C (p-nitrophenyl method-130-200 Units/mL. [1 unit enzyme (PNP unit) is the amount of enzyme, which in the presence of excess levels of B-glucosidase, will release 1 μ mole p-nitrophenyl from p-nitrophenyl β -maltosidase/min at 40°C)

The only enzyme which has been found to be significantly contaminated with interfering activities is amyloglucosidase. Thermostable α -amylase and protease from commercial sources have been found to be generally free of interfering enzymes. Low levels of β -glucanase have been detected in protease preparations, at levels well below that which would interfere with total dietary fiber analysis. The major contaminant in amyloglucosidase endo-cellulase and resulted in oats, endo-depolymerization of mixed-linkage β -glucan from barley and oats, with resultant underestimation of this dietary fiber component. The contamination of amyloglucosidase with endo- cellulase (β -glucanase) can be easily detected.

Alternatively, there are kits containing all three enzymes (pretested) available from a number of companies.

(h) Sodium hydroxide solution -0.275 M. Dissolve 11.00 g NaOH ACS in ca 700 mL H₂O in 1 L volumetric flask. Dilute to volume with H₂O.

(i) Hydrochloric acid solution - 0.325 M. Dilute stock solution of known titer, e.g., 325 mL 1 M HCl, to 1 L with H₂O.

(j) Celite Acid-washed.

D. Enzyme Purity

To ensure absence of undesirable enzymatic activity in enzymes used in this procedure, run materials listed in through entire procedure each time lot of enzymes is changed, or at maximum interval of 6 months to ensure that enzymes have not degraded.

E. Test Portion Preparation

Determine total dietary fiber on dried test sample. Homogenize test sample and dry overnight in 70°C vacuum oven, cool in desiccator, and dry-mill test sample to 0.3-0.5 mm mesh. If test sample cannot be heated, freeze-dry before milling. If high fat content (>10%) prevents proper milling, defat with petroleum ether (3 times with 25 mL portions/g test sample) before milling. Record loss of weight due to fat removal and make appropriate correction to final % dietary fiber found in determination. Store dry-milled test sample in capped jar in desiccator until analysis is carried out.

F. Determination

Run blank through entire procedure along with test portions to measure any contribution from reagents to residue.

Weigh duplicate 1 g test portions, accurate to 0.1 mg. into 400 mL tall-form beakers. Test portion weights should not differ >20 mg. Add 50 mL pH 6.0 phosphate buffer to each beaker. Check pH and adjust to $\text{pH } 6.0 \pm 0.2$ if necessary. Add 0.1 mL Termamyl solution. Cover beaker with Al foil and place in boiling water bath 15 min. Shake gently at 5 min intervals. Increase incubation time when number of beakers in boiling water bath makes it difficult for beaker contents to reach internal temperature of 95-100°C. Use thermometer to indicate that 15 min at 95-100°C is attained. Total of 30 min in water bath should be sufficient.

Cool solutions to room temperature. Adjust to $\text{pH } 7.5 \pm 0.2$ by adding 10 mL 0.275 M NaOH solution. Add 5 mg protease. [Protease sticks to spatula, so it may be preferable to prepare enzyme solution (50 mg in 1 mL phosphate buffer) and pipet 0.1 mL to each sample just before use]

Cover beaker with Al foil. Incubate 30 min at 60°C with continuous agitation. Cool. Add 10 mL 0.325 M HCl solution. Measure pH and dropwise add acid if necessary. Final pH should be 4.0-4.6. Add 0.3 mL amyloglucosidase, cover with Al foil, and incubate 30 min at 60°C with continuous agitation. Add 280 mL 95% ethyl alcohol preheated to 60°C (measure volume before heating). Let precipitate form at room temperature for 60 min.

Weigh crucible containing Celite to nearest 0.1 mg, then wet and redistribute bed of Celite in crucible by using stream of 78% ethyl alcohol from wash bottle. Apply suction to draw Celite onto fritted glass as even mat. Maintain suction and quantitatively transfer precipitate from enzyme digest to crucible.

Wash residue successively with three 20 mL portions of 78% ethyl alcohol, two 10 mL portions of 95% ethyl alcohol, and two 10 mL portions of acetone. Gum may form with some products, trapping liquid. If so, break surface film with spatula to improve filtration. Time for filtration and washing will vary from 0.1 to 6 h, averaging 0.5 h per sample. Long filtration times can be avoided by careful intermittent suction throughout filtration.

Dry crucible containing residue overnight in 70°C vacuum oven or 105°C air oven. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine weight of residue. Analyze residue from 1 test portion of set of duplicates for protein by using a conversion factor, except in cases where N content in protein is known,

Incinerate second test portion of duplicate 5 h at 525°C, Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash.

G. Calculations

Determination of blank:

$$B = \text{blank, mg-weight residue} - P_B - A_B$$

where weight residue = average of residue weights (mg) for duplicate blank determinations; and P_B and A_B = weight (mg) of protein and ash, respectively, determined in first and second residues.

Calculate TDF as follows:

$$\text{TDF \%} = [(\text{weight residue} - P - A - B) / \text{weight test portion}] \times 100$$

4.5.5 SUGAR

where weight residue = average of weights (mg) for duplicate blank determinations, and P and A = weights (mg) of protein and ash respectively, in first and second test portion residues; and weight test portion = average of two test portion weights (mg).

Weigh accurately about 5 g sample, transfer to a 200 ml volumetric flask dissolve in warm water, dilute to about 150 ml. In case solution is not clear, add 5 ml of Carrez 1 solution followed by 5 ml of Carrez 2 solution. Make up to 200 ml. Filter through a dry filter paper. Titrate the solution obtained as such to determine % Reducing sugars.

PRELIMINARY TITRATION:

Pipet 5 ml each of Fehling A and B into 250 ml conical flask. Mix and add about 10 ml water and a few boiling chips or glass beads. Dispense solution. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Continue the addition of solution dropwise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

FINAL TITRATION:

Pipet 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration duplicate and take the average.

CALCULATIONS:

Reducing sugars (%) = Dilution x Factor of Fehling (in gm) / Weight of sample x Titre value *100

4.5.6 CALCIUM

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 per cent sodium hydroxide solution using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass = $V_1 \times 0.04008 \backslash M$

Where V_1 = Volume in ml of EDTX solution in titration; and

M = Mass in g of the sample in the solution for the test

CHAPTER-5

RESULT AND DISCUSSION

Bottle gourd, also known as *calabash* or *lauki*, is a versatile vegetable commonly used in various culinary preparations due to its mild flavor and high-water content. To enhance its culinary potential, this study explores the incorporation of banana into bottle gourd jam. By combining these two ingredients, we aim to create a jam with improved texture, flavor complexity, and nutritional value.

5.1 TEXTURE:

Adding banana to bottle gourd jam results in a smoother texture due to the natural binding properties of bananas. The jam will be less likely to have a stringy or fibrous texture commonly associated with bottle gourd.

5.2 FLAVOUR:

The addition of banana introduces a natural sweetness to the jam, reducing the need for excessive amounts of sugar. The flavor profile becomes more complex, with hints of both bottle gourd and banana complementing each other.

5.3 COLOUR:

The use of beetroot as a natural colorant in jam production offers a myriad of advantages, including visual enhancement, stability, nutritional enrichment, and flavor enhancement. Further research into optimal formulations and processing techniques may unlock additional opportunities for innovation in the development of natural food colorants.

5.4 CONSISTENCY:

Banana contributes to the jam's consistency, helping it to thicken naturally without the need for additional pectin or thickeners. However, it's important to ensure the jam reaches the desired thickness through proper cooking and reduction.

5.5 NUTRITIONAL BENEFITS:

The addition of banana not only enhances the flavor and texture but also increases the nutritional value of the jam. Bananas are rich in potassium, vitamin C, and dietary fiber, adding to the overall health benefits of the jam.

5.6 SWEETNESS CONTROL:

By incorporating banana, you can control the sweetness of the jam more effectively. This is particularly useful for those looking to reduce their sugar intake or for individuals with dietary restrictions.

5.7 BALANCING FLAVOURS:

The combination of bottle gourd and banana offers a balanced flavor profile, with the mild sweetness of bottle gourd complemented by the richer, sweeter taste of banana. This makes the jam appealing to a wider range of palates.

5.8 RECIPE VARIATIONS:

Experimentation with different ratios of bottle gourd to banana can yield various flavor profiles and textures. Additionally, incorporating other complementary ingredients such as citrus zest can further enhance the jam's complexity.

5.9 MICROBIAL ANALYSIS:

Microbial analysis of the bottle gourd jam prepared with the addition of banana revealed the following findings:

Total Plate Count (TPC): The TPC of the jam samples ranged from 10^{-2} to 10^{-4} colony-forming units per gram (CFU/g). This indicates a relatively low level of microbial contamination, well within the acceptable limits for preserved food products.

Yeast and Mold Count: Yeast and Mold counts were below the detection limit in all tested samples, indicating effective control of fungal contamination during the preparation and storage of the jam.

Coliform Count: Coliform bacteria were absent in all samples, indicating proper sanitation practices and minimal risk of faecal contamination during production.

Pathogen Detection: No pathogenic bacteria, such as *Salmonella* spp. or *Escherichia coli*, were detected in any of the jam samples, confirming the safety of the product for consumption.

The microbial analysis results demonstrate that the bottle gourd jam prepared with the addition of banana meets microbiological safety standards for preserved food products. Several factors contribute to the low microbial counts observed in the samples:

Acidic pH: The natural acidity of fruits, including bottle gourd and banana, contributes to the inhibition of microbial growth by creating an unfavourable environment for pathogens and spoilage microorganisms.

High Sugar Content: The high sugar content of jam acts as a preservative by reducing water activity and inhibiting microbial growth. Additionally, the osmotic pressure exerted by sugar helps to prevent the proliferation of spoilage organisms.

Heat Treatment: Proper cooking and heat treatment during the jam-making process contribute to the reduction of microbial load by killing or inactivating bacteria, yeasts, and Molds present in the raw ingredients.

Hygienic Practices: Strict adherence to hygienic practices, including handwashing, sanitization of equipment, and clean working environments, minimizes the risk of cross-contamination and ensures the safety of the final product.

Overall, the microbial analysis results provide confidence in the safety and quality of the bottle gourd jam prepared with the addition of banana. However, it is essential to maintain proper storage conditions, such as refrigeration or hot water bath canning, to prolong shelf life and prevent microbial spoilage during storage. Regular monitoring of microbial quality is also recommended to ensure continued compliance with food safety standards.

Fig 5.1: Test report of microbial analysis



CIN: U93000TN2000PTC043869




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TEST REPORT

Test Report No & Date	CTL/CH/F-1401/2023-24 & 28.02.2024
Sample Number	F-1401/23-24
ULR No	TC613324000001284F
Name of the Customer	Ms. Bharathi.J
Address	Roary Illum - 447 (Girls Hostel), Sathyabama Institute of Science and Technology, Semmancheri, Chennai - 600 119.

Sample Drawn by	Customer
Sample Name	Bottle Guard Banana Jam
Sample Description	Bottle Guard Banana Jam
Sampling Location	NA
Sample Drawn on	NA
Sample Collected by	NA
Sampling Plan & Procedure	NA
Sample Quantity	200g
Sample Condition	Good & Received in Packed Condition.
Environmental Conditions	NA
Equipment used for Sampling	NA
Sample Received on	22.02.2024
Analysis Started on	23.02.2024
Analysis Completed on	28.02.2024

Test Results:

The above sample tested as received, and results are as follows:

S. NO	PARAMETERS	METHOD	UNITS	RESULTS	REQUIREMENTS*
1	Total Plate Count	IS 5402 : 2012 (RA.2018)	CFU/g	6900	NMT 10,00,000
2	Escherichia Coli	IS 5887 (Part 1):1976 (RA.2022)	CFU/g	< 10	NA
3	Coliform Count	IS 5401 (Part 1):2012 (RA.2022)	CFU/g	< 10	NA
4	Yeast & Mould Count	IS 5403:1999 (RA.2018)	CFU/g	< 10	NMT 100
5	Salmonella	IS 5887 (Part 3):1999 (RA.2022)	Per 25g	Absent	Absent

Note : CFU- Colony Forming Unit

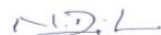
Verified by



END OF REPORT



For Chennai Testing Laboratory Pvt Ltd



Authorised Signatory

N. RAJENDRAKUMAR
Head - Quality & Microbiology Division
(MICROBIOLOGY)

Fig 5.2: Test report of nutrient analysis



CIN: U93000TN2000PTC043869




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TEST REPORT

Test Report No & Date	CTL/CH/F-1400/2023-24 & 28.02.2024
Sample Number	F-1400/23-24
ULR No	TC613324000001283F
Name of the Customer	Ms. Bharathi.J
Address	Roary Illum - 447 (Girls Hostel), Sathyabama Institute of Science and Technology, Semmancheri, Chennai - 600 119.

Sample Drawn by	Customer
Sample Name	Bottle Guard Banana Jam
Sample Description	Bottle Guard Banana Jam
Sampling Location	NA
Sample Drawn on	NA
Sample Collected by	NA
Sampling Plan & Procedure	NA
Sample Quantity	100g
Sample Condition	Good & Received in Plastic Box.
Environmental Conditions	NA
Equipment used for Sampling	NA
Sample Received on	22.02.2024
Analysis Started on	23.02.2024
Analysis Completed on	28.02.2024

Test Results:

The above sample tested as received, and results are as follows:

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1	Energy (By Calculation)	FAO Method	kcal/100g	189
2	Carbohydrates (By difference)	CTL/SOP/FOOD/262 - 2014	g/100g	40.3
3	Total Fat	AOAC 20th Edn.2016, 954.02	g/100g	2.53
4	Protein (Nx 6.25)	AOAC 20 th Edn.2016, 986.25	g/100g	1.26
5	Dietary fibre	AOAC 20th Edn.2016, 985.29	g/100g	BDL(DL:1.0)
6	Total Sugars	FSSAI Manual 2015- Beverages, Sugar and confectioneries	g/100g	29.7
7	Sodium as Na	AOAC 20 th Edn.2016, 969.23	mg/100g	17.9
8	Calcium as Ca	IS 5949:1990 (RA.2010)	mg/100g	29.0
9	Iron as Fe	AOAC 20th Edn.2016, 999.11	mg/100g	1.88

Note : BDL - Below Detection Limit; DL - Detection Limit;

Verified by



END OF REPORT



For Chennai Testing Laboratory Pvt Ltd



Authorised Signatory
K. KESAVAN
Senior Chemist
(CHEMICAL)

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SUMMARY AND CONCLUSION

In conclusion, the research paper on bottle gourd and banana jam provides a comprehensive analysis of its nutritional composition, microbial safety, preparation process, and potential health benefits. Through meticulous examination of the final product, valuable insights have been gained regarding the quality, safety and nutrient analysis of the jam. The nutritional analysis revealed that bottle gourd and banana jam is a rich source of essential vitamins, minerals, and dietary fiber, making it a nutritious addition to the diet. The presence of vitamins A, C, and potassium from bottle gourd, combined with vitamins B6 and C from bananas, highlights the diverse array of nutrients present in the jam. These nutrients play crucial roles in supporting various bodily functions, including immune health, digestion, and cardiovascular function.

The Microbial analysis confirmed the safety of the jam for consumption, with low levels of bacterial and fungal contamination detected. Proper sanitation and cooking techniques were found to be effective in minimizing microbial risks and ensuring the quality and safety of the final product.

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FORMULATION AND STANDARDIZATION OF FUSION BITES USING “DIOSCOREA ALATA”

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Clinical Nutrition & Dietetics

By

KAVYA SHREE .S (41736007)

SATHIYA PRIYA. M (41736015)



**DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDECERTIFICATE

This is to certify that this Project Report is the Bonafide work of SATHIYAPRIYA M (41736015) KAVYA SHREE S (41736007) who has the project work entitled **FORUMULATION AND STANDARDIZATION OF NUTRITION BITES USING "DIOSCOREA ALATA"** under my supervision from JUNE 2023 to MAY 2024.

Internal Guide
(Ms. PRIYA S, M.Sc., NET)

External Guide
Dr.L.STANLEY ABRAHAM

Head of the Department
(Dr. L. INBATHAMIZH)

Submitted for Viva – voce Examination held on 28.04.24

Internal Examiner

External Examiner

DECLARATION

We Sathiya priya M and Kavya Shree S hereby declare that the project entitled **"FORMULATION AND STANDARDIZATION OF FUSION BITES USING DIOSCOREA ALATA"**. Done by me under the guidance of Ms. PRIYA .S is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

PLACE: CHENNAI

S. Kavya Shree

SIGNATURE OF THE CANDIDATES

DECLARATION

We Sathiya priya M and Kavya Shree S hereby declare that the project entitled "FORMULATION AND STANDARDIZATION OF FUSION BITES USING DIOSCOREA ALATA". Done by me under the guidance of Ms. PRIYA .S is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

PLACE: CHENNAI

M. Sathiya priya
SIGNATURE OF THE CANDIDATES

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ABSTRACT

The study aims to comprehensively investigate *Dioscorea alata*, commonly known as purple yam, through various analytical methods and assessments. The research integrates techniques such as Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) to identify bioactive compounds present in *D. alata*. Additionally, the study conducts a nutritive analysis to evaluate its nutritional composition, shedding light on its potential health benefits. Furthermore, the immunomodulatory activity of *D. alata* is explored, aiming to understand its potential in enhancing immune response. Lastly, the research extends to product development, exploring innovative ways to incorporate *D. alata* into consumer products, potentially leveraging its nutritional and immunomodulatory properties. This interdisciplinary approach provides a holistic understanding of *D. alata*'s potential applications in both health and food industries.

KEYWORDS:

Dioscorea alata, Nutritive, purple yam, Health

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CHAPTER -1

INTRODUCTION

"Dioscorea alata: Nature's masterpiece, blending beauty and nutrition in a single tuber." An economical approach to put on weight-Alanine, cyaniding, and peonidin make up the bulk of its over 85% carbohydrate/starch content (Moriya, 2015). For small-scale farmers in many nations, yam is a vital crop since it can provide food security and serve as a source of revenue. For instance, about 60 million people consume yam, and West African nations grow almost 90% of the world's supply. A hydrophilic polymer that is biodegradable, starch is extracted from roots, legumes, grains, and tubers like purple yams (Adewale, 2022). The primary constituents are amylose and amylopectin, whose ratios vary depending on the source of the vegetable. Additionally, starch is a cheap substance that is frequently employed in the pharmaceutical and food industries. (Paul *et al*, 2022).

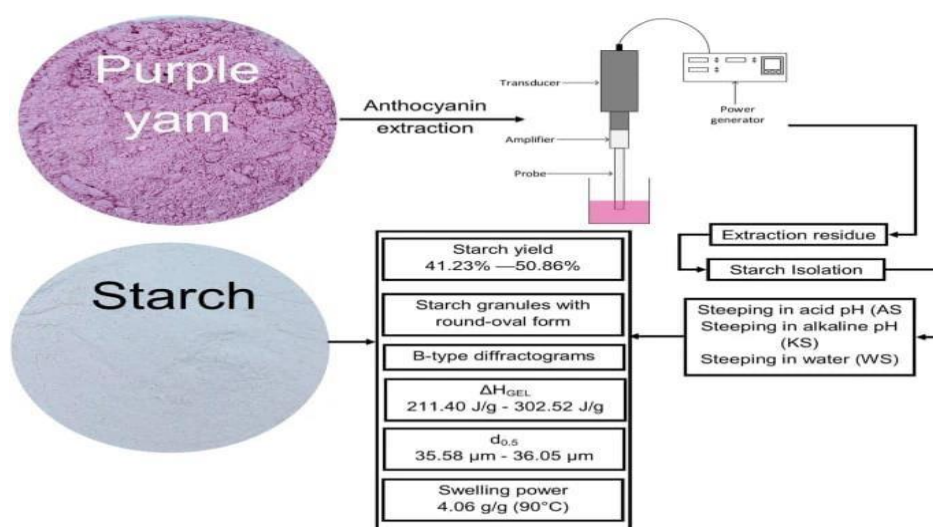


FIG: 1.1EXTRACTION OF STARCH FROM YAM

Planting season spans from May to June. It takes nine ten months for greater and white yams to be ready for harvest. Once collected, the outstanding nutritional characteristics of yam tubers can be stored under ambient tropical conditions for 46 months without experiencing any degradation. (Noora, 2019)The maturation period of to a lesser yam is 8–9 months. Without harming anyone, delicately remove the tubers and with an average annual temperature of 300C and a rainfall of 12002000

mm, the plant grows best in warm, humid climates with sandy loam soil that has a pH of 6.0–6.5. (Barooah, 2014).

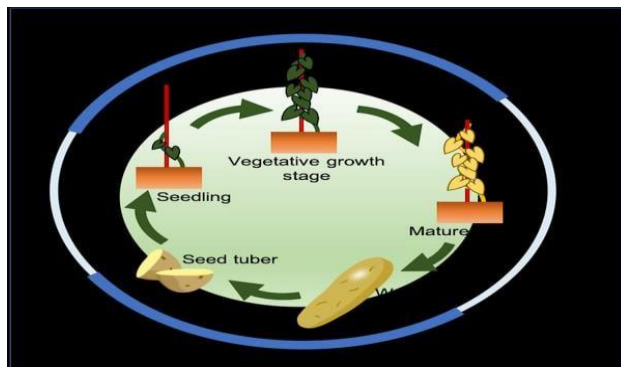


FIG: 1.2 GROWING STAGES OF DIOSCOREA ALATA

The purple colouring of these yams is believed to be mostly caused by the presence of powerful antioxidants called anthocyanins, which are coloured brilliantly. (Davies, 2017)



FIG: 1.3 COLLECTION OF PURPLE YAM FROM THENI

Several bioactive compounds have been associated with the anticancer properties of the *Dioscorea* species, including dioscin, diosgenin, dioscorin, and anthocyanins (Schwinn, 2017). Our data indicate that these chemicals are not the cytotoxic agents causing the anti-proliferative action in the acetonic and ethanolic extracts of *D. alata*, despite the fact that they are abundant and anti-cancer—antiproliferative (Hong.sh, 2019). The cooked tubers are crushed in a mortar to create a thick, elastic paste, the result is pounded yam, or fufu, which is consumed

as balls with meat and sauce in other areas and Consumer sensory testing has identified several characteristics of high-quality boiled yams, including their white shade, crumbly texture that sticks to fingers, non-fibrous and simple to chew, sweet aroma, and pleasant aroma (Honfozo, 2021). But due to growing urbanisation, diets are quickly shifting, and consumption of highly processed yam-based meals is becoming popular. Examples include ready-to-use flours and ready-to-cook peeled tuber pieces in plastic bags that have been vacuum-sealed. Due to its affordability and versatility, starch finds extensive application in the food, pharmaceutical, textile, medicinal, and biotechnology sectors (Ashri, 2014) .

Starch is utilised in the food business for a variety of purposes, including thickening, gelling, emulsion stabilisation, and water binder. (Agudelo, 2014). The pigment that gives purple yams their distinctive purple colour is called anthocyanins, and purple yams are filled with these antioxidants.

Aside from their potential to prevent heart disease and stroke, anthocyanins have anti-inflammatory and anti-cancer qualities and Due to their low glycaemic index, purple yams don't raise blood sugar levels (Wallace, 2021). They are therefore a fantastic option for those who have diabetes or prediabetes and the high fibre content of purple yams is beneficial to digestive health in addition to promoting regularity, fibre can nourish the beneficial microorganisms in your stomach and Purple yams' anthocyanins have been shown in studies to enhance cognitive performance and shield brain cells from harm (Dhanabhal, 2021). And Along with potassium, manganese, and copper, purple yams are also a good source of vitamins A, C, and E. For general health and wellbeing, these nutrients are essential and also Because of their capacity to improve blood vessel function and have antiinflammatory properties, anthocyanins and other substances found in purple yams may help lower blood pressure (Chaudhuri, 2021). Salts known as oxalates are eliminated through urine because they are not metabolised by humans. Its high content in urine, however, raises the possibility of nephrolithiasis, or calcium oxalate kidney stones, since it forms insoluble compounds when it combines with calcium ions. The process of cooking encourages tissue rupture, which releases soluble oxalate and lowers the oxalate concentration in food so consuming this yam will reduces the harm occurs to our body. (Bhandari, 2003).

CHAPTER-2

LITERATURE REVIEW

There are not very many studies that explored the pharmacological properties of yams, although abundant information and traditional knowledge on the medicinal values of yams are prevalent in the tropical countries. Almost all *Dioscorea* Comparison Nutritional properties of yams with other root and tuber crops Chandrasekara and (Padhan, 2020); Bhattacharjee species contains high amounts of secondary metabolites that help in modulating metabolic processes in humans and animals, thus exhibiting beneficial health effects such as anti-oxidative, antihypertensive, anti-inflammatory, and antidiabetic activities as well as inhibition of receptor activities and triggering enzyme activities (Chaudhuri, 2021) (. A wide range of other compounds with medicinal properties as well as anti-nutritional properties are also present in yams such as phenolic, flavonoids, allantoin, dioscin, dioscorin, diosgenin, polyphenols, tannins, oxalate, saponins, and alkaloids have also been reported in yams by various researchers (Wallace, 2021)

Saponins are glycosidic compounds with triterpenoid and steroidal properties, of which steroidal saponins are mainly present in different *Dioscorea* spp. (Adewale, 2022) Steroidal saponins can be further classified based on biological activities as Water-soluble polysaccharides from yams are reported to reduce blood glucose (Honfozo, 2021) and cholesterol levels especially the LDL cholesterol while improving the immune system. The presence of flavonoids has also been reported in several species of yams (Dhanabhal, 2021) spirostane, stigmastane, furostane, cholestane, ergostane, and pregnane families (agudelo, p. 2019) predominantly yams contain spirostane steroidal saponin known as diosgenin (Iachman, 2019)

2.1 ANTI-NUTRITIONAL PROPERTIES OF YAMS

The presence of anti-nutritional factors influences the bioavailability of dietary nutrients such as proteins, minerals, and vitamins. Yam tubers are acrid in nature representing several anti-nutritional properties associated with skin irritation and inflammation of throat after consumption (Kumar, 2017). The presence of some

bioactive compounds such as phenolic acids (Zhao) tannins, phytates, (continued)

Species name	Plant extract	Medicinal properties
<i>D. Oppositifolia</i>	Rhizomes/ tuber	Relief of menopausal syndromes and rejuvenation of early mothers
<i>D. panthaica</i>	Rhizome	Gastric diseases, bone injuries, rheumatic arthritis, and cardiovascular diseases
<i>D. (Moriya, 2015) pentaphylla</i>	Leaf/vine	Treatment of paralysis
	Tuber/flower/ young shoot	Rheumatism Tuber Pain relief and reduce swelling, stomach disorders
<i>D. polystachya</i>	Rhizome	Consumptive cough and dysentery, aid for digestion and gastric motility, and for restraining nocturnal emissions
<i>D. Septemloba</i>	Rhizome	Rheumatism, urethra, and renal infection
<i>D. sylvatica</i>	Tuber	Decoction used to treat cuts, wounds, and sores
<i>D. Trinervia</i>	Tuber	Chronic diarrhoea, asthma, and diabetes
<i>D. villosa</i>	Rhizome	Rheumatism, menstrual complaints, and perimenopause symptoms
<i>D. zingiberensis</i>	Rhizome	Cough, anthrax, rheumatic heart disease, rheum, arthritis, tumefaction, and sprain

Designing Dioscorea Genomes for Improved Nutritional and oxalates have been reported in different species of yams, which are referred to as anti-nutritional because of their toxic properties and bitterness associated with them (Ravi Shankar, 2019)

2.2 NUTRITIONAL AND PHARMACOLOGICAL PROPERTIES OF YAMS

However, over the years, additional tuber characteristics targeted to consumer preferences (tuber size and shape), higher dry matter, tuber flesh colour, tuber flesh oxidation, and starch properties are being routinely measured for wider acceptance of newly developed varieties (Darkwa, 2020)

Recently, there has been some progress on application of advanced or omics tools to several species of Dioscorea. Currently based on short-reads, long reads, and chromosome conformation sequencing, good-quality reference genomes have been released for four Dioscorea species such as *D. Rotundata* (Tamiru, 2017) *D. alata* This has enabled to develop high-quality linkage maps and identification for several agronomic and quality determination systems anthracnose disease, tuber oxidative browning, tuber starch content , and postharvest hardening (Ladjeu, 2020).

2.3 HEALTH BENEFICIAL POTENTIALS

All the mice of the experimental sets (control and treated) did not show any sign of allergic lesion, sickness, and unusual stool and urine discharge and appeared healthy and fit throughout the feeding experimentation; hence, the treated samples are not toxigenic to the animal model. (Sartor, 2019) . Despite any significant differences in total food intake among the mice, the growth (body weight) was better in the treatment sets than in the control. (Salvan, 2019) The weight gain was maximum in the synergistic symbiotic setup comprising both the probiotics,, and FOS fractions recognizable body weight increment was also observed in the other treatment sets .The fermentable fibres in the carbohydrate fractions have been reported to influence the nutrient uptake and mineral absorption among the treated mice, as fermentable inulin-type fructans can increase the mineral absorption and positively influence endocrine function related to appetite and satiety. (Ashri, 2014). The blood sugar level in all treatment sets was average or significantly decreased, showing the FOS fractions' hypoglycaemic effects. (Respondex, 2019).

The lipid profile analysis of treated mice shows that the concentration of total serum cholesterol, serum LDL, serum VLDL, and triglyceride lowered significantly in prebiotics treated sets individually or in combination, compared to the commercial prebiotic Inulin. (Sherkat, 2017) The symbiotic combinations of FOS from hot water and 80% ethanol fractions with probiotics also showed a significantly lower cholesterol profile than untreated control mice. At the same time, serum HDL level increases significantly in all the treatment cases compared to untreated control. (Wallace, 2021)These results also prove more pronounced hypocholesterolaemia effects in symbiotic sets of the carbohydrate fractions than other sets, comparable to the inulin treated set. Therefore, our study explored that the FOS from the hot water and 80% ethanol fractions isolated from *D. Alata L.* contain some prebiotics that has a symbiotic effect on the serum lipid profile of mice. Lowering high serum cholesterol levels contributes to the prevention of atherosclerosis. Kim & Shin reported that administration of inulin, a commercially established prebiotic, for 30 days' treatment increased serum HDL in rats compared to control, which is mainly attributed to the reduced absorption of cholesterol and by the higher rate of catabolism in the liver to the hypocholesterolaemia effect. Wolthuis (Cheng, 2021)

Russo et al. proved the efficacy of inulin against gastrointestinal mobility and lipid and glucose metabolism. The blood sugar level in implicated no abrupt changes in blood sugar level in all treatment sets compared to the untreated control set when checked randomly. (Zaky, 2019)

Dioscorea plants have been used in traditional medicine for centuries to treat various diseases and represent an invaluable source of pharmaceuticals. Certain species of the *Dioscorea* genus have a rich history of traditional use and have been extensively studied (Tiwaeri, 2018) currently, the following species have welldocumented records and are widely employed

2.4 ANTI-TUMOUR ACTIVITY

Recently, there has been a surge of interest in studying the pharmacological potential of *Dioscorea* plants as antitumor agents. Research has demonstrated their effectiveness in inhibiting the growth of various cancer cells, including those associated with liver, lung, ovary, colon, and breast cancers as well as with melanomas and leukaemia. These anti-tumour effects have been observed to occur through multiple pathways, (Jiao, 2021)

2.5 SUGARS

Glucose and sucrose are the main free sugars reported in yam tubers (Kouassi, 2019) Hari Prakash and Namibian, 1996). The presence of maltose and fructose were reported after tubers were stored for some days other sugars found are pentose and mannose (Omijeh, 2018)

Hydrolysed to different sugars (especially when sprouting starts) such as maltose and fructose thus elevating the level of sugars and decreasing starch content consequently. (Harprakash, 2020)The organoleptic properties such as taste and mouthfeel are reported to improve during storage probably due to the increase in sugar content. Differences in species, variety and growth environment also influence the level of sugars in yam tubers (Mensah, 2018)

CHAPTER-3

AIM AND OBJECTIVES

3.1 AIM

The aim of this study is to comprehensively evaluate the phytochemical composition of *Dioscorea alata*, commonly known as purple yam, and explore its potential immunomodulatory properties. Additionally, this study seeks to conduct a thorough nutritive analysis of *D. alata* to better understand its nutritional profile. Furthermore, the research aims to utilize these findings to develop innovative products derived from *D. alata*, potentially harnessing its health-promoting properties for various applications. Through this investigation, we aim to contribute valuable insights into the medicinal and nutritional potential of *D. alata*, ultimately fostering the development of novel therapeutic and dietary interventions.

3.2 OBJECTIVES

- To evaluate the phytochemical composition from *dioscorea alata*
- To examine immunomodulatory properties from *dioscorea alata*
- To evaluate nutritive analysis from *dioscorea alata*
- Innovative product from *dioscorea alata* flour

CHAPTER-4 METHODOLOGY

4.1 MATERIALS-INGREDIENTS USED:

Flour [dried dioscorea alata yam], dates, sesame seeds, peanut, flaxseed.

4.1.1 PRE-PREPARATION:

The fresh yam has been collected, washed and boiled and the skin of yam was peeled, chopped into fine small cubes then the cubes are kept for complete sundry.



FIG: 4.1 (YAM SAMPLE BEFORE DRYING)

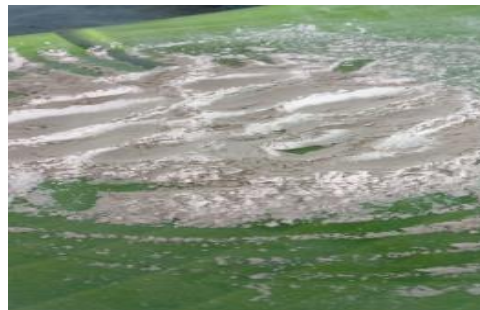


FIG: 4.2(PREPARATION OF FLOUR)

4.1.2 PREPARATION:

- First the yam flour has been roasted in the pan, and then transferred into cleaned container.
- Flax seeds, sesame seeds, and peanuts roasted for in low flame using pan.
- After roasting of peanuts, dates, sesame seeds and flaxseed have been blended up to half-rough textured consistency.

- The vessel which the flour is kept, this mixture has been added and mixed and made into fine balls.

This combination provides the customer with a wonderful sensory experience in addition to possible health benefits.

4.2 FORMULATION

TABLE 4.1 FORMULATION EPISODES

Materials used	Formulation - 1	Formulation -2	Formulation -3	Formulation -4
Flour	50g	75g	150g	150g
Dates	35g	45g	100g	200g
Peanuts	70g	75g	100g	100g
Sesame seeds	5g	6g	15g	20g
Flax seeds	3g	10g	5g	5g

The fourth formulation, which utilizes flour, dates, peanuts, sesame seeds, and flax seeds, was selected for preparation due to the optimal consistency of the resulting balls.

FLOW CHART OF BITES PREPARATION

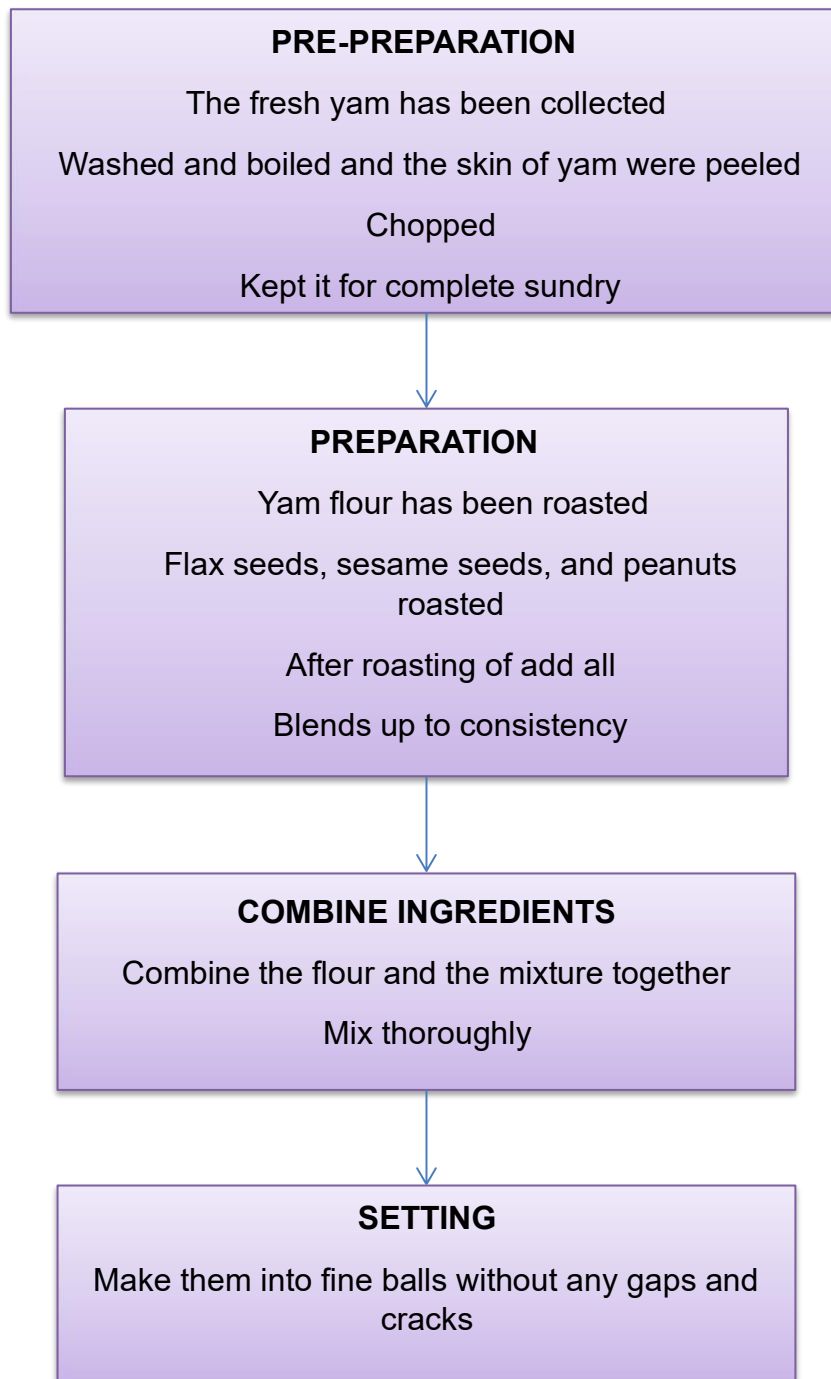


FIG: 4.3 PREPARATIONS OF BITES

4.3 THIN LAYER CHROMATOGRAPHY (TLC):

Separating and analysing biomolecules from a complicated mixture is done via chromatography . An immobile phase and a stationary phase make up this separation procedure. Mixture to be separated percolates through stationary phase, forming mobile phase. Gas-liquid, liquid-liquid, or solid-liquid phases can be these two states. Polar absorbents can be used as the stationary phase in Thin Layer Chromatography (TLC), a solid-liquid chromatography technique, whereas a single solvent or a combination of solvents can be used as the mobile phase.

SOLVENT PHASE:

PHASE -1: hexane (75%) and acetone (25%).

PHASE-2: hexane (7:2) and ethyl acetate (3:8).

PHASE-3: chloroform (6:4), acetic acid (3:2) and methanol (1:2) and water (0:8).

PHASE-4: diethyl ether (65%), hexane (30%) and acetone (10%).



FIG: 4.4 PREPARATION OF MOBILE PHASE-1



FIG: 4.5 PREPARATION OF MOBILE PHASE -2



FIG: 4.6 PREPARATION OF MOBILE PHASE-3



FIG: 4.7 PREPARATION OF MOBILE PHASE -4

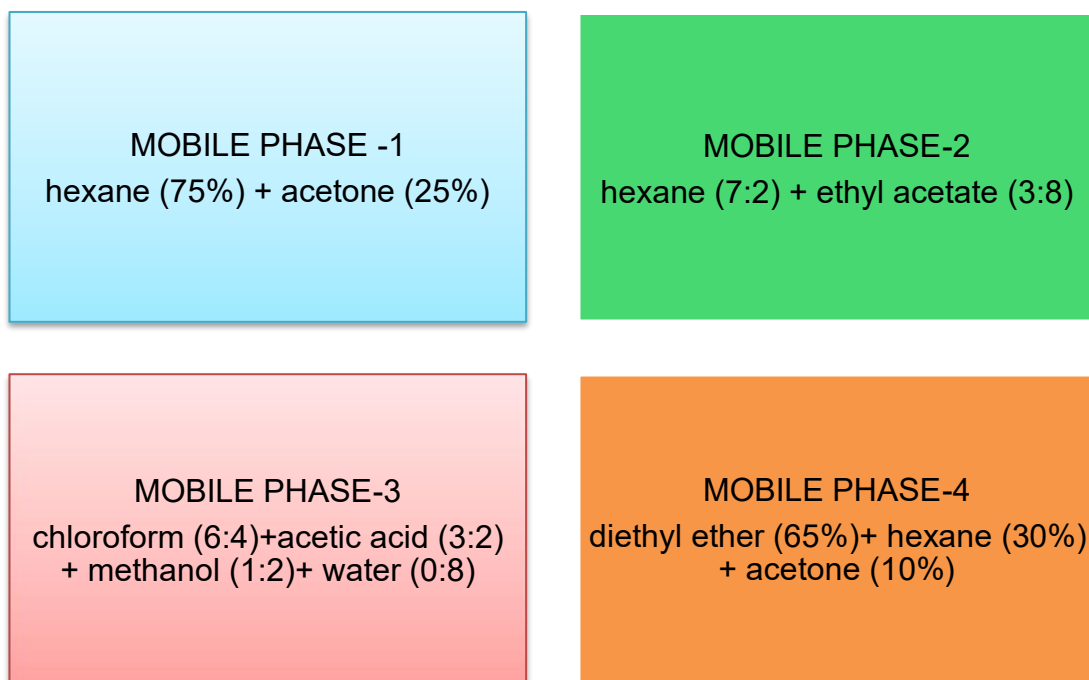


FIG: 4.8 COMBINATION OF MOBILE PHASE

4.4 PHYTOCHEMICAL ANALYSIS:

The acetone and ethanolic extracts were utilised to evaluate the phytochemicals for flavonoids, alkaloids, tannins, sterols, glycosides, phenols, saponins, terpenoids, carboxylic acid, and resins. The signs for presence and absence were denoted by plus (+) and minus (–), respectively. The presence of two plusses (++) suggested strong reactivity, or a high phytochemical concentration. (lebot, 2023)

4.1.3 TOTAL PHENOLIC CONTENT (TPC):

Standards were created with 1 µg, 2 µg, 4 µg, 6 µg, 8 µg, and 10 µg of Gallic acid diluted in 200 µL of volume. A quadruplicate of 20 µL of standard was applied to each well. Each well that held 20 µL of standards and unidentified samples received around 80 µL of 7% calcium carbonate added to it. Each well received 100 µL of a 1:10 diluted Folin–Ciocalteu reagent in deionized water. Following the addition of the Folin–Ciocalteu reagent, the absorbance was measured at 700 nm. Gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) were the measure used to express the extract's total phenolic content. (lachman, 2019).

$$Y = mx + c$$

$$1.737 = 0.6736x + 0.3112$$

$$= 0.6736x + 0.3112 - 1.737$$

$$x = -1.4258 / 0.6736$$

$$= -2.1166$$

4.5 GAS CHROMATOGRAPHY/MASS SPECTROMETRY:

An electron impact mode operating at 70 eV was employed for the investigation. A 0.75-minute injectable purge was conducted while using the split less mode. The transfer line temperature was kept at 280°C while the injector temperature was adjusted to 250°C. The DB-1701 column, measuring 30 metres in length, 0.25 millimetres in inner diameter, and 0.25 millimetres in film thickness, was utilised. An 80°C start point was maintained for two minutes, after which a ramping temperature programme was implemented, increasing the temperature by 20°C per minute for ten minutes. An extra ten minutes were then spent maintaining the temperature. Helium was employed as the carrier gas, flowing at a rate of 1.2 mL min⁻¹, with a solvent delay of three minutes. Scan mode was utilised for the operations. (Sheela, 2019).

4.6 IMMUNOMODULATORY ACTIVITY:

The ability of a drug to either stimulate or inhibit the immune system's reaction is known as immunomodulatory activity. It refers to the ability of plant extracts to either favourably or unfavourably affect immunological function. (Riaz, 2021).

MATERIALS USED:

1. Dioscorea alata extract
2. Peripheral blood mononuclear cells (PBMCs)
3. RPMI-1640 medium

4. Fetal bovine serum (FBS)
5. Penicillin-streptomycin solution
6. Phorbol 12-myristate 13-acetate (PMA)
7. Lipopolysaccharide (LPS)
8. Enzyme-linked immunosorbent assay (ELISA) kits for cytokine quantification
9. Trypan blue solution
10. Haemocytometer
11. Cell culture plates and incubator
12. Pipettes, tips, and other laboratory consumables

Protocol:

1. Isolation and Culture of PBMCs:

- a. Collect peripheral blood from healthy volunteers using standard venipuncture techniques.
- b. Isolate PBMCs using density gradient centrifugation with Ficoll-Paque solution.
- c. Wash the isolated PBMCs with RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin.
- d. Adjust the cell concentration to 1×10^6 cells/mL.

2. Treatment Groups:

- a. Prepare different concentrations of *Dioscorea alata* extract in culture medium (e.g., 10 µg/mL, 50 µg/mL, 100 µg/mL).
- b. Prepare positive control groups with known immunostimulants such as PMA (10 ng/mL) and LPS (1 µg/mL).
- c. Include negative control groups with only culture medium.

3. Cell Stimulation and Incubation:

- a. Plate the PBMCs in 96-well cell culture plates (100 μ L/well).
- b. Add the *Dioscorea alata* extract and control substances to appropriate wells.
- c. Incubate the plates at 37°C in a humidified atmosphere with 5% CO₂ for 24 to 48 hours.

4. Assessment of Immunomodulatory Activity:

- a. After incubation, collect the supernatants for cytokine analysis using ELISA kits according to the manufacturer's instructions.
- b. Measure the levels of pro-inflammatory (e.g., interleukin-6, tumour necrosis factor-alpha) and anti-inflammatory (e.g., interleukin-10) cytokines.
- c. Evaluate cell viability using trypan blue exclusion assay and calculate the percentage of viable cells.

5. Data Analysis:

- a. Analyse the cytokine levels and cell viability data using appropriate statistical methods (e.g., ANOVA followed by post-hoc tests).
- b. Compare the immunomodulatory effects of *Dioscorea alata* extract with positive and negative controls.

4.7 SENSORY EVALUATION:

QUALITY ANALYSIS:

Via sensory evaluation with a broad group of 100 individuals, this study explores how consumers perceive yam bites. This research aims figure out the optimum yam bites features across various age demographics by assessing their comments on aspects like look, texture, flavour, aroma, and mouthfeel.

TABLE: 4.2 TOTAL MEAN AND STANDARD DEVIATION OF RATINGS

CATEGORIES	MEAN $\pm$ STANDARD DEVIATION
APPEARANCE	9.9 $\pm$ 0.438
TEXTURE	9.65 $\pm$ 0.538
FLAVOUR	9.75 $\pm$ 0.5
AROMA	9.65 $\pm$ 0.538
MOUTHFEEL	9.74 $\pm$ 0.504
OVERALLACCEPABILITY	9.65 $\pm$ 0.588

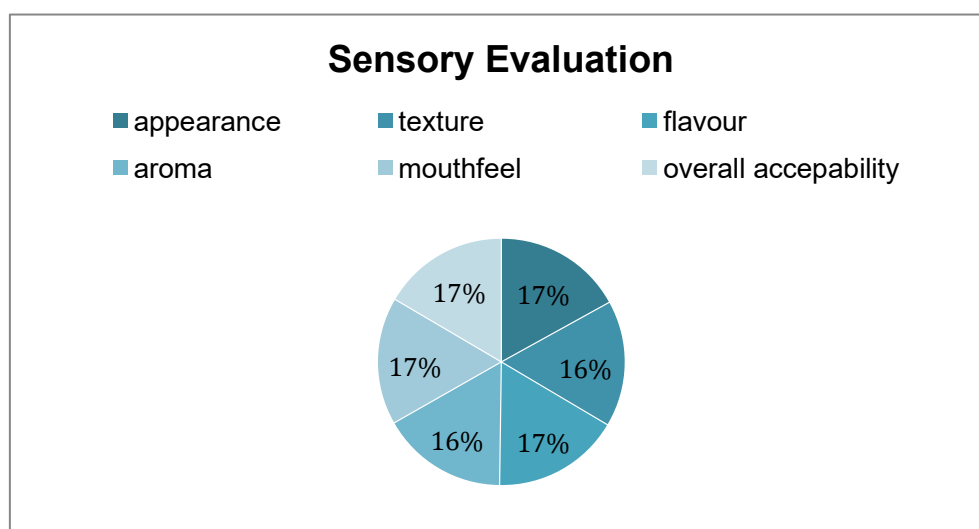


FIG: 4.8 REPRESENTING RATING OF SENSORY EVALUATION

The above figure shows the sensory evaluation results from 100 individuals

QUESTIONNAIRE:

1. APPEARANCE:

TABLE: 4.3 RATING SCALE AND NO. OF RESPONSES

Rating scale(1-10)	No. of responses
1-4	0
5-8	5
9-10	95

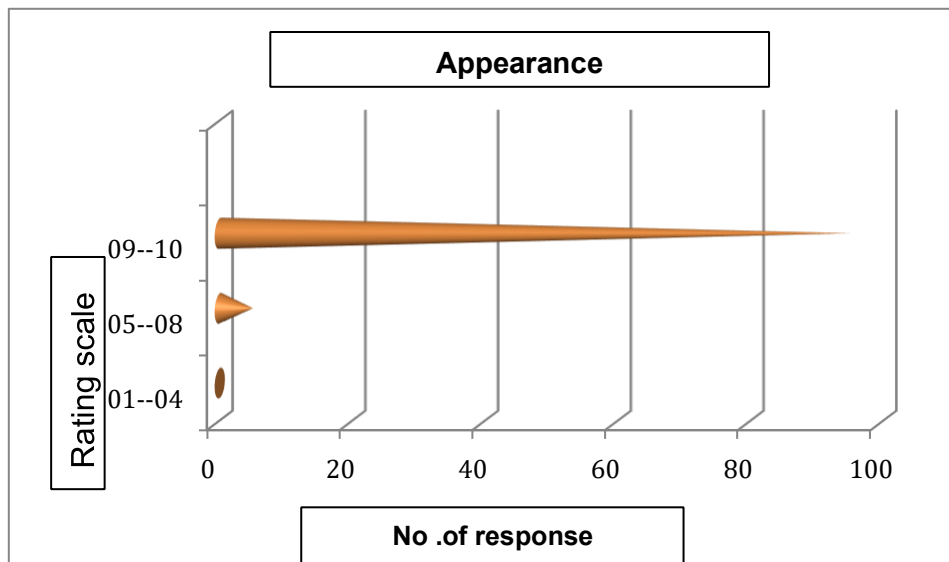


FIG: 4.9 REPRESENTING THE RATING FOR APPEARANCE

INTERPRETATION:

It was found that majority of the people liked the appearance of the product.

2. TEXTURE:

TABLE: 4.3 RANGE OF RATING AND NO. OF RESPONSE

RANGE OF RATING	NO.OF RESPONSES
1-4	0
5-8	3
9-10	97

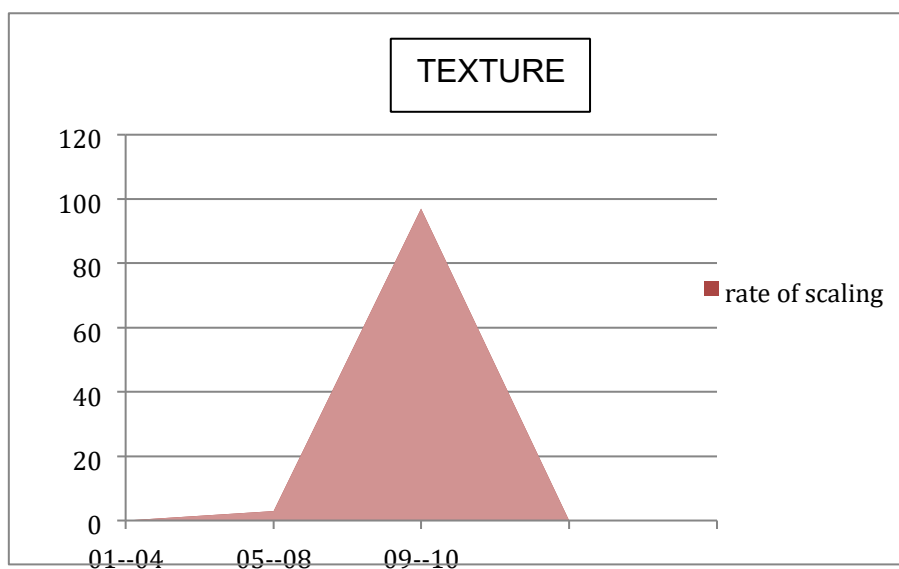


FIG: 4.10 REPRESENTING THE RATING OF TEXTURE

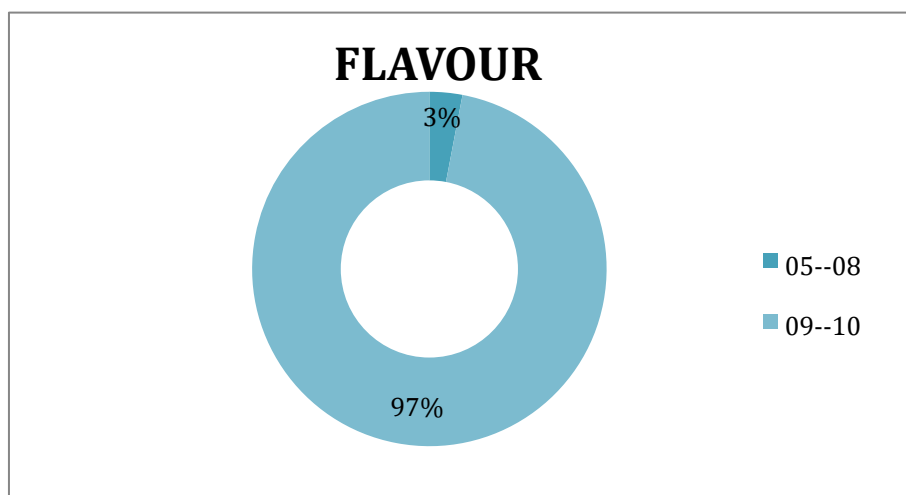
INTERPRETATION: It was found that majority of individual liked the texture.

3. FLAVOUR:

TABLE: 4.3 RANGE OF RATING AND NO. OF RESPONSE

RANGE OF RATING(1-10)	NO.OF.RESPONSE
0-4	0
5-8	3
9-10	97

FIG:



4.11

REPRESENTING THE RATING OF FLAVOUR

INTERPRETATION: It was found that majority of individual liked the flavour.

4. AROMA:

TABLE: 4.4 REPRESENTING THE RATING SCALE FOR AROMA AND ITS RESPONSE

Rating scale	NO.OF RESPONSES
1-4	0
4-8	3
9-10	97

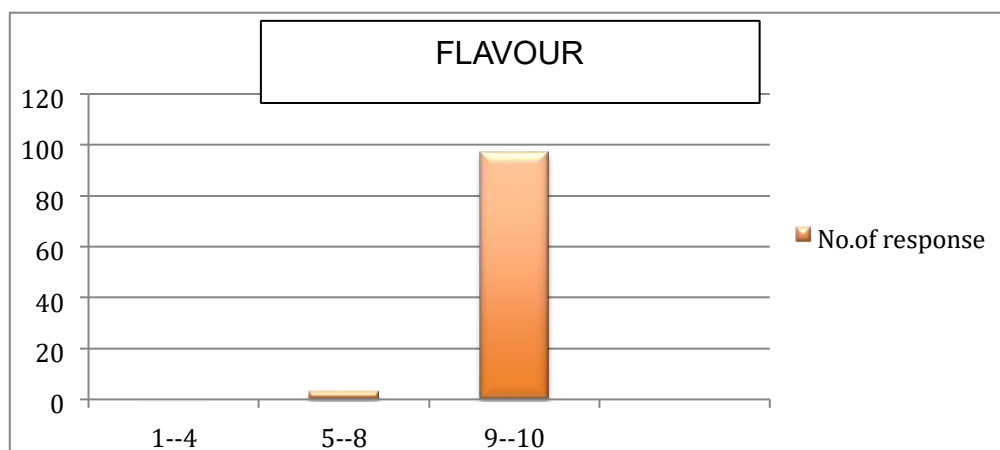


FIG: 4.12 REPRESENTING THE RATING OF AROMA

INTERPRETATION:

It was found that majority of individuals liked the flavour of the product.

5. MOUTHFEEL:

TABLE: 4.5 REPRESENTING THE RATING SCALE FOR MOUTHFEEL AND ITS RESPONSE

RATE OF SCALING(1-10)	NO.OF RESPONSES
1-4	0
5-8	3
9-10	97



FIG: 4.13 REPRESENTING THE RATING OF MOUTHFEEL

INTERPRETATION:

It was found that the majority of individual liked the mouthfeel of the product.

6. OVERALL ACCEPTABILITY:

TABLE: 4.6 REPRESENTING THE RATING SCALE FOR OVERALL ACCEPTABILITY AND ITS RESPONSES

RATE OF SCALING(1-10)	NO.OF RESPONSES
1-4	0
5-8	3
9-10	97

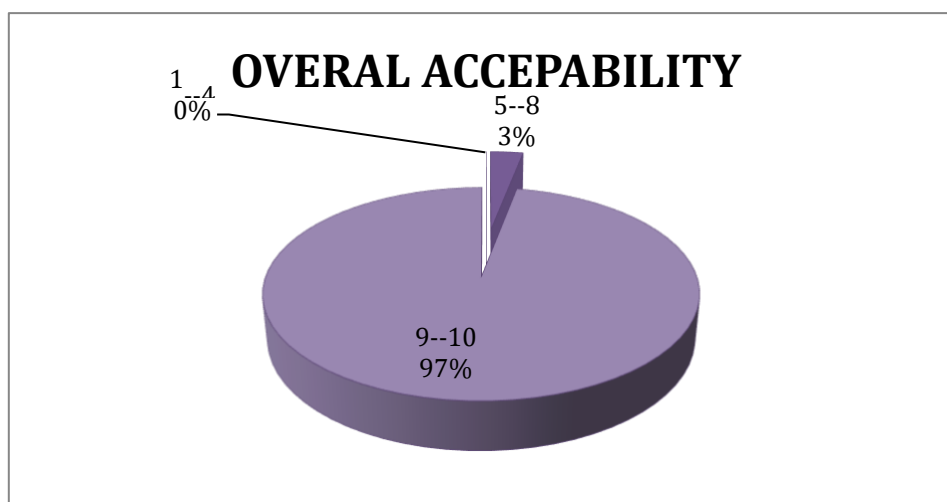


FIG: 4.14 REPRESENTING THE RATING FOR OVERALL ACCEPTABILITY

INTERPRETATION:

It was found that majority of individual liked the overall acceptability of the product.

CHAPTER-5

RESULT AND DISSCUSSION

The results and discussion pertaining to the study of nutritional analysis KASA FUSION BITES (Gluten free) using Dioscorea Alata is discussed under the following headings.

5.1 MACRO –NUTRIENT COMPOSITION

TABLE: 5.1 MACRO- NUTRIENT COMPOSITION TABLE

S.NO	PARAMETERS	UNITS	RESULTS
1.	Energy	Kcal/100g	419
2.	Carbohydrates	g/100g	67.3
3.	Total Fat	g/100g	12.5
4.	Protein	g/100g	9.22

From the table it is observed that the macro – nutrient composition of 100g of Nutri fusion bar using (Disocorea alata, dates, peanuts, and sesame seeds) had 419 kcals of Energy, 67.3g of Carbohydrates, and 12.5g of Protein and 9.22g of Total Fat.

5.2 MICRO – NUTRIENTS COMPOSITION

TABLE: 5.2 MICRO NUTRIENT COMPOSITION TABLES

S.NO	PARAMETERS	RESULTS
1	Dietary Fibre	4.43
2	Sodium	72.6
3	Calcium	126
4	Iron	2.65

Micro minerals like Dietary Fibre, Sodium, Calcium and Iron content from 100g in KASA FUSION BITES is contain 4.43g of Dietary Fibre, 72.6mg of Sodium, 126mg of Calcium and 2.65mg of Iron.

5.3 SENSORY EVALUATION OF FUSION BITES:

The appearance, texture, flavour, aroma, mouthfeel and the overall acceptability is very much liked by the majority of individuals.

5.5 GCMS ANALYSIS:

TABLE: 5.3 PEAK REPORT

Peak	Reading Time	Area	Area%	Name
1	41.424	894400	0.19	Hexadecanoic acid, 1-(hydroxyethyl)-1,2-ethyl ester
2	44.261	1864209	0.40	9,12-Octadecadienoyl chloride, (Z,Z)-
3	44.354	5585105	1.19	9-Octadecenoic acid, 1,2,3-propanetriyl ester,
4	44.475	1016815	0.22	3-HEPTYL-1-CYCLOPENTENE
5	44.761	699426	0.15	Glycidol stearate
6	46.060	2429068	0.52	OCTADECANOIC ACID, 2,3-BIS[(1-OXOTETRAHYDRO-2H-PYRAN-2-YL)METHOXY]
7	46.551	28843334	6.16	DODECANOIC ACID, 1,2,3-PROPANETRIYL ESTER
8	46.654	22891935	4.89	DODECANOIC ACID, 1,2,3-PROPANETRIYL ESTER
9	47.167	359579136	76.83	DODECANOIC ACID, 1,2,3-PROPANETRIYL ESTER
10	48.269	44240962	9.45	DODECANOIC ACID, 1,2,3-PROPANETRIYL ESTER
		468044390	100.00	

The Gas Chromatography-Mass Spectrometry (GCMS) analysis revealed a significant presence of lauric acid, indicating high lauric acid content in the sample." It uses in making skincare products like soaps, lotions, and creams for its antimicrobial and moisturizing properties.

5.6 IMMUNOMODULATORY PROPERTY:

Immunomodulatory Activity Testing

Name of the User: Dr. Stanley

No. of Compounds: 1 (Crude Extract)

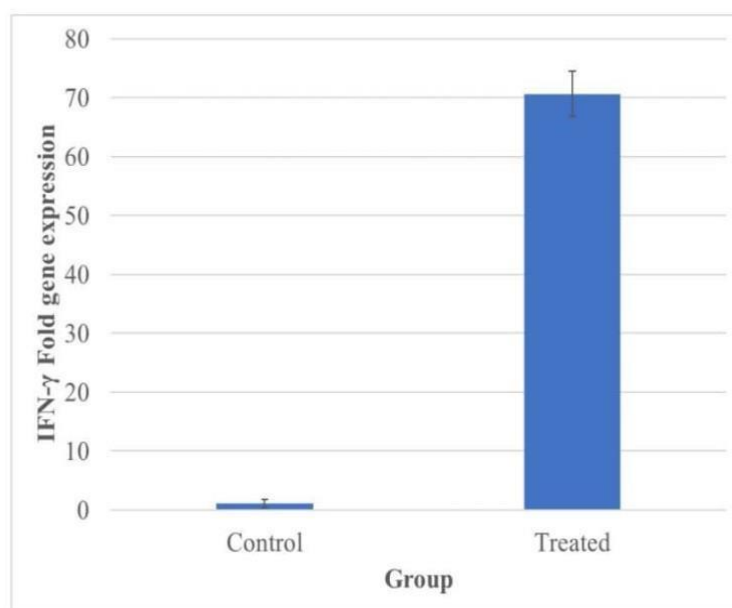
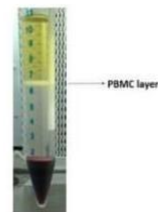
Given Concentration: 166 mg/ml

Target Gene: IFN- γ

Method: RT-PCR

Cells: PBMCs

Compound Test Concentration: 30 μ g/ml (1/2 of MIC50). RT-PCR was done in triplicates.



Inference: The given crude extract induces IFN- γ expression in PBMCs in the absence of any other external stimulant.

FIG: 5.1 REPORT OF IMMUNOMODULATORY ACTIVITY

CHAPTER-6

SUMMARY AND CONCLUSION

The thesis aims to explore the phytochemical composition, immunomodulatory properties, and nutritive analysis of *Dioscorea alata*, commonly known as purple yam, and develop innovative products from it. The process involves using dried yam flour, dates, sesame seeds, peanuts, and flaxseeds to create nutritious balls. Through formulation experiments, the optimal combination of ingredients is determined. Thin-layer chromatography (TLC) is employed to separate and analyse biomolecules from the mixture, utilizing various solvent phases. Phytochemical analysis involves evaluating the presence of compounds like flavonoids, alkaloids, tannins, and more. Total phenolic content (TPC) is measured using Gallic acid equivalents. Gas chromatography/mass spectrometry (GC/MS) is utilized to analyse chemical components. Immunomodulatory activity is assessed by stimulating or inhibiting the immune system's reaction using PBMCs and ELISA kits. Sensory evaluation is conducted to assess the quality of the yam bites, with majority favorably rating appearance, texture, flavor, aroma, mouthfeel, and overall acceptability.

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**DETERMINATION OF PHYTOCHEMICAL PROPERTIES AND ANTICANCER
ACTIVITY OF SOLANUM BETACEUM EXTRACT AND FOOD FORMULATION
OF ITS EDIBLE PART**

PROJECT REPORT

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science in
CLINICAL NUTRITION AND DIETETICS

By

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**DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS
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**SATHYABAMA
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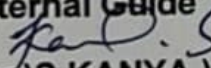
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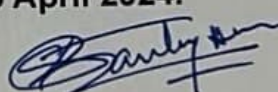
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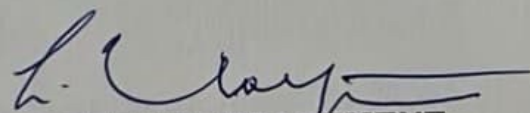
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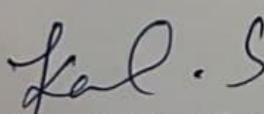
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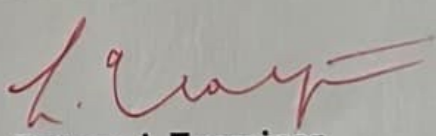

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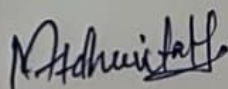

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ABSTRACT

Tamarillo has a unique flavor and rich history. South American fruit is popular in New Zealand. Tamarillo is commercially grown in New Zealand and South America. It grows best under sub-tropical areas. It matures in 18 months. It's 2 m tall and has a lifespan of about 7 years. For propagation, seeds or cuttings are employed, and plant trimming for effective output varies according to the propagation method. Tamarillo plants are wind-sensitive and need cover or windbreaks. It's a beautiful fruit with smooth, shining skin. Yellow, red, and purple fruits are available. This fruit contains vitamins, minerals, fiber, and antioxidants. It has a very low calorie count. Breeding focuses on fruit quality through selection, hybridization, and biotechnological treatments for plantation and post-harvest management. Diseases, pests, viruses, and physiological abnormalities can be treated with plant protection techniques. Like other fruits, it's edible after harvesting. Made into juices, concentrates, jams, gelatins, and sweets. If processing facilities and transport are available, it can be exported as pulp or concentrate. The tamarillo can diversify sub-tropical fruit production as a high-value cash crop, with excellent fruits commanding premium prices in Europe, North America, and Japan.

Keywords: Botany, breeding, biotechnology, cultivation, composition, diseases, origin, production, processing, pests, trade, value addition.

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LIST OF ABBREVIATIONS

USA	United States Of America
Cm	Centimeter
Mm	Millimeter
Kg	Kilogram
Ha	Hectare
E.G.	Exempli Gratia (For Example)
Cv.	Cultivar
Spp.	Species (Plural)
Fig.	Figure
Ø	Diameter
G	Gram
Cm ³	Cubic Centimeter
¹⁴ C	Radiocarbon
Et Al.	Et Alii (And Others)
Cv.	Cultivar
F1	First Filial Generation (Hybrid Offspring)
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
In Vitro	In Glass
Aux	Auxin (Plant Hormone)
GUS	B-Glucuronidase (A Reporter Gene)
NEP25	A Gene Name
Rpm	Revolutions Per Minute
µm	Micrometer
Tamv	Tamarillo Mosaic Virus
GE	Genetic Engineering
Rnai	RNA Interference
ACC	1-Aminocyclopropane-1-Carboxylic Acid (Ethylene Precursor)
Ha-1	Per Hectare
DW	Dry Weight

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CHAPTER 1

INTRODUCTION

The tree tomato (*Solanum betaceum* Cav., syn. *Cyphomandra betaceum* (Cav. Sendt.)) is a lesser-known tiny shrub or semi-woody tree that grows at elevations of 500–2500 m. The tamarillo, sometimes known as a ‘tree tomato’ because its flesh resembled that of a tomato, is a member of the Solanaceae (nightshade) family (Correia *et al.*, 2009). The tree tomato is related to a group of taxa that used to be classified under the *Cyphomandra* genus.

1.1. SPECIES OF TAMARILLO

The species of the genus *Cyphomandra* were reassigned to the genus *Solanum*, subgenus *Bassovia*, based on morphological and genetic data (Bohs *et al.*, 1994) (Bohs *et al.*, 1995). The scientific community now uses the *Solanum* designations to refer to tree tomatoes and wild cousins. In New Zealand, the term “Tamarillo” has become the usual commercial identification for the fruit (Morton *et al.*, 1987). It can be found in subtropical and mild temperate climates all over the world. Commercially, it is grown in New Zealand and a few parts of South America. Although some high-performing uniform lines have been produced, commercial plantings are of seed-propagated populations. Although it is a largely unexploited species, it presents a wonderful chance to diversify fruit production as a high-value income crop in many subtropical and mild temperate production locations. It comes in two colors red and yellow, with red being the more prevalent. It's a vivid red egg-shaped fruit with yellow-orange flesh and black seeds encased in purple gelatin. The red color comes from anthocyanin pigments, while the yellow-orange color comes from carotenoids. Tree tomatoes at the fruit-bearing stage require support to keep their branches from breaking off as they get packed with fruit. Because of its shallow root system, they are readily blown over by the wind. The plant is not affected by the duration of the day. *Cyphomandra* species such as *C. hartwegimi*, *C. sibundoyensis*, and *C. amanuensis* produce edible fruits in the wild. Other *Cyphomandra* species are employed as colors and in medical formulations. This category of plants is becoming more economically important, and it may have a lot of future promise.

1.2. AREA AND PRODUCTION

Tamarillo popularly known as arboreal tomato is a fruit tree of Andean origin and is currently grown in California, Argentina, Colombia, Ecuador, Venezuela, and New Zealand (Ordóñez *et al.*, 2010) for use in the fresh fruit market and for food processing industry. Tamarillo is also produced less widely in Zambia, Zimbabwe, Uganda, Sri Lanka, and India (Stangeland *et al.*, 2009). The fruit's distinctive flavor and nutritional benefits are causing an uptick in interest today, which is keeping prices high. New Zealand and the United States have developed extensive plant breeding programs to develop new cultivars that are more appealing to customers (Correia *et al.*, 2011). In Colombia, tamarillo orchards cover a total cultivated area of 7646 ha distributed in 18 provinces. However, Antioquia and Cundinamarca alone account for two-thirds of the total production (50 and 14%, respectively). In New Zealand, about 2000 tons are produced on 200 ha of land and exported to the United States, Japan, and Europe. New Zealand's horticultural practices, handling, storage, and transportation methods have all been enhanced via research there. Some better cultivars and shipping containers and circumstances for overseas exports have resulted from these investigations. This crop is limited by factors such as a lack of clear differentiation among varieties, low fruit quality (heterogeneity and phytosanitary issues), the use of ineffective local and foreign variety substitutions, and the fact that the tree tomato is frequently a subsistence crop and not included in genetic conservation programs.

1.3. MARKETING AND TRADE

New Zealand is one of only three countries to grow tamarillos commercially, the others being Colombia and Australia. New Zealand leads in the production and export followed by Colombia (Acosta-Quezada *et al.*, 2011). Tamarillos from New Zealand are exported to the United States, Japan, and Europe. For the export, the existing marketing channels developed for the kiwifruit are used (Prohens J *et al.*, 2000) which has greatly benefited the farmers. Due to a lack of international market and knowledge of the fruit, the export potential of this fruit crop has not been yet achieved to its level. There are relatively few barriers to trade and very few countries specifically refer to tamarillos in their tariff schedules. The United States of America continues to be the main export market for tamarillos. In Colombia, the majority of the produce is consumed locally, but some parts are also exported to the Netherlands, France,

Canada, Germany, and Spain. It is important to mention here that standards have been established among commercial growing countries that market tamarillo fruits (New Zealand, Ecuador, and Colombia), and in other countries, there is no such regulation and therefore no commercialization capacity (Vasco *et al.*, 2009) (Schotsmans *et al.*, 2011). Fresh tree tomatoes are in high demand in foreign markets, particularly in the United Kingdom, the Netherlands, and Spain, where they are especially popular if they are grown without the use of pesticides. Fair Trade accreditation also helps to open up new markets for the product. Imported fruit is processed into juices, syrups, and other beverages as well as gelatins and other treats. It might also be exported in the form of fruit pulp or concentrate if processing facilities and suitable transportation were available.

1.4. COMPOSITION AND USES

This fruit is found to be a good source of vitamins A, C, B6, and E, and antioxidants (MertzCet *et al.*, 2009). Tamarillo has a relatively high content of vitamins A and C. Levels of vitamin A are intermediate between those of tomato and carrot (Rodriguez-Amaya *et al.*, 1983), while the ascorbic acid content is similar to that of citrus fruits. Tamarillo is rich in anthocyanins and carotenoids which are responsible for their color (Kou *et al.*, 2009). The presence of anthocyanins and carotenoids shows their biological, therapeutic, and preventative properties (Kong *et al.*, 2013). [using spectroscopic analyses revealed that tamarillo fruits are a rich source of natural pigments with potential antioxidant activity, giving them a remarkable added-value. Phenolics are the main antioxidants found in the tamarillo fruit pulp (Vasco *et al.*, 2008). The seed of the fruit is consumed together with flesh (Morton JF, 2013). Tamarillo seeds made up 1.0–1.5% wet basis of the fruit (50–80 g). The seeds of tamarillo were examined for their proximate components (Ramakrishnan *et al.*, 2013). Protein content was discovered to be 22.63%, fat content to be 21.13%, and ash to be 3.15%, with a total carbohydrate content of 43.87%, in the seeds. Tamarillo seed oil's fatty acid profile revealed that linoleic acid (70.47%), oleic acid (14.93%), palmitic acid (9.41%), stearic acid (2.23%), and linolenic acid were the most prevalent fatty acids (1.73%). Apart from these, the oil included arachidic acid (0.23%), phenolic acid (0.22%), and lignoceric acid (0.23%). The fruits are very low in calories (only about 40 calories per fruit). These fruits are available in both red and yellow varieties. However, the red varieties are more popular and more common (Lister, 2005). (Acosta-Quezada *et al.*, 2011) assessed fruits of

purple and yellow/orange cultivars and did not find any relevant differences among them.

1.4.1 Nutritional composition of tamarillo fruits

Tamarillo is grown mainly for its edible fruits and to a lesser extent as an outdoor ornamental. The tamarillo fruit has been described as “brazenly beautiful” and the aroma “unusual and attractive”. They have several culinary uses and can be eaten raw in salads or as desserts but preferably cooked. The flesh can be eaten fresh or made into a range of sweet and savory dishes and condiments. Tamarillos are tangy and usually sweet with a bold and complex flavor that differs by variety. The fruit can be stewed to use on cereal or as a pie or crumble filling, added to stews, or made into a delicious chutney, which is especially good with chilies. In Jamaica and the West Indies, the fruits are considered to have beneficial effects in relieving disorders of the liver. Tamarillo is an important component in Rwanda’s exotic fruit industry and consumption of the fruit is traditionally recommended for people suffering from stomach ailments. It is processed into jams, juices, and jellies canned in syrup or prepared in combination with milk products like yogurts, milkshakes, and ice creams. The fruit has a high level of pectins, which makes it especially suited for jams and preserves. It is also used for canning in syrup and for producing pulp, chutney, sauce, baby food, and in combination with milk products like yogurt, milkshakes, and ice. The fruit can be used much as a regular tomato, but it has less moisture, so more water, stock, or gravy is needed for most cooked dishes.

1.5. ORIGIN AND DISTRIBUTION

The tamarillo *C. betaceum*(Cav.) is native to the Andean region of South America where most *Cyphomandra* species are found in cultivated states. It is also grown in New Zealand, Brazil, Argentina and Colombia. The area where *C. betaceum* originated is not known, but some wild or naturalized populations have been reported in southern Bolivia and northeastern Argentina and may give an indication of its area of origin .It is found that *S. betaceum* is closely related to *S. unilobum*, *S. roseum*, and in particular to *S. maternum*, all of which are found in Bolivia in wild status (Lester, 2001). Little information is available on the domestication of the tree tomato, and at present it is unknown when and where this process took place. In New Zealand, it is cultivated as a minor fruit crop. It was introduced to New Zealand as early as the

late 1800s and there have been several further introductions (Fletcher , 1975). It is cultivated and naturalized in Venezuela and grown in the highlands of Costa Rica, Guatemala, Jamaica, Puerto Rico, and Haiti. Tamarillo was adopted on January 31, 1963, by the growers of New Zealand as the official common trade name for *Cyphomandra betacea*. The tree tomato was named as tamarillo in New Zealand in 1967 (Hewett *et al.*, 1993). Tamarillo is a Maori word that indicates leadership, and “hello” comes from the Spanish word for yellow, “Amarillo” which was the original type of tamarillo to be grown, and only in the 1920s was a new red variety developed (Tamarillo Growers Association., 2018) . It is possible to grow it successfully in areas with Mediterranean climates, where it has good prospects as a developing new fruit crop (Grau *et al.*, 1994). In India it is found growing in Sikkim, Darjeeling hills of West Bengal, Tamil Nadu, Meghalaya, and in other northeastern states. In Nepal during the survey, it was found that it is grown in home gardens for vegetable purposes. It has been grown in Queensland and Australia in home gardens for many years and is a practical crop in the highlands of the Australian part of New Guinea. In the USA it is grown in California and occasionally in Florida in pots and indoors. The plants fruit satisfactorily in greenhouses. In Malaysia, *C. betaceum* is cultivated in Cameron Highland (Peninsular Malaysia), and Kundasang (Sabah) and is locally known as “Pokok Tomato” or “Tamarillo” in Peninsular Malaysia, and as “Buah Cinta,” “Moginiwang,” or “Tamarillo” in Sabah.

1.6. BOTANY AND TAXONOMY

The plant is a small shrub or half-woody, evergreen or partially deciduous, attractive growing, brittle tree, shallow-rooted reaching 3 m to 5.5 m in height rarely as much as 7.5 m. The dichasial branching is responsible for the shrubby habit of the tree, although seedling-grown plants do go through a juvenile phase and the initial branching pattern may not occur until after 30 nodes growth (Richardson *et al.*, 1993). The plant is not tolerant to drought stress and can be damaged by strong winds because of the shallow root system. The leaves are perennial, simple, 10–35 cm long and 4–12 cm broad, evergreen, alternate, more or less heart-shaped at the base, ovate and pointed at the apex, thin, softly hairy, with prominent veins, and have a musky smell and slightly tinged purple when the leaves are young. Flowers with fragrance are borne in small, loose clusters near the branch tips, 1.25–2 cm wide, and have 5 pale-pink or lavender, pointed lobes with 5 prominent yellow stamens and

green-purple calyx. The long-stalked, smooth, egg-shaped are borne singly or in clusters of 3–12, pointed at both ends, and capped with the persistent conical calyx. In length, it can be anywhere from 5 to 10 cm long, and in breadth, it can be anywhere from 4 to 5 cm wide. Red, orange, yellow, or red-and-yellow skin colors are all possible, as well as a variety of shades in between. Species-dependent variations in flesh color include shades of orange-red, yellow, and cream-yellow. In the two longitudinal compartments of dark-purple and red fruits, as well as yellow and orange fruits, a soft, juicy, subacid to sweet pulp surrounds the seeds. The exterior layer of flesh is luscious and bland, whereas the skin is rough and unpleasant to eat. Despite their diminutive size, the seeds are tough and bitter. A weak or under-ripe tomato with a subtle resinous aftertaste has a resinous scent and flavor. The plant bears in the first or second year after planting, but peak production is reached after 4 years and has a life expectancy of only 5–12 years. In general, it forms a large spreading crown at a height of 1.5–2 m from the base of a single upright woody trunk.

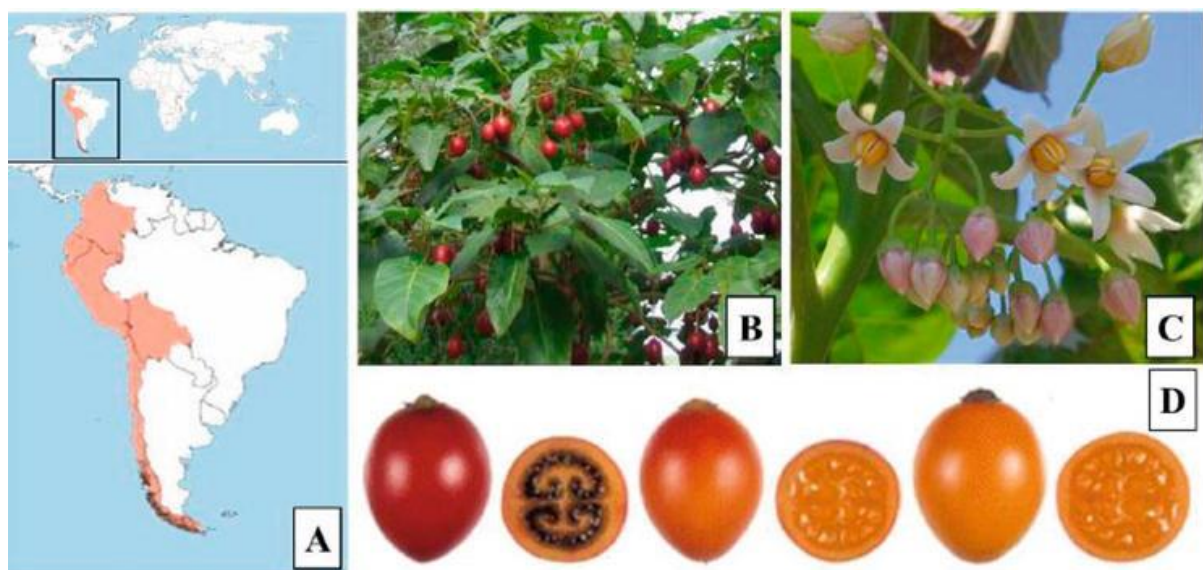


Figure 1.

Tamarillo origin and general aspects (A) Andean region from where tamarillo is native; (B) tamarillo tree with red fruits; (C) Tamarillo's flowers; (D) Tamarillo's fruits range of colors.

1.7. VARIETIES AND CULTIVARS

There are no named cultivars, but there are local preferences according to fruit color. Tamarillo have been described as three strains yellow, red (red skin, yellow-orange flesh), and purple (red-purple skin, light orange flesh), with the red being more popular and more common but no named varieties have been analyzed. In Europe and the USA, the red and purple cultivars are preferred by consumers due to their attractive color, flavor, and nutritional properties. In Malaysia, the red variety of tamarillo can be easily grown at Cameron Highlands, Pahang. It is an egg-shaped bright red fruit with yellow-orange flesh and black seeds that are surrounded by a purple seed coat. In Kenya, the main varieties grown are the Gold-mine, Inca red, Rothamer, Solid gold, and Ruby red. Red fruits are chosen for the fresh fruit markets because of their appealing color. Different selections have been developed in New Zealand from time to time. Commercial plantings of New Zealand's current dominant "black" type were selected in the early 1920s as a variation of the yellow and purple variants that had been used in the past. A reselection process resulted in the creation of this massive, higher-quality red variety. New Black was chosen by William Bridge in 1927 for its huge fruit. Ruby Red has been a staple of the New Zealand economy for decades. Heart-shaped fruit with a deep crimson color and a savory flavor were selected in 1970. Inca Gold is a canning favorite because of its amber hue and oval shape. If you are looking for something a little more vibrant, Ecuadorian Orange is a great choice. In 1979, Kaitaia Yellow was chosen for its flavor and sweetness. New Zealand is testing a new cultivar named Goldmine. Rothemer is an 85-gram fruit from San Rafael, California, with a bright red exterior and a golden yellow pulp. Solid Gold is an orange, luscious fruit with a sweet flavor. Red Delight, Yellow, and Oratia Round are also made in New Zealand.

1.8. BREEDING AND CROP IMPROVEMENT

Tamarillo's chromosomes are diploid ($2n = 2x = 24$). However, spontaneous tetraploids, aneuploids, and triploids have all been documented. The flowers are autogamous and self-compatible but flowers need to be shaken for pollination. Traditional plant breeding has largely ignored the tamarillo, and few crop improvement techniques have been applied. Most attempts to transfer this crop into new areas have failed since they have relied on a single cultivar. As a result, the ability to use variety

for local adaptation has been limited. On the other hand, the loss of genetic diversity in this crop and allied wild species is a major worry . Plantation management, fruit quality, and postharvest management are all improved through research and breeding. Breaking seed dormancy, improving fruit taste, and increasing yields are all key breeding goals. Little “stones” of sodium and calcium that occasionally form in the fruit skin offer difficulty for industrial applications and must be eradicated through breeding. The following are the most important breeding procedures in this crop.

1.8.1 Selection

Although the tamarillo is essentially autogamous, there is some variation among plants of the same accession, particularly in traditional growing methods. This variance is caused by spontaneous mutations or by the inadvertent introduction of genetic material from different populations through genotype crossover . This variation could be used to find a favorable variant that could be used in a future breeding effort for crop improvement. As a result, screening valuable genetic varieties in traditional growing areas for conservation, selection, and crop improvement for this underutilized fruit crop is critical.

1.8.2 Hybridization

Pollen tubes pierce the ovary and set fruits in most interspecific matings with *Cyphomandra*, according to , but seeds do not mature. Pringle and Murray experimented with interspecific hybridization of *Cyphomandra* with nine different *Cyphomandra* species and discovered that the majority of the crosses failed after fertilization. However, a species hybrid between the two Brazilian species *C. corymbiflora* and *C. diploconos* was obtained very easily. Most species’ combinations had reciprocal variances in compatibility. The results of these crosses indicate that the *S* locus is not involved in the regulation of interspecific incompatibility.

1.8.2.1 Intraspecific hybridization

There are no technical issues in crossing between different varieties of *C. betacea*. Tamarillo individuals have a high degree of homozygosity due to their autogamy, and crossing genetically dissimilar individuals produces homogeneous offspring. Commercial production of the hybrid tamarillo is simple because each fruit contains more than 300 seeds . The agronomic behavior of F1 hybrids, on the other hand, is

completely unknown. Most solanaceous horticulture crops have heterotic yield features , and the tamarillo is no exception. Obtaining segregant generations allows for recombination and segregation, allowing for the emergence of new superior genetic combinations. There is, however, a scarcity of investigations on the degree of diversity in segregant generations. Even though it takes several years to evaluate, tamarillo cannot be regarded as a standard fruit crop, as individual genotype evaluation can take up to 20 years. Nonetheless, evaluating each individual takes 3–5 years, making yearly species breeding schemes impracticable, as it could take up to 10 generations before an improved cultivar is released. It will be impossible to determine the best effective breeding strategy for this crop until studies are conducted to determine the corresponding value of the additive and dominant components of genetic variation for each of the primary interest traits. Breeders are interested in developing pure lines or F1 hybrids that maximize heterozygoses. Vegetative propagation, on the other hand, allows for the propagation of the most valuable genotypes that may arise in segregant generations, such as an F2 produced by complementary or transgressive crossings. Cloning permits the entire genotype to be preserved, which might be useful in the production of novel cultivars. Micropropagation for tamarillo provides for vegetative propagation without the risk of viral transmission.

1.8.2.2 Interspecific hybridization

Interspecific hybridization could be effective for transferring desirable traits from wild species to cultivated forms, such as disease and worm resistance. Crossings between *C. acuminata* Rusby and *C. uniloba* Rusby have proven successful. These species are morphologically quite similar to *C. betacea*. Fertility is low in Tamarillo hybrids with *C. acuminata*. Those obtained using *C. Manitoba*, on the other hand, are vigorous and prolific . Other *Cyphomandra* species, such as *C. hartwegii* (Miers) Sendt. Ex Walp. and *C. sibundoyensis* Bohs, are edible as well and may have some future potential as stand-alone plants or sources of genetic variety for tamarillo breeding. There is no publicly available enhanced breeding material for tree tomatoes. Nonetheless, numerous hybrids between *S. betaceum* and *S. unilobum* are being tested in Colombian fields as a result of breeding operations .

1.8.3 Polyploidy breeding

Triploid and tetraploid seedlings are found at low frequency among commercial plantings of the diploid tamarillo and may arise from the union of unreduced gametes. Further, reported successful induction of tetraploids in the tamarillo (*Cyphomandra, betacea* (Cav.) Sendt.) by the application of colchicine to the germinating seed. Triploids and aneuploids are produced from interploidy crosses. Most aneuploids produce primary trisomics ($2n = 2x + 1 = 25$), but some possess 26 chromosomes and few may be hyperpolyploids. Aneuploidy with 25 chromosomes, instead of 24 as the diploid types, shows good fertility and gives a similar or even higher yield and fruit size than their diploid counterparts. The pollen fertility of these plants can vary from 10 to 90%. The morphological traits of aneuploids and diploids were the same. For commercial use, aneuploids have fruit and seed sizes comparable to those of diploids. Tamarillo polyploids have been proven to have low fertility and poor agronomic properties, whether they are spontaneous or manufactured .

1.8.4 Biotechnological methods

Traditional techniques have revealed themselves inadequate for improving cultivars because of the low success of cross-pollination, the high incidence of incompatibility, and phytosanitary issues. A valid alternative for this plant's breeding is biotechnological methods such as in vitro cloning and genetic transformation .

1.8.4.1 In vitro regeneration systems

Tamarillo micropropagation methods have been described in various assays. In vitro cloning can be achieved through (1) axillary shoot proliferation, which was the first method to be applied (2) organogenesis, obtained on leaf explants and (3) somatic embryogenesis, first obtained from mature zygotic embryos and hypocotyls cultures, and later from other explants.

1.8.4.1.1 Somatic embryogenesis

Plant multiplication has the potential to be revolutionized by somatic embryogenesis, a powerful biotechnology tool. Auxin-rich media is used to induce somatic embryogenesis in tamarillo, where embryogenic callus is first generated (induction phase) and subsequently developed into embryos after being switched to a medium free of auxin (development phase). Several explants of tamarillo have the potential to

initiate embryogenic cultures including, mature zygotic embryos, young leaves, cotyledons, and hypocotyls. Tamarillo mature zygotic embryos were one of the first explants tested for somatic embryogenesis. The formation of embryogenic tissue offers great potential for the large-scale production of plantlets and is also useful in plant genetic transformation. Somatic embryos pass through different morphological phases similar to those occurring during zygotic embryogenesis. The subculturing of somatic embryos, on an auxin-free medium for a further 4–5-week period gives rise to green normal plantlets.

1.8.4.2 Genetic transformation

Several viral infections impact the health and vigor of tamarillo trees, as well as the appearance of the fruit. The tamarillo mosaic virus, the most important pathogenic virus, has no known resistance in *Cyphomandra* species (TaMV). There has been little success in using traditional breeding programs to increase tamarillo's virus resistance. Plants can now be genetically modified to be resistant to a variety of viruses, thanks to recent advances in molecular biology, including the tobacco mosaic virus. *Agrobacterium*-mediated transformation of tamarillo has been successfully achieved and protocols for transformation are now available. The use of genetic transformation offers a great opportunity for the improvement of many characters for which there is not enough genetic variation in the local germplasm. Genetic transformation is also being used as a functional genomics tool, helping to better understand the process of somatic embryogenesis. The most successful strategy so far has involved constitutive expression in the host plant of the coat protein gene of the target virus. Recently, the coat protein gene for TaMV has been cloned and sequenced which may allow TaMV resistance to be engineered into tamarillo. The application of the *Agrobacterium*-mediated transformation method to obtain genetically modified tamarillo plants regenerated via organogenesis was used to introduce the pKIWI110 binary vector into leaf disks by using a virulent LBA4404 and some of them also expressed the *b*-D-glucuronidase (*gusA*) reporter gene and chlorsulfuron resistance. *Agrobacterium*-mediated transformation was also used to obtain tamarillo plants resistant to tamarillo mosaic virus (TaMV) that were regenerated by shoot proliferation. The NEP25 gene has been silenced in tamarillo using *Agrobacterium*-mediated genetic transformation procedures. After undergoing somatic embryogenesis, these plants have been regenerated and are currently being

evaluated to see if they have the same capacity for somatic embryogenesis as untransformed plants.

1.8.5 Breeding for yield

At present, the tamarillo is being introduced as a promising crop in a variety of environments, e.g., regions with a Mediterranean climate. However, most attempts to introduce tamarillo culture have been based on a single cultivar and this has restricted the opportunity to exploit variation for local adaptation. For this purpose, germplasm screening is essential to select the most adapted types in local environments. Several evaluations of tamarillo germplasm have shown a high degree of genetic variation for yield and fruit weight. Differences among different plant genetic materials can be as high as two-fold for fruit weight. The fruit yield is highly affected by environmental components and exploitation of the most adapted material could maximize the selection for yield.

1.8.6 Breeding for quality

Among fruit quality parameters fruit shape is of interest because it is directly related to consumer acceptance because it affects fruit attractiveness and is important in terms of packaging and presentation. The fruit shape varies from round to elongated, with a ratio length/width higher than two among accessions. Round to oval shapes appear to be preferred. Fruit color varies from yellow types to purple and it may have stripes or not. Differences among cultivars have been found in organoleptic characters like soluble solids, titratable acidity, ascorbic acid, and other characters. Cultivars with a higher sugar/acid ratio are probably more suited for the processing industry. Sugar/acid ratio and sweetness were found to be higher in one of the seven accessions studied by that had reduced acidity (between 15% and 23% and similar levels of soluble solids). Recently developed 'Oratia Red' and 'Andys Sweet Red' have a high sugar/acid ratio (Boyce and Strubi, 1997). Breeding for nutritive value is also possible as a lot of variation for ascorbic acid content and vitamin A have been found among genotypes. Aromatic compounds of tamarillo have been and the selection of high aromatic cultivars is of interest. However, aroma is a complex character, in which many interactions among different compounds are involved and in which environmental influence is considerable.

1.8.7 Breeding for disease resistance

Few studies have been devoted to tamarillo breeding for disease resistance. Most work has dealt with TaMV resistance. Resistance to TaMV has not been achieved yet, even in wild species belonging to the genus *Cyphomandra*. Genetic transformation is being used to develop tamarillo cultivars resistant to TaMV. Strategies used up to now have mostly involved the use of genetic constructs which include sequences of the coat protein of this virus. The use of mutagenic agents, such as nitrous acid for the production of defective TaMV strains, which could be used for cross-protection has not been successful. Resistance to anthracnose is also being attempted by in vitro selection of cells capable of growing in the presence of crude filtrate of the fungus. However, there are no reports of the efficacy of this strategy in developing mature plants resistant to this disease.

1.8.8 Breeding for early harvesting

Early harvesting is of significance since different cultivars differ in how quickly their fruit ripens. This could be exploited to select the earliest cultivars. There is also quantitative variation in the response to postharvest applications of ethylene in some cultivars so it is possible to achieve successful postharvest ripening. Despite being considered a non-climacteric fruit ethylene applications stimulate ripening. Fruit can be harvested during the turning stage and allowed to ripen after harvest in materials where ethylene induces ripening. Fruits that are turning may be stored in this way and matured as needed.

1.8.9 Breeding for parthenocarpy

Occasionally, parthenocarpic (seedless) fruit-producing trees can be discovered in orchards. These trees require vegetative propagation because they are the result of spontaneous mutations. Fruits from parthenocarpy are ovoid-shaped, red to orange in color, and have stripes that range from green to coffee in hue. Orange is the color of the flesh. Fruit weighs normally only 20 g and is smaller than other fruit. Due to their lack of seeds, parthenocarpic tamarillos would be very intriguing; nevertheless, before this form of fruit can become well-known, low-weight and poor yield issues must be resolved.

1.9. SOIL AND CLIMATE

Tamarillo plants grow best in light, deep, fertile, and rich in organic matter soils. However, soils must be well-drained, since the plants are not tolerant to water logging. They grow naturally on soils with a pH of 5–8.5. The tamarillo requires full sun and freedom from competition with roots or shade from other plants. The tamarillo prefers a subtropical climate, they grow in many parts of the world with rainfall between 600 and 4000 millimeters and annual temperatures between 15°C and 20°C. Although species can also thrive in colder climates, in areas with temperatures not lower than 10°C and where extreme freezing does not occur, but it is intolerant to frost (below –2°C) and drought stress. Even though extreme cold could severely damage tamarillo plants, often the plant can recover. During the first year of planting the cuttings and seedlings should be protected from frost as plants are highly susceptible to frost and can readily be killed. Frost kills the small branches and foliage of mature trees but not the largest branches and main stem. It is assumed that the fruit set is affected by night temperatures. Areas where citrus is cultivated provide good conditions for tamarillos. Tree tomatoes cannot survive in areas with prolonged drought. They must have ample water during the dry season. The best way to retain moisture in a tree tomato plantation is to apply mulch, which also reduces weed growth. Branches of tamarillos are fragile, and brittle, and break easily when laden with fruit so windbreaks should be established before the actual planting of the fruit plants in an area where wind may be a problem. Further, these plants have a shallow root system and can be blown over by strong winds if not protected sufficiently. Hailstones can also damage the leaves and break the brittle branches. However, damage to fruit is not as severe as in other fruit crops due to their thick skin and strong attachment to the plant.

1.10. PROPAGATION AND ROOTSTOCK

The plant is mostly multiplied through seeds, cuttings, or grafting as well as more contemporary methods of in vitro clonal propagation, which include axillary branch proliferation, organogenesis, and somatic embryogenesis and are commonly referred to as micropropagation. Seed propagation is simple and best done in protected areas. Seeds produce a high-branched, erect tree, ideal for sheltered locations. Seeds for planting are first washed, dried in the shade, and then placed in a freezer for 24 hours to accelerate germination. They are then planted in boxes of rich soil by keeping a

30 cm distance between seeds and 60 cm between rows and virtually 100% will germinate in 4–6 days. Seedlings should be kept in the nursery until they reach a height of 1–1.5 m for efficient growth. Cuttings should be taken from healthy plants that are free from pathogenic viruses from the basal or aerial suckers and should be of 1- to 2-year-old wood with thickness ranging from 10 to 25 mm and length of 45–75 cm. They are planted directly in the field until they reach a height of 1–1.5 m. Cuttings develop into a shorter, bushy plant with low-lying branches, suitable for exposed, windy sites. Tamarillo can be grafted on several closely related rootstock species. In Java, *Cyphomandra costaricensis* is sometimes used as a rootstock to attain a longer-lived plant. The cuttings can also be grafted on wild tobacco trees (*Solanum mauritianum*). The Solanaceae family, which includes many tamarillo cousins, has strong and frequently undesirable alkaloids that can transmit to scions and into fruits grafted on such roots. This is important to remember. It is not advisable to integrate tamarillo on an unknown or untested rootstock.

Propagation through seeds is not recommended as this method is known to produce a high degree of genetic variability that negatively affects fruit color resulting in the rejection of fruits in the international market. Thus, methods of vegetative propagation are usually used to obtain uniform plants. Vegetative propagation by cuttings has been found to transmit deadly viral diseases. Tissue culture offers a feasible solution to produce large numbers of disease-free planting materials. It is also known that in vitro propagated Tamarillo plants produce higher yields and shorter gestation periods compared with traditional methods. Another advantage offered by tissue culture methods is the large-scale availability of planting materials at any time of the year irrespective of the season.

Nodal explants are surface sterilized, added to Murashige and Skoog media containing Benzyl amino purine (BAP), and then incubated in a growth environment with a temperature of 25°C and a 16-h photoperiod. Rooting occurs without the use of an exogenous source of auxin 2 weeks after micro shoot development, and they grow to a height of 40 mm in 4 weeks. After that, the rooted plantlets are brought to the greenhouse to begin weaning. In vitro, propagation of Tamarillo using axillary buds has been reported. Somatic embryogenesis and direct organogenesis have also been reported by. Direct organogenesis has the potential to keep regenerated plants' genomes stable, whereas regeneration through an intermediary callus phase raises

the probability of somaclonal changes. A very effective and repeatable in vitro regeneration technique is moreover an absolute requirement for creating transgenic plants.

1.11. LAYOUT AND PLANTING

The seedlings are transplanted in the field when they reach 5–7 cm in height, spaced 80 cm apart in rows and 2 m apart between rows. In New Zealand, the trees are kept 2.5–3 m apart in paired rows 2.5 m apart with 4.25 m between each pair. In mechanized production, single-row planting distances of 1–1.5 m between plants and 4.5–5 m between rows are recommended. In unprotected areas where winds are strong closer planting is recommended by keeping 1.5–1.8 m between the plants and 2.5–3 m between the rows and the trees may be staked to prevent swaying and disturbing of roots. In India, the trees are planted in pits 1.2–1.5 m apart. On poorly drained soils, plants should be planted on ridges. In poor soils or soils where nematode infestations or virus infections are possibly prevalent, replanting with clean stock will be necessary and is recommended after a period of 3 or 4 years.

1.12. IRRIGATION

To maximize and stabilize production, water and nutrient inputs should be provided when needed. The plants need a continuous supply of water due to their shallow root system. Drought stress results in a decrease in plant growth, fruit size, and productivity. The tree tomato cannot tolerate prolonged drought and must have an ample water supply during extremely dry periods. A mulch may be very beneficial in conserving moisture at such times.

1.13. NUTRIENT MANAGEMENT

The suggested fertilizer rates per hectare for intensive orchard production techniques are 170 kg of nitrogen, 45 kg of phosphorus, and 130–190 kg of potassium. While potassium and phosphorus are sprayed at the beginning of the growth season, nitrogen is applied all year round. It is recommended to apply 0.25–1.0 kg of NPK fertilizer per tree evenly between early spring and summertime. The producer should give the plants in their fifth or sixth year a special feeding of 2 parts superphosphate, 1 1/2 parts nitrate of soda, and 1 part sulfite of potash in late winter or early spring at a rate of 1–1.5 kg per plant or 100 kg per hectare.

1.14. TRAINING AND PRUNING

The plant should be trained by pruning back long shoots and pinching shoot tips to induce compact growth and the production of the fruit clusters near the center of the tree. Seedling trees are pruned back the first year after planting to a height of 0.9–1.2 m to encourage branching. Annual pruning thereafter is advisable to eliminate branches that have already fruited and induce ample new shoots close to the main branches since branches that have already carried fruits will produce smaller fruits with lower quality the next time. Annual pruning is also a must otherwise, the tree will develop a broad top with fruits only on the outer fringe, and wide-spreading branches are also subjected to wind damage. Pruning also facilitates harvesting and if done timely and appropriately can extend the total fruiting period. Light pruning facilitates the production of medium-sized fruits and heavy pruning to large-sized fruits. Under protected cultivation pruning can be helpful to prevent excessive vegetative growth. Early spring pruning of some trees brings about early maturity and fall pruning of other trees delays fruit maturity to the following fall. It is best to cut the roots on one side of the tree and lean it to the other when it is between one and one and a half meters tall (in the direction of the midday sun at about 30–45 degrees). As a result, fruiting branches can develop along the entire trunk as opposed to simply at the top.

1.15. INTERCROPPING AND INTERCULTURE

In plantations, tamarillo is frequently intercropped with citrus so that, for the first 4–6 years of its rapid growth to a productive size, it can be produced economically. Thereafter, the tamarillo plants are removed. Mulching can aid in preserving soil moisture because plants are susceptible to drought stress. As traditional soil management techniques like plowing are impractical due to the thin and delicate root system, it can also be used as a tactic to control weeds. Because of the shallow root system, deep cultivation is not possible, but light cultivation is desirable to eliminate weeds until there is sufficient vegetative growth to shade them out. The plants have to be protected from wind. Their shallow root system does not provide enough stability and the lateral branches are fragile and break easily when carrying fruits. The tree has to be protected from the wind because its roots are weak and it can easily be blown over. It is also fragile, and strong winds can easily break its branches, especially when they are heavy with fruit. Before laying out the plantation, it is advised that windbreaks

be built to protect the young plants for each 1/2 acre (1/5 hectare). In the New Zealand region of North Auckland, *Albizia lophantha* Benth. and *Hakea saligna* hedges are maintained clipped, narrow, and well-liked.

1.16. FLOWERING AND FRUIT SET

Tamarillo shows sympodial growth habit and each sympodial unit consists of 3–4 leaves and a terminal inflorescence. New season's growth begins in October with shoots that originate in the axils of the previous season's leaf scars. Before inflorescence commencement and branching, shoot extension advances up to 15 nodes at the start of the growth season. At the tip of the stalk, inflorescences develop, and later, continuation shoots develop, typically in the axils of the two most recent leaves. Monochasial and trichasial branches are also seen, despite dichasial branching being prominent. Although they begin at the shoot apex, inflorescences can eventually be found 30–40 mm above the branch fork. At maturity, the flowers and inflorescences hang pendulously, with the anthers and stigma facing downward. Typically, the inflorescence is a mono- or dichasial with flowers arranged alternately along the rachises. Up to 15 flowers per rachis and 50 in total could be seen in each inflorescence. At 2–3-day intervals, flowers open in an acropetal succession. As a result, the same inflorescence may contain fruits, flowers, and flower buds. Particularly if a fruit had already set inside the inflorescence, apical flower buds on each rachis frequently abscise before flower opening. Inflorescences formed late in the season also lose their immature flower buds, and occasionally just one or two blooms fully open.

In climates with little annual variation, tamarillo trees can flower and set fruit throughout the year. In climates with pronounced seasons (such as New Zealand), fruits ripen in autumn. The flower is pentamerous, radially symmetrical, stellate and hermaphrodite, and 24 mm in diameter when the petals are fully reflexed. The petals have a variety of colors, ranging from white to pale pink to white with purple flecks, and are relatively lengthy and meaty. Each stamen is united to a petal and forms a cone around the style. The stamens have two anther sacs on either side of a wide, yellow connective tissue, as well as a short filament and an anther. The style extends 2–4 mm beyond the anther cone and is long and thin. Small, flat, and papillate describe the stigma. Pollen is formed in large quantities in anthers (800,000 pollen). Pollen grains in their

desiccated state are oval and trilobed with a pitted exine bearing numerous fine spines. Individual flowers generally open before midday and the petals close around the anthers and style again in the evening. The following morning, each flower bloomed once more, and this pattern persisted for another 2–3 days before the petals fully closed. The number of days a flower is open has an impact on pollination. Nearly a day earlier than unpollinated blooms, pollinated flowers closed for the last time on average after 2.25 days (3.05 days). Either at the flower opening or right before it, an anther opens. Pollen is not released spontaneously but anthers release a cloud of pollen via the apical pore if touched or squeezed. If the anthers are not disturbed the pollens remain in the anthers beyond final flower closure. All flowers bear stigmatic exudate on the day of flower opening and remain present on the stigma until Day 5, even though the petals have closed. On the stigma, pollen grains and tubes can be seen from days three to four, but their abundance is greatest on days zero and two. On Day 0, pollen grain germination is at its highest, and it gradually decreases over the following days. On any given day, there are observed to be comparable numbers of pollen tubes in the style and pollen grains on the stigma. After 24 h, pollen tubes are about two-thirds of the way down the style, and after 48 h, they are present surrounding the ovules, according to observations of the pollen tube growth rate.

It is self-compatible and usually autogamous, but the flowers need to be shaken by the wind or visited by insects for pollination to take place. If grown in conditions where flower vibration is limited, such as in a greenhouse, fruit set can be very low. Flowers pollinated on Days –3-1 had the highest probability of fruit set. Fruit set then declined sharply until no fruit set on flowers pollinated on Day 4. Fruit set is strongly influenced by treatments designed to modify the pollen source and the vector. On average, two fruit sets in open-pollinated inflorescences and 0.6 in inflorescences bagged to exclude external sources of pollen have been found. The flower pollination is carried out by honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris* or *B. hortorum*). Although fruit set is lower in both cases than in open-pollinated flowers, the quantity of fruits set per inflorescence is unaffected by the pollen source, whether “self” or “cross” (by hand pollination). Self-pollinated or cross-pollinated flowers produce between 0.7 and 0.9 fruit sets per inflorescence on average, as opposed to 2.0 from open-pollinated blooms.

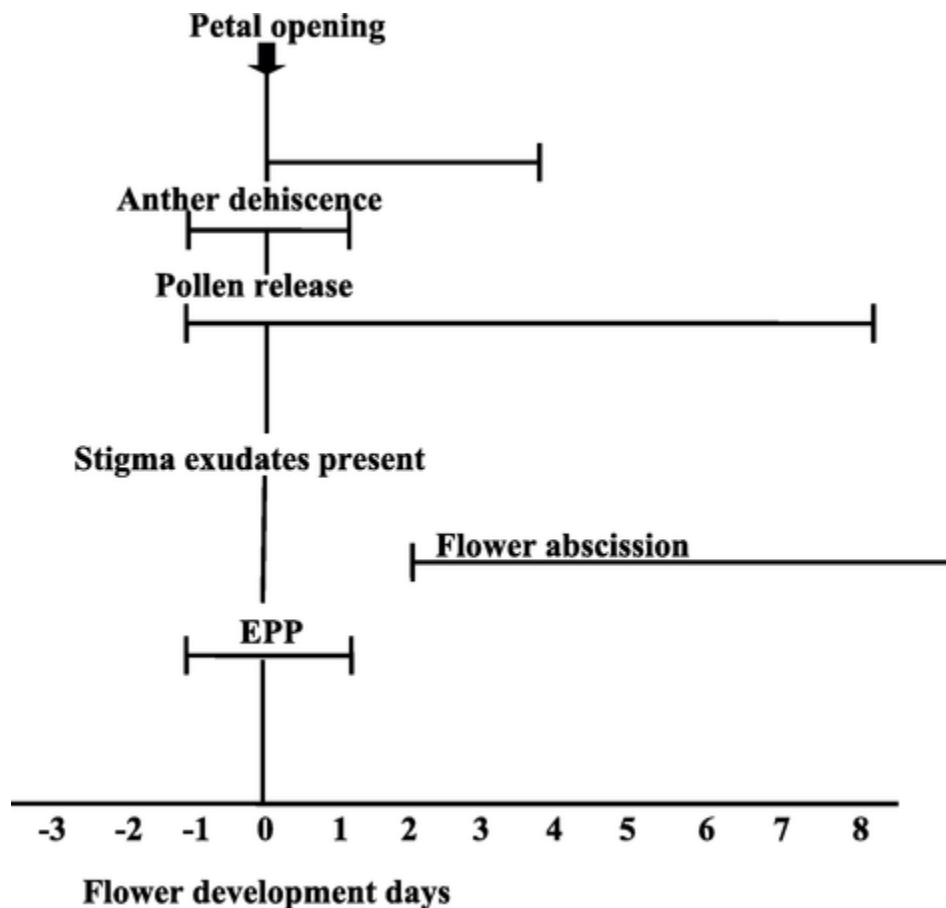


Figure 2.

Floral phenology of the tamarillo flower based on observations from 20 flowers grown under glasshouse conditions (EPP—effective pollination period) [73].

1.17. FRUIT GROWTH DEVELOPMENT AND RIPENING

Fruit growth and development involves changes in its morphology, anatomy, and physiology whilst fruit ripening is associated with dramatic changes in rind texture, color, juice composition, increase in softness due to changes in the cell walls, the metabolism of organic acids, and the development of compounds involved in flavor and taste. During development, it follows a simple sigmoid growth curve and during ripening it behaves as a non-climacteric fruit. Fruit growth shows an increase of fresh weight (and volume) fast and linear between the sixth and sixteenth week after this period the growth ceases. However, the weight dryness of the fruit continues to increase until reaching a maximum in the twentieth week after anthesis. Tamarillos are commercially mature at 21–24 weeks after anthesis. Maturity is indicated by color, firmness, juice content, and soluble solids content. Heatherbell *et al.* reported that

tamarillo fruits grew rapidly and reached full size within 16 weeks after anthesis and maturity is attained 11 weeks later i.e., at about 27 weeks. Marked changes in the skin and pulp color occur during development. After 15 weeks the first purple coloration of the skin appears and thereafter increases in intensity. The green chlorophyll underlay disappears from the fruits between 18 and 22 weeks. Tamarillo is a non-climacteric fruit; therefore, it does not exhibit an adequate self-stimulated increase in ethylene production and a consequent respiratory increase as part of its ripening behavior . Pratt and harvested red and yellow tamarillos from 5 to 23 weeks of age and they monitored their respiration rate, demonstrating that the respiratory rate decreases as the age of the fruit increases. The ripe fruits presented a relatively high respiratory rate immediately after harvest ($35 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at 20°C), which then decreased gently until the beginning of the senescence. In this study, the ethylene production of the fruits harvested was measured and it was shown to be negligible length of fruit development (less than $0.10 \text{ } \mu\text{l kg}^{-1} \text{ h}^{-1}$ at 20°C) until the beginning of the senescence, when it increases abruptly together with the respiratory rate.

Tamarillos of red cultivars remain green until the fruit grows around the sixteenth week after anthesis. From that moment, it begins to appear a violet color at the apical end subsequently, it will spread over the whole fruit. Around the nineteenth week after anthesis, the green base color begins to turn yellow and the apparent color purple is revealed to be red. Violaceous coloring of the tissue surrounding the seeds is evident around the 12th week after anthesis and intensifies until the Twenty-first week when it begins to decrease in intensity again. The fruits of yellow cultivars present a pattern of similar color change, except that the red coloration is very slight, resulting finally in a light orange color, and without that red pigmentation appears around the seeds. Changes in skin color are due to the degradation of chlorophyll and an increase in the concentration of anthocyanins and carotenes. However, the increase in the concentration of anthocyanins in the skin is very small in the 4–6 weeks before commercial maturity. The concentration of anthocyanins, carotenes, and chlorophylls from the tissue that surrounds the seeds is maximum when the fruit is in the violet state and then decreases.

The immature fruits have a high Starch content (14% of fresh weight) that, before the end of the stage of active growth, begins to decrease until reaches less than 1% in ripe fruits. The decrease in starch content is accompanied by an increase in the

concentration of soluble solids and sugars. Sucrose is the predominant sugar in tamarillos. The citric and the malic are the organic acids predominant in tamarillo throughout the fruit development. The concentration of citric acid increases rapidly during the period of active fruit growth, reaching more than 2% of the fresh weight; the decreases up to 1.4% in mature fruits. Malic acid concentration is relatively low during the entire period of fruit development (0.2% of fresh weight in mature fruits). The pectin content decreases throughout the fruit development. The lysis of unions between pectin and hemicelluloses increases the solubilization of pectins during ripening. Tamarillo's soluble solid content appears to rise to 10–12°B during ripening (normally, the values range between 10.0 and 13.5°B), while the titratable acidity gradually declines (typically, the values range between 1.0 and 2.4%), increasing the SSC/TA ratio and, as a result, the sensory flavor rating. The stems also change when the fruit ripens, turning yellow instead of green as a result of increased water loss and chlorophyll degradation. Further, according to an SSC value over 12% can qualify tamarillo either for raw consumption or industrial processing.

1.18. FRUIT RETENTION AND FRUIT DROP

Self-pollination occurs naturally in tree tomato blossoms. Unless there are bees to transmit the pollen, pollination may suffer if the wind is fully stopped so as not to move the branches. Flowers that have not been pollinated will wilt quickly.

1.19. HARVESTING AND YIELD

The tree typically starts bearing when it is 1 1/2 to 2 years old and keeps producing for another 5 or 6 years. It may continue to bear fruit for 11–12 years if given appropriate nutrition. The crop does not ripen simultaneously and several pickings are necessary. Tamarillos are picked when fully colored. The optimum time to harvest the tamarillos of red cultivars is when they are violet in color. Other recommended indices to determine the maturity of the fruit with greater accuracy are the firmness, the content of juice, and the content of soluble solids. The color of the pedicel in combination with the skin color was also suggested as the Maturity index. Fruit can also be picked when they are at the turning stage (when the green color of the skin begins to change and the characteristic skin color begins to show) and then treated with ethylene to stimulate ripening. This early picking and subsequent postharvest ripening reduce the risk of crop failure, increases earliness and concentrates harvesting as it allows harvesting

to be advanced by up to 1 month The fruits are clipped, leaving about 12.5 cm of stem attached. They are collected in bags worn by the harvesters. A single tree can produce more than 30 kg of fruits per year. A regular orchard yields 15–17 tons per hectare.

1.20. PROTECTED/HIGH TECH CULTIVATION

Tamarillos are suitable for growing as indoor container plants, though their swift growth, their light, water, and humidity requirements, and their large leaves can pose a challenge within a limited space. Plants grown in the greenhouse are heavily pruned to prevent excessive vegetative growth. Greenhouse tamarillo production can be somewhat inconvenient due to its long life and extended growing cycle .If grown in conditions where flower vibration is limited, such as in a greenhouse, fruit set can be very low .

1.21. DISEASE, PEST, AND PHYSIOLOGICAL DISORDERS

1.21.1 Diseases

1.21.1.1 Powdery mildew and leaf spot

If left unchecked, this fungus can produce a significant amount of defoliation. It is possible to remove powdery mildew by using commercial pesticide washes and neem oil sprays (*Oidium* sp.).

1.21.1.2 Bacterial blast

It is caused by *Pseudomonas syringae* and *P. solanacearum*. It affects the shoot and leaves. Anthracnose (*Colletotrichum gloeosporioides*).

1.21.1.3 Anthracnose (Colletotrichum gloeosporioides)

The damage it inflicts is immense. Good orchard management can help lower the risk of pest and disease control in markets that demand immaculate fruit. As long as the nursery is kept clean, predator insects can reduce the need for chemical pesticides.

1.21.2 Viral diseases

1.21.2.1 Tamarillo mosaic virus (TaMV)

TaMV symptoms include a mosaic mottling on the leaf and ugly irregular spots on the fruit skin that are a darker red than the cultivar's typical skin color. Inside the fruit, there are no visible symptoms, and the eating quality is unaffected. Due to the milder

background color typical of this variety of fruit, the darker red splotch on golden tamarillos is particularly ugly. The plants grow stunted as a result. TaMV can only be controlled after symptoms occur by removing trees that are seriously infested .

1.21.2.2 Cucumber mosaic virus and potato virus Y

These not only diminish the plant's strength and health but also distort the leaves, produce skin blemishes, and lower the plant's marketability. They result in fruit mottling and yield loss. The severity of the symptoms will be greater if multiple viruses have simultaneously attacked the plant and will be worse on young or sickly plants. In the valleys of Ecuador's Pichincha region, PLRV, ToRSV, PVY, and AMV were the most often discovered viruses linked to symptoms of viral diseases . Alfalfa mosaic virus (AIMV), tomato spotted wilt virus (TSWV), Arabis mosaic virus (ArMV), tobacco streak virus (TSV), and tomato aspermy virus (TAV) are other viruses that have affected the tamarillo, albeit the losses they cause are not as severe as those produced by TaMV . The Colombian tamarillo crop's declining yield is caused by a virus complex. The phytosanitary problem, among which anthracnose (*Colletotrichum acutatum*) and viruses (PLCR, CMV, Potyvirus, and ToMV) and others, apparently less influential (AMV, ToRSV, and TSWV) has a major effect on expansion plans of the tamarillo cultivation in Colombia . Good orchard hygiene, pruning and burying infected plants, and a good pest management program will help to reduce pests and diseases in the crop. A suitable spraying schedule can also be helpful. However, there is no cure for a virus once it has infected a plant; the only option is prevention. Since aphids are the primary transmitters of viruses, it is crucial to have effective control over them. Since there is no proof that viruses affecting the tamarillo may be propagated by seed, seed-based reproduction is a strategy for restricting their spread.

1.21.3 Nematodes

The plant is also harmed by *Pseudomonas solanacearum* wilt, root-knot (*Meloidogyne* sp.), root rot, crown rot, and other diseases. Good cultural norms ought to help prevent these issues.

1.21.4 Physiological disorders

1.21.4.1 Abnormality

To make jam, small, hard, irregular, semi-transparent stones that are present in the flesh of tree tomatoes must be strained out. If these resemble the two grit-filled bumps in the fruit's wall, that is unknown. Probably in the form of silicates, borates, aluminum-magnesium-oxygen complexes, aluminates, or magnesium oxides, these stones are highly concentrated sources of sodium and calcium.

1.21.4.2 Fruit scarring

Tamarillo fruit scarring is a cosmetic condition that costs New Zealand's tamarillo growers significantly lost revenue. According to estimates, the condition affects 10–20% of the fruit, which will result in considerable economic losses. When the scars are about 3 cm long, they first appear as little, dark lines on the skin of young fruit. As the fruit grows, the scars turn into corky lesions. Phillips *et al.* reported that a physical injury caused the scarring. A dark, uneven, and crazy corky scar was routinely produced when the epidermal layer was scratched with a toothbrush. Physical injury such as wind rub has been reported to cause corky scarring in other fruits also, such as avocado . Wounding fruitless by scratching or removing a patch of epidermis resulted in the characteristic scarring, which suggests that any type of physical epidermal damage incurred early in fruit development may result in scarring.

1.22. PACKAGE AND TRANSPORT

Fruits are sorted by size as small, medium, and large and packed in paper-lined wooden boxes for marketing. Polythene films can also be used to reduce water loss and maintain fruit quality. Packaging usually consists of wooden or cardboard boxes containing one tray of tamarillos or preformed plastic trays with 3–8 fruits. Because of its firm flesh and tough skin, the fruit can be transported over long distances without bruising. However, it deteriorates rather rapidly under ordinary storage conditions.

1.23. POSTHARVEST HANDLING AND STORAGE

Different studies carried out to improve post-harvesting ripeness showed that the application of ethylene or ethephon helps decrease the risk of crop failure and an earlier delivery to the consumer, thereby enhancing the marketability of tamarillo The fragile lateral branches can break easily when loaded with fruits, so premature harvest helps to reduce this risk and allows storage of fruits for up to 20 days at room temperature. Tamarillos can be stored for about 12–14 weeks at 3.5–4.5°C. A cold

water dipping process, developed by the New Zealand Department of Scientific and Industrial Research also allows further storage of 6–10 weeks. At higher temperatures, postharvest diseases multiply rapidly. One of the main causes of postharvest loss is bitter rot caused by a *Colletotrichum* sp. Applications of postharvest fungicides greatly reduce the number of fruits affected. Another alternative for postharvest disease control is to dip the fruit in water at 5°C for 10 min. Temperatures below 7°C will reduce softening, weight loss, TA reduction, and color change, among other postharvest handling characteristics that might impact quality. On the other hand, extremely low temperatures (between 0 and 2°C) raise the danger of chilling injury and worsen stem and calyx discoloration.

1.24. PROCESSING AND VALUE ADDITION

Pomegranate can be used in both savory and sweet dishes, and it can even be eaten raw. Fruits can be used to make preserves because of their high pectin content. However, if they are left untreated, they will oxidize and eventually fade. The yellow fruit is preferred for industrial production. As it turns out, it can be utilized in the same way as tomatoes are used in many other cuisines. Simply slice the tomatoes in half, sprinkle them with sugar, then consume the pulp and flesh by scooping it out. Chopped bacon and cream cheese go on top of sandwiches. Diced fruits, bread crumbs, butter, and seasonings are used as a stuffing for roast lamb. Dessert in the form of a pie sliced tree tomatoes, either on their own or accompanied by an apple, are available. They may be filled with water or sugar syrup, then packed into plastic containers with 50% sugar syrup and quickly frozen for later use as pie fillings or puddings. In a blender or on the stove, puree the peeled fruits, remove the seeds, and freeze in containers. Before blending, add some lemon juice to the purée to enhance the flavor even further. Several slices of fresh tree tomato are placed on top of the gelatin, milk, sugar, and lemon juice-cooked fruits. Cooking tree tomatoes with sugar, lemon zest, and juice results in a jam or chutney; the tomatoes can also be cooked with onions and apples for chutney, depending on the desired consistency. Chutney for the commercial market is made in an Auckland, New Zealand, facility. Pectin in the fruit makes it simple to turn into jelly, but the fruit quickly oxidizes and discolors if not treated further. Using whole, peeled fruits and sugar, a thick sauce is cooked to serve with ice cream. The peeled fruits can be used for tomatoes in tomato sauce. Tamarillo jelly can be made from tomatoes with a 50°C Brix sugar concentration, sucrose, and 2% pectin.

CHAPTER 2

LITERATURE SURVEY

Tamarillo is a unique fruit with a distinctive flavor and an interesting history. Despite being native to South America, it is very popular in New Zealand. Tamarillo thrives in a subtropical climate but can also be grown in areas where citrus crops are cultivated. While it is grown in various countries, commercial cultivation is mainly in New Zealand and some parts of South America. It does not typically bear fruit in low-lying tropical regions. The plant grows rapidly and produces fruit in approximately 18 months, reaching a height of about two meters and living for around seven years. Propagation is commonly done using seeds or cuttings, with trimming techniques varying depending on the method used. Tamarillo plants are sensitive to wind and should be planted in sheltered areas or protected by windbreaks. The fruit has a glossy skin and is rich in vitamins, minerals, fiber, and antioxidants, with a low-calorie content. It can be consumed fresh or used to make juices, concentrates, jams, gelatins, and desserts. If processing facilities and suitable transportation are available, tamarillo can be exported as pulp or concentrate. This fruit offers an opportunity to diversify fruit production in subtropical regions, as high-quality fruits can fetch premium prices in niche markets in Europe, North America, and Japan.

Tamarillo is primarily cultivated for its edible fruits and is also grown as a decorative plant outdoors to a lesser degree. The tamarillo fruit has been described as noticeably beautiful and its scent as unique and appealing. It is versatile in the kitchen and can be enjoyed raw in salads or desserts, but it is usually preferred cooked. The flesh of the tamarillo can be consumed fresh or used to create a variety of sweet and savory dishes and condiments. Tamarillos have a tangy and usually sweet flavor with a bold and intricate taste that varies depending on the variety. The fruit can be stewed for use in cereals, pies, or crumbles, added to stews, or turned into a tasty chutney, particularly good with a hint of chili. In Jamaica and the West Indies, the fruit is believed to have positive effects in the treatment of liver disorders. Tamarillo plays a significant role in Rwanda's exotic fruit industry, and the fruit is traditionally recommended for individuals with stomach ailments. It is processed into jams, juices, jellies, or canned syrup, and is also used in combination with dairy products like yogurt, milkshakes, and ice cream. The fruit contains a high level of pectin, making it

especially suitable for making jams and preserves. It is also utilized for canning in syrup and for producing pulp, chutney, sauce, baby food, and in combination with dairy products. The fruit can be used similarly to a regular tomato, but it has less moisture, so more liquid is needed in most cooked dishes.

"This fruit is rich in antioxidants and vitamins A, C, B6, and E. Tamarind has high levels of vitamins A and C compared to other fruits (Table 2). Its ascorbic acid content is similar to citrus fruits, while its vitamin A levels fall between that of tomatoes and carrots. The color of tamarind comes from its high levels of carotenoids and anthocyanins, which also contribute to its biological, medicinal, and preventive properties. Researchers like Osorio et al. have found that tamarillo fruits are valuable due to their natural pigments with antioxidant potential, confirmed by spectroscopic analyses. The primary antioxidants in the fruit pulp are phenols. Both the fruit flesh and seeds are consumed together, with seeds making up one-third to one-half of the fruit's weight (50-80 g). An analysis of tamarillo seeds showed a total carbohydrate content of 43.87 percent, protein content of 22.63 percent, fat content of 21.13 percent, and ash content of 3.15 percent. The most common fatty acids in tamarind seed oil are linoleic acid (70.47 percent), oleic acid (14.93 percent), palmitic acid (9.41 percent), stearic acid (2.23 percent), and linolenic acid (1.73 percent). In addition, lignoceric acid (0.23 percent), phellonic acid (0.22 percent), and arachidic acid (0.23 percent) are also present in the oil. Tamarind fruits are low in calories."

The growth and development of fruits involve changes in their morphology, anatomy, and physiology, while the ripening of fruits is characterized by significant changes in texture, color, juice composition, softness, organic acids metabolism, and the development of compounds responsible for flavor and taste. A simple sigmoid growth curve is typically followed during development, while ripening is non-climacteric. An increase in fresh weight and volume occurs rapidly and linearly between the sixth and sixteenth week before growth ceases, although the dry weight of the fruit continues to increase until reaching a maximum in the twentieth week after anthesis. Tamarillos are considered commercially mature at 21-24 weeks after anthesis, indicated by color, firmness, juice content, and soluble solids content. Changes in skin and pulp color occur as the fruit develops, with purple coloration appearing on the skin around 15 weeks and the disappearance of green chlorophyll between 18 and 22 weeks. Tamarillos are non-climacteric fruits, showing a decrease in respiratory rate as they

age. Ethylene production in these fruits remains low until the onset of senescence when it abruptly increases along with the respiratory rate.

Fruits of red cultivars remain green until around the sixteenth week after anthesis when a violet color starts at the apical end before spreading throughout the fruit. In the nineteenth week, the green base color turns yellow, and the violet color transitions to red. Fruits of yellow cultivars undergo a similar color change pattern, ending in a light orange color without the red pigmentation around the seeds. Skin color changes are due to chlorophyll degradation and an increase in anthocyanins and carotenes. The concentration of anthocyanins in the skin peaks during the violet state and decreases thereafter.

Immature fruits have a high starch content that decreases to less than 1% in ripe fruits, accompanied by an increase in soluble solids and sugars. Citric acid is the primary organic acid in tamarillos, increasing during active growth but decreasing in mature fruits. Malic acid levels remain relatively low throughout fruit development. Pectin content decreases during fruit development, resulting in increased pectin solubilization during ripening. The soluble solids content of tamarillos typically reaches 10-12°B during ripening, while titratable acidity declines, leading to an increase in the SSC/TA ratio and improved sensory flavor. Stems also change color when fruits ripen, turning yellow due to water loss and chlorophyll degradation. An SSC value above 12% qualifies tamarillos for raw consumption or industrial processing.

2.1.HARVESTING AND PRODUCTION

The tree usually begins to bear fruit when it is 1 1/2 to 2 years old and continues to produce for another 5 or 6 years. With proper nutrition, it may bear fruit for up to 11-12 years. Since the crop does not all ripen at once, multiple harvests are necessary. Tamarillos are harvested when they are fully colored, with red cultivars being best picked when they are violet in color. Other indicators of fruit maturity include firmness, juice content, and soluble solids. The color of the stem in combination with the skin color has also been suggested as a maturity indicator. Fruit can be harvested when they are starting to change color and then treated with ethylene to promote ripening. This early picking and postharvest ripening reduces the risk of crop failure, increases early availability, and concentrates harvesting, allowing for harvest to be advanced by up to 1 month. Fruits are cut with a stem of about 12.5 cm attached and collected in

bags. A single tree can yield over 30 kg of fruit per year, while a regular orchard can produce 15-17 tons per hectare.

2.2.ORIGINS AND CULTIVATION OF TAMARILLO

Tamarillo has a rich history rooted in South and Central American cultures. Indigenous peoples of these regions have long utilized the fruit for its nutritional properties and medicinal benefits. Tracing the historical cultivation and consumption of tamarillo provides insights into its cultural significance and traditional uses.

2.3.CULTIVATION PRACTICES

The cultivation of tamarillo involves specific agricultural practices tailored to its tropical origins. From soil requirements to climate considerations, understanding the optimal conditions for tamarillo cultivation is essential for maximizing yield and quality. Additionally, exploring modern cultivation techniques and challenges faced by tamarillo growers sheds light on the fruit's commercial production.

2.4.MACRO-NUTRIENT COMPOSITION OF TAMARILLO

2.4.1.Carbohydrates

Tamarillo is predominantly composed of carbohydrates, with simple sugars such as glucose and fructose being the primary constituents. Analyzing the carbohydrate profile of tamarillo elucidates its role as a source of energy and its implications for blood sugar regulation.

2.4.2.Proteins

While tamarillo is not a significant source of protein, it still contains essential amino acids necessary for various physiological functions. Examining the amino acid composition of tamarillo provides insights into its nutritional value and potential contributions to dietary protein intake.

2.4.3.Fats

Tamarillo contains minimal amounts of fat, primarily in the form of healthy unsaturated fats such as omega-3 and omega-6 fatty acids. Investigating the lipid profile of tamarillo highlights its role in promoting heart health and overall well-being.

2.4.4.Fiber

One of the standout features of tamarillo is its high fiber content, which includes both soluble and insoluble fiber. Exploring the fiber content and composition of tamarillo underscores its importance for digestive health, satiety, and blood cholesterol management.

2.5.NUTRITIONAL VALUE OF TAMARILLO

2.5.1.Vitamins

Tamarillo is rich in various vitamins, particularly vitamin C and vitamin A. Analyzing the vitamin content of tamarillo reveals its antioxidant properties and potential benefits for immune function, skin health, and vision.

2.5.2.Minerals

In addition to vitamins, tamarillo contains essential minerals such as potassium, magnesium, phosphorus, and calcium. Investigating the mineral composition of tamarillo elucidates its role in supporting electrolyte balance, bone health, and muscle function.

2.6.HEALTH BENEFITS OF TAMARILLO

The antioxidant properties of tamarillo play a crucial role in protecting against oxidative stress and inflammation. Exploring the mechanisms of antioxidant action in tamarillo provides insights into its potential role in preventing chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders.

2.6.1.Heart Health

Tamarillo's fiber, potassium, and antioxidant content contribute to its cardiovascular benefits. Examining the impact of tamarillo consumption on cholesterol levels, blood pressure regulation, and arterial health sheds light on its role in promoting heart health and reducing the risk of cardiovascular disease.

2.6.2.Digestive Health

The high fiber content of tamarillo makes it particularly beneficial for digestive health. Investigating the effects of tamarillo fiber on bowel regularity, gut microbiota

composition, and digestive disorders provides a comprehensive understanding of its digestive health benefits.

2.6.3.Weight Management

Tamarillo's fiber content and low-calorie density make it a valuable addition to weight management diets. Exploring the satiety-promoting effects of tamarillo fiber and its potential role in reducing calorie intake and promoting weight loss offers insights into its practical applications for individuals seeking to manage their weight.

2.6.4.Immune Support

The vitamin C content of tamarillo plays a crucial role in supporting immune function. Investigating the mechanisms of vitamin C action in tamarillo and its potential benefits for immune response modulation provides a scientific basis for its traditional use as an immune-boosting fruit.

2.7.CULINARY VERSATILITY OF TAMARILLO

2.7.1.Traditional Uses

Tamarillo is a versatile ingredient in various culinary traditions, from savory dishes to sweet treats. Exploring traditional recipes and cooking methods involving tamarillo offers insights into its cultural significance and culinary applications.

2.7.2.Modern Culinary Trends

In addition to traditional uses, tamarillo is gaining popularity in modern cuisine, particularly in gourmet cooking and health-conscious recipes. Investigating innovative tamarillo recipes and culinary trends highlights its potential for culinary creativity and culinary tourism.

2.7.3.Nutritional Considerations in Cooking

Understanding the impact of cooking methods on the nutritional composition of tamarillo is essential for maximizing its health benefits. Exploring the effects of cooking, processing, and storage on tamarillo nutrients offers practical guidance for preserving its nutritional value in culinary applications.

Secondary metabolites are organic compounds produced by plants that are not directly involved in their growth, development, or reproduction. Instead, these

compounds often serve various ecological functions, such as defense against herbivores, attraction of pollinators, and resistance to pathogens. Secondary metabolites found in tamarillo encompass a diverse array of chemical classes, including alkaloids, flavonoids, phenolic compounds, terpenoids, and glycoalkaloids. Each class of secondary metabolites in tamarillo contributes to its unique flavor, aroma, color, and potential bioactivity.

Alkaloids are nitrogen-containing compounds known for their diverse pharmacological properties. In tamarillo, alkaloids such as solanidine and tomatidine have been identified. These alkaloids exhibit cytotoxic effects against cancer cells and may also possess anti-inflammatory and antioxidant properties. However, their concentrations in tamarillo are relatively low compared to other alkaloid-rich plants within the Solanaceae family.

Flavonoids are a class of polyphenolic compounds that contribute to the pigmentation of fruits and flowers. Tamarillo contains various flavonoids, including quercetin, kaempferol, and rutin. These flavonoids possess antioxidant activity, which helps protect cells from oxidative damage caused by free radicals. Additionally, they exhibit anti-inflammatory, antimicrobial, and cardiovascular protective effects, making them beneficial for human health.

Phenolic compounds are another group of antioxidants present in tamarillo. These include phenolic acids such as chlorogenic acid and caffeic acid, as well as other polyphenols like resveratrol. Phenolic compounds contribute to the fruit's bitterness and astringency while offering potential health benefits such as reducing the risk of chronic diseases like cancer, diabetes, and cardiovascular disorders.

Terpenoids are a diverse class of compounds synthesized from isoprene units and are abundant in plants. Tamarillo contains terpenoids such as β -carotene, lycopene, and phytosterols. β -carotene is a precursor of vitamin A and is essential for vision, immune function, and skin health. Lycopene is a red pigment with potent antioxidant properties, known for its potential role in reducing the risk of prostate cancer and cardiovascular disease. Phytosterols, structurally similar to cholesterol, help lower blood cholesterol levels and may reduce the risk of heart disease.

Glycoalkaloids are nitrogen-containing compounds found predominantly in the Solanaceae family, known for their bitter taste and potential toxicity. Tamarillo contains

glycoalkaloids such as solanine and tomatine, albeit in lower concentrations compared to other Solanaceae species like potatoes and tomatoes. These compounds serve as natural defenses against pests and pathogens but may pose health risks if consumed in large amounts.

In addition to their potential health benefits, secondary metabolites from tamarillo have attracted interest for their industrial applications. For example, the pigments derived from tamarillo, such as lycopene and β -carotene, are used as natural colorants in food and cosmetic products. The antioxidant properties of tamarillo extracts make them valuable ingredients in dietary supplements and functional foods aimed at promoting health and wellness.

The extraction and purification of secondary metabolites from tamarillo involve various techniques such as solvent extraction, chromatography, and spectroscopy. These methods allow researchers to isolate specific compounds of interest and evaluate their bioactivity and potential applications. Furthermore, advances in biotechnology offer opportunities to enhance the production of desired secondary metabolites through genetic engineering and metabolic engineering of tamarillo plants or microbial systems.

However, despite the promising health benefits and industrial potential of tamarillo secondary metabolites, further research is needed to fully understand their mechanisms of action, safety profiles, and potential interactions with other compounds. Additionally, efforts to optimize extraction methods, scale up production, and ensure sustainable sourcing of tamarillo resources are essential for maximizing the commercial viability and societal impact of these valuable natural compounds.

Recent scientific research has unveiled the diverse array of bioactive compounds present in tree tomatoes, which contribute to their potential health benefits. These bioactive compounds, collectively known as secondary metabolites, include alkaloids, flavonoids, phenolic compounds, terpenoids, and glycoalkaloids. Accumulating evidence suggests that tree tomato possesses antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, and neuroprotective properties, making it a promising candidate for various therapeutic applications.

This review aims to provide a comprehensive overview of the bioactivity of tree tomatoes, focusing on their phytochemical composition, biological activities,

mechanisms of action, and potential therapeutic applications. Furthermore, it discusses the challenges and opportunities for further research and the development of tree tomato-derived products for nutraceutical, pharmaceutical, and cosmeceutical purposes.

2.8.PHYTOCHEMICAL COMPOSITION OF TREE TOMATO

Alkaloids are nitrogen-containing compounds known for their diverse pharmacological properties. Tree tomatoes contain several alkaloids, including solanidine and tomatidine. These alkaloids exhibit cytotoxic effects against cancer cells and may also possess anti-inflammatory and antioxidant properties (Mhlongo *et al.*, 2018) .

Flavonoids are polyphenolic compounds known for their antioxidant and anti-inflammatory properties. Tree tomatoes contain various flavonoids, including quercetin, kaempferol, and rutin. These flavonoids contribute to the fruit's color and offer potential health benefits such as reducing the risk of chronic diseases like cancer and cardiovascular disorders (Yahia *et al.*, 2018).

Phenolic compounds are antioxidants found in abundance in tree tomatoes. These include phenolic acids such as chlorogenic acid and caffeic acid, as well as other polyphenols like resveratrol. Phenolic compounds contribute to the fruit's bitterness and offer potential health benefits such as reducing oxidative stress and inflammation (Fuentes *et al.*, 2015).

Terpenoids are a diverse class of compounds synthesized from isoprene units. Tree tomatoes contain terpenoids such as β -carotene, lycopene, and phytosterols. These compounds exhibit antioxidant properties and may contribute to the fruit's color and flavor. Additionally, they offer potential health benefits such as reducing the risk of cancer and cardiovascular diseases (Castro *et al.*, 2016).

Glycoalkaloids are nitrogen-containing compounds found predominantly in the Solanaceae family. Tree tomatoes contain glycoalkaloids such as solanine and tomatine, albeit in lower concentrations compared to other Solanaceae species like potatoes and tomatoes. These compounds serve as natural defenses against pests and pathogens (Jiménez *et al.*, 2019)

2.9.BIOLOGICAL ACTIVITIES OF TREE TOMATO

2.9.1.Ant-ioxidant activity

Tree tomato exhibits significant antioxidant activity attributed to its high content of flavonoids, phenolic compounds, and carotenoids. Antioxidants scavenge free radicals and prevent oxidative damage to cells, thereby reducing the risk of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases

2.9.2.Anti-inflammatory Activity

Several studies have demonstrated the anti-inflammatory properties of tree tomato extracts. Flavonoids and phenolic compounds present in tree tomatoes inhibit pro-inflammatory cytokines and enzymes, thereby alleviating inflammation and associated symptoms. These anti-inflammatory effects make tree tomatoes potentially beneficial for conditions such as arthritis and inflammatory bowel diseases (Rodríguez *et al.*, 2011)

2.9.3.Antimicrobial Activity

Tree tomato extracts possess antimicrobial properties against a wide range of pathogenic microorganisms, including bacteria, fungi, and viruses. These antimicrobial effects are attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and phenolic compounds. Tree tomato extracts have shown inhibitory activity against foodborne pathogens and antibiotic-resistant bacteria, highlighting their potential as natural antimicrobial agents (Quispe, C., *et al.* , 2017)

2.9.4.Anticancer Activity

Several studies have investigated the potential anticancer effects of tree tomato extracts against various cancer cell lines. The cytotoxic effects of tree tomato extracts are attributed to their alkaloids, flavonoids, and phenolic compounds, which induce apoptosis (programmed cell death) and inhibit the proliferation of cancer cells. Additionally, tree tomato extracts exhibit anti-angiogenic properties, suppressing the formation of new blood vessels that supply nutrients to tumors .

2.9.5.Cardioprotective Activity

Tree tomato extracts have shown promising cardioprotective effects in preclinical studies. Flavonoids and phenolic compounds present in tree tomatoes exert antioxidant and anti-inflammatory effects, which help prevent cardiovascular diseases

such as atherosclerosis, hypertension, and myocardial infarction. Additionally, tree tomato extracts improve lipid metabolism and endothelial function, contributing to their cardioprotective properties (Hurtado *et al.*, 2014)

2.9.6.Neuroprotective Activity

Emerging evidence suggests that tree tomato extracts possess neuroprotective properties, potentially beneficial for neurological disorders such as Alzheimer's disease and Parkinson's disease. Flavonoids and phenolic compounds present in tree tomatoes exhibit antioxidant and anti-inflammatory effects, which protect neurons from oxidative stress and neuroinflammation. Additionally, tree tomato extracts enhance cognitive function and promote neurogenesis (the formation of new neurons) in preclinical models (Barrera *et al.*, 2016)

2.9.7.Mechanisms of Action

The bioactive compounds present in tree tomatoes exert their therapeutic effects through various mechanisms of action:

Antioxidant compounds scavenge free radicals and inhibit oxidative stress, thereby protecting cells from damage and reducing the risk of chronic diseases.

Anti-inflammatory compounds suppress the production of pro-inflammatory cytokines and enzymes, alleviating inflammation and associated symptoms.

Antimicrobial compounds inhibit the growth and proliferation of pathogenic microorganisms, preventing infections and promoting health.

Anticancer compounds induce apoptosis (programmed cell death) and inhibit the proliferation of cancer cells, thereby suppressing tumor growth and metastasis.

Cardioprotective compounds improve lipid metabolism, endothelial function, and antioxidant defenses, reducing the risk of cardiovascular diseases.

Neuroprotective compounds protect neurons from oxidative stress and neuroinflammation, enhancing cognitive function and promoting neurogenesis.

Given its diverse biological activities, tree tomato holds promise for various therapeutic applications. Some potential uses of tree tomato-derived products include:

Nutraceuticals: Tree tomato extracts can be formulated into dietary supplements or functional foods with antioxidant, anti-inflammatory, and cardioprotective properties. These products may help promote overall health and prevent chronic diseases.

Pharmaceuticals: Bioactive compounds isolated from tree tomatoes can serve as lead compounds for the development of novel pharmaceutical drugs. For example, anticancer compounds may be formulated into chemotherapy drugs, while neuroprotective compounds may be used for the treatment of neurological disorders.

Cosmeceuticals: Tree tomato extracts can be incorporated into skincare products and cosmetics for their antioxidant and anti-aging properties. These products may help reduce oxidative damage to the skin, improve complexion, and prevent premature aging.

Functional Ingredients: Tree tomato extracts can be used as functional ingredients in various food and beverage products to enhance their nutritional value and health benefits. For example, tree tomato juice may be fortified with antioxidants to boost immune function and protect against oxidative stress.

2.10. CHALLENGES AND OPPORTUNITIES

Despite the promising therapeutic potential of tree tomato, several challenges need to be addressed for its widespread utilization:

Standardization: There is a need for standardized extraction and purification methods to ensure consistent quality and bioactivity of tree tomato-derived products.

Safety: The safety profile of tree tomato extracts needs to be thoroughly evaluated, especially regarding potential toxicity and adverse effects.

Bioavailability: The bioavailability of bioactive compounds in tree tomato extracts needs to be optimized to ensure their efficacy in vivo.

Sustainability: Sustainable cultivation practices need to be implemented to ensure the long-term availability of tree tomato resources without harming the environment.

Regulatory Considerations: Regulatory approval and compliance with safety and quality standards are essential for the commercialization of tree tomato-derived products.

Despite these challenges, there are also significant opportunities for further research and development:

Exploring Synergistic Effects: Studying the synergistic interactions among different bioactive compounds in tree tomatoes may lead to the development of more potent and effective therapeutic formulations.

Clinical Trials: Conducting well-designed clinical trials to evaluate the efficacy and safety of tree tomato-derived products in humans is essential for their validation and acceptance in mainstream healthcare.

Personalized Medicine: Tailoring tree tomato-based therapies to individual genetic and metabolic profiles may enhance their efficacy and minimize adverse effects.

Biotechnological Approaches: Harnessing biotechnological tools such as genetic engineering and metabolic engineering may allow for the production of tree tomato varieties with enhanced bioactivity and nutritional value.

Value-Added Products: Exploring innovative applications of tree tomato extracts in sectors such as pharmaceuticals, cosmetics, and nutraceuticals can create new market opportunities and economic benefits.

2.11.ANTI-CANCER ACTIVITY

Research on the potential anticancer activity of tree tomatoes (*Solanum betaceum*) is still ongoing and relatively limited compared to other fruits and vegetables. However, preliminary studies suggest that tree tomatoes contain bioactive compounds such as antioxidants, polyphenols, carotenoids, and flavonoids, which are known for their potential anticancer properties.

Antioxidants play a crucial role in neutralizing free radicals and reducing oxidative stress, which is linked to the development of cancer. Carotenoids, such as lycopene and beta-carotene found in tree tomatoes, have been studied for their ability to inhibit the growth of cancer cells and reduce the risk of certain types of cancer, including prostate and breast cancer.

Additionally, flavonoids present in tree tomatoes have been associated with anti-inflammatory and anticancer effects. Some studies have suggested that flavonoids

may help inhibit the growth of cancer cells, induce apoptosis (programmed cell death), and prevent the formation of blood vessels that supply tumors with nutrients.

While these preliminary findings are promising, more extensive research, including in vitro and in vivo studies and clinical trials, is needed to fully understand the anticancer potential of tree tomatoes and their bioactive components. Furthermore, it's essential to consider tree tomato consumption as part of a balanced diet and a healthy lifestyle, rather than a standalone cancer treatment. Always consult with a healthcare professional for personalized medical advice and treatment options.

CHAPTER 3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

The research aims to explore the phytochemical composition of tree tomatoes (*Solanum betaceum*) and their potential anti-cancer activity, with a focus on implications for food formulation. Tree tomato, a tropical fruit belonging to the Solanaceae family, is rich in bioactive compounds such as antioxidants, polyphenols, carotenoids, and flavonoids. These compounds have been studied for their potential health benefits, including anti-inflammatory, antioxidant, and anti-cancer properties. Antioxidants like vitamin C and E help neutralize free radicals and reduce oxidative stress implicated in cancer development, while carotenoids such as lycopene and beta-carotene are associated with a reduced risk of prostate and breast cancer. Flavonoids such as quercetin and kaempferol exhibit anti-inflammatory and anti-cancer effects through mechanisms like inhibiting cancer cell proliferation and inducing apoptosis.

Tree tomato (*Solanum betaceum*) is a tropical fruit rich in bioactive compounds.

Bioactive compounds include antioxidants, polyphenols, carotenoids, and flavonoids.

Antioxidants like vitamins C and E help neutralize free radicals and reduce oxidative stress implicated in cancer development.

Carotenoids such as lycopene and beta-carotene are associated with a reduced risk of prostate and breast cancer.

Flavonoids such as quercetin and kaempferol exhibit anti-inflammatory and anti-cancer effects through mechanisms like inhibiting cancer cell proliferation and inducing apoptosis.

The research aims to explore the anti-cancer potential of tree tomatoes and their implications for food formulation.

CHAPTER 4

METHODOLOGY

4.1 PREPARATION OF TAMARILLO JAM

4.1.1 *INGREDIENTS USED:*

Tamarillo, palm sugar.

4.1.2 *PRE- PREPARATION METHOD:*

- Cut the tips of the tree tomatoes
- Slit at the bottom and blanch for 3-4 minutes
- Grind the tree tomatoes and de-seed the puree with strainer.
- Now tree tomato puree is ready

4.1.3 *PREPARATION:*

- Add palm sugar in a pan and allow it to melt, then add the puree to it.
- Bring to a boil over medium heat, stirring often.
- Reduce heat and simmer, stirring occasionally, until mixture has consistency of thick paste. Then cool to room temperature

4.1.4 *Formulation 1:*

Our 1st try was done with tamarillo puree and white sugar as. It was with seeds. It has a crunchy texture, the seed was hard to chew.

Materials used	Formulation 1
Tree tomato	200g
sugar	170g

Table no. 1 – formulation 1

4.1.5 *Formulation 2:*

Our 2nd try was with tamarillo puree and white sugar but de-seeded this time to improve the texture of the jam.

Materials used	Formulation 2
Tree tomato	250g
sugar	190g

Table no. 2 – formulation 2

4.1.6 Formulation 3:

Third formulation was done by substituting white sugar with jagerry with equal amount of tamarillo puree.

Materials used	Formulation 2
Tree tomato	250g
jagerry	250g

Table no. 3 – formulation 3

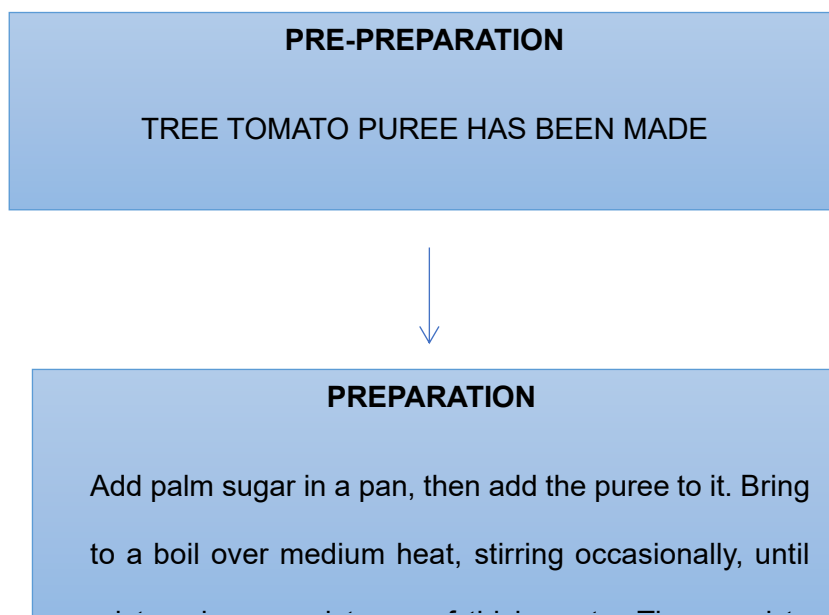
4.1.7 Formulation 4:

Fourth formulation was done by using palm sugar with equal amount of tamarillo puree.

Materials used	Formulation 2
Tree tomato	250g
Palm sugar	250g

Table no. 4 – formulation 4

4.1.8 FLOW CHART OF JAM PREPARATION



4.2 THIN LAYER CHROMATOGRAPHY (TLC):

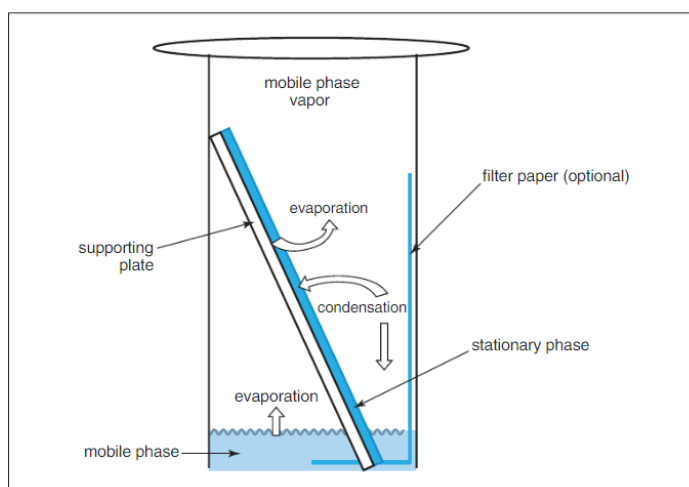


Fig no. 3 - Tlc set up

Thin layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. It involves a stationary phase (usually a thin layer of silica gel or alumina) and a mobile phase (a solvent mixture). The sample is spotted near the

bottom of the plate and placed in a developing chamber with the mobile phase. As the mobile phase moves up the plate by capillary action, components of the mixture separate based on their affinity for the stationary phase and mobile phase. Polar absorbents can be used as the stationary phase in Thin Layer Chromatography (TLC), a solid-liquid chromatography technique, whereas a single solvent or a combination of solvents can be used as the mobile phase.



Fig no. 4 - Process of TLC

SOLVENT MOBILE PHASE:

PHASE -1: Ethyle acetate and methanol

PHASE-2: hexane and ethyl acetate and acetic acid

PHASE-3: hexane and ethyle acetate and acetic acid.

PHASE-4: chloroform and methanol and hexane

Solute used was methanol tree tomato extract.

4.3 GAS CHROMATOGRAPHY/MASS SPECTROMETRY:

Gas Chromatography-Mass Spectrometry, or GCMS for short, is a potent analytical method for separating, identifying, and quantifying constituents in a mixture. It blends the detection and identification powers of mass spectrometry with the separation capabilities of gas chromatography. This method is frequently applied in many different disciplines, including forensics, environmental analysis, medicines, and food and beverage testing.

GC-MS analysis of the whole plant extract of *Evolvulus alsinoides* was performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film. The carrier gas used is Helium with a flow of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min.

4.4 DNA FRAGMENTATION:

DNA fragmentation research has become pivotal in various fields, including genetics, forensics, and medical diagnostics. This phenomenon, characterized by the breakdown of DNA molecules into smaller fragments, offers insights into cellular processes, disease mechanisms, and evolutionary patterns. Understanding DNA fragmentation has profound implications, from unraveling the intricacies of apoptosis to developing novel diagnostic tools for cancer detection. For instance, studies by Kumar et al. (2018) have elucidated the role of DNA fragmentation in cancer progression, shedding light on potential therapeutic targets. This introduction sets the stage for further exploration into the multifaceted realm of DNA fragmentation and its far-reaching implications.

DNA FRAGMENTATION: (Wyllie, 1980)

Procedure:

- Colo 320 cells were plated in 6 well plate and kept in CO₂ incubator to attain confluency.
- 1ml of Sample was added in to the well (IC₅₀ concentration) and incubated for 24 hrs. After this, cells were harvested using TPVG

- Centrifuge cells at 200xg at 4°C for 10 min.
- Add to the pellet 0.5 ml of TTE Solution and Vortex Vigorously. This procedure allows the release of fragmented chromatin from nuclei, after cell lysis (due to the presence of Triton X- 100 in the TTE solution) and disruption of the nuclear structure (following Mg ++ chelation by EDTA in the TTE Solution).
- To separate fragmented DNA from intact chromatin, centrifuge tubes at 20,000xg for 10 min at 4°C.
- Carefully remove the supernatants and add 500µl of TTE solution into the pellet.
- Add 500µl of Ice-cold NaCl and vortex vigorously. The addition of the Salt should be able to remove histones from DNA.
- Add 700µl of ice-cold isopropanol and vortex vigorously.
- Allow precipitation to proceed overnight at -20°C.
- After, precipitation, recover DNA by pelleting for 10 min at 20,000x g at 4°C.
- Rinse the pellets by adding 500-700µl of ice-cold 70% ethanol.
- Centrifuge tubes at 20,000x g for 10 min at 4°C.
- Dissolve DNA by adding to each tube 20-50 µl of TE buffer and place the tubes at 4°C.
- Mix the samples of DNA with loading buffer .The addition of loading buffer to samples allows to load in wells more easily and to monitor the run of samples.
- Run the electrophoresis in standard TE buffer after setting the voltage to the desired level. During electrophoresis it is possible to monitor the migration of samples by following the migration of bromophenol blue dye contained in the loading dye.
- Stop the electrophoresis when the dye reaches about 3 cm from the end of the gel.
- To visualize DNA, place the gel on a UV Trans illuminator.

4.5 Quantification of Total phenolic content

The Folin-Ciocalteu reagent method (Mc Donald et al., 2001) was used to determine total phenol. After mixing 0.1 ml of Folin-Ciocalteu reagent (1N) with a 100 µl plant extract sample, it was incubated at room temperature. After adding 5 ml of Na₂CO₃,

the mixture was allowed to sit at room temperature for 30 minutes. Using a UV-VIS spectrophotometer (Biospectrometer, Eppendorf, Germany) set to 760 nm, the total phenolic content was calculated. The unit of measurement for total phenol was gallic acid equivalent (mg/g of the extracted component), with gallic acid serving as the standard.



Fig no. 5 sample placed at 96 well plate for reading

4.6 Anti cancer activity

Cell line and culture:

MCF 7 Cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37 °C.

***In Vitro* assay for anti cancer activity: (MTT assay) (Mosmann, 1983)**

Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation,

1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \text{A570 of treated cells} / \text{A570 of control cells} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

4.7 QUALITY ANALYSIS

This done by the review of 100 consumers having our product tamarillo jam by sensory evaluation. The data has been taken as appearance, texture, flavour, aroma, mouthfeel. This tells the experience of customers after having our product.

CATEGORY	MEAN±STANDARD DEVIATION
APPEARANCE	9.44±0.987
TEXTURE	9.73±0.446
FLAVOUR	9.56±0.977
AROMA	9.62±0.8012
MOUTH FEEL	9.65±0.914
OVER ALL ACCEPTABILITY	9.57±0.923

Table No. 5 – Sensory Evaluation

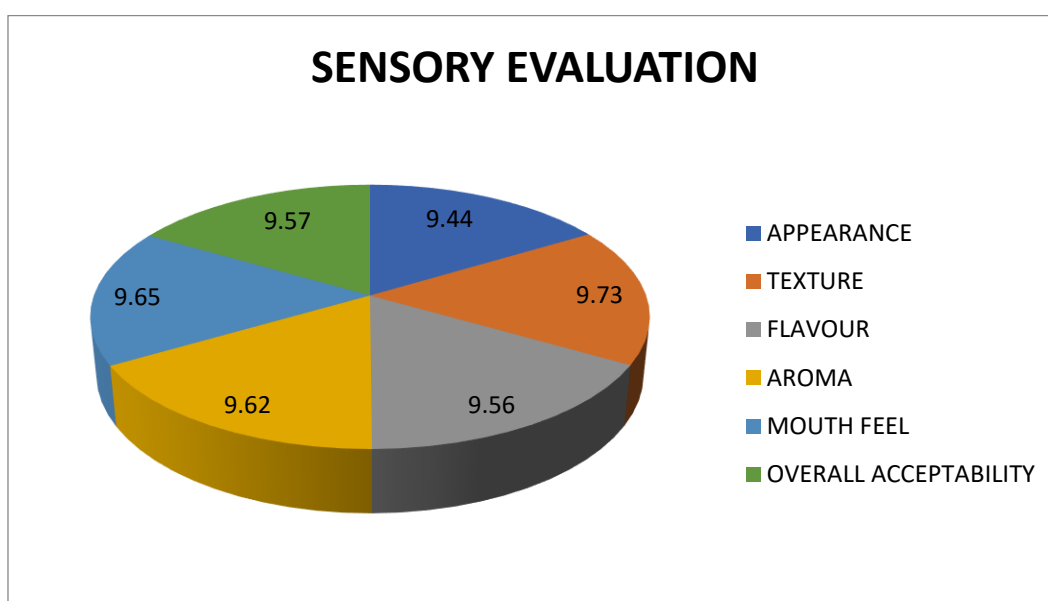


Fig no. 6 – sensory evaluation

1. INTERPRETATION : The above pie chart tells about the sensory evaluation results given by 100 consumers.

QUESTIONNAIRE :

1. Appearance :

Rating range (1-10)	No. of responses
Below 10	28
10	72

Table No. 6 – Appearance

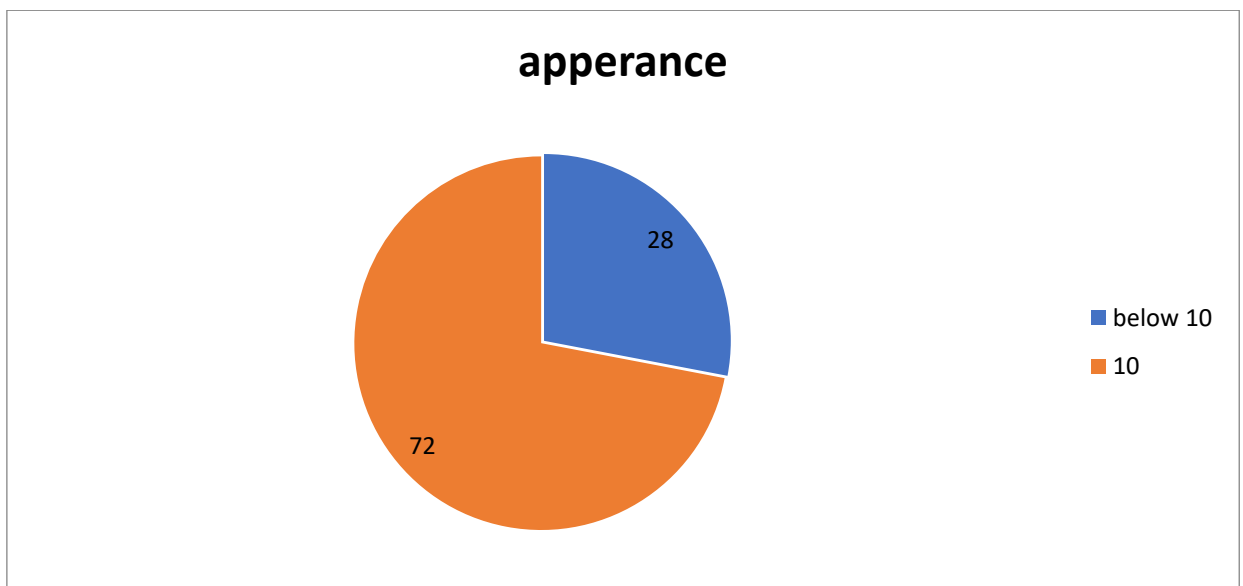


Fig no. 7 - Appearance

Intrepratation:

72 people are fully satisfied with our product and 28 members have responses below 10.

2. Texture :

Rating range (1-10)	No. of responses
Below 10	22
10	78

Table no. 7 - Texture

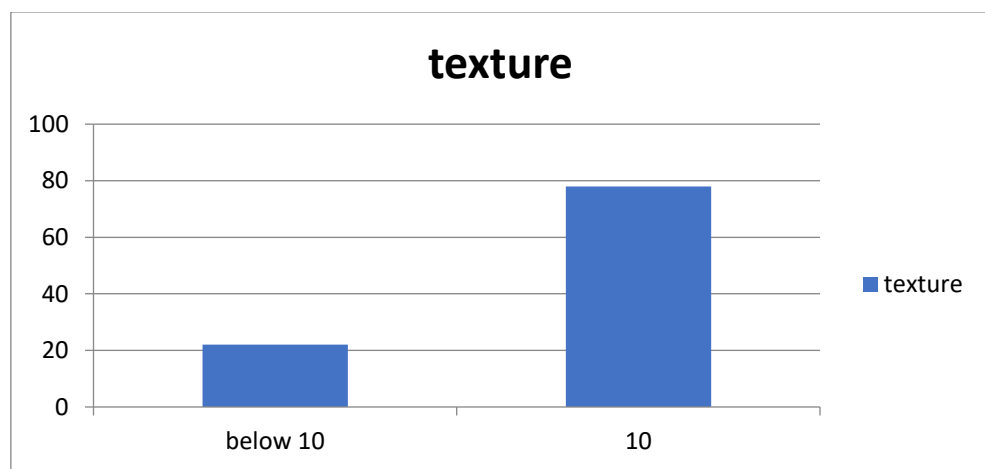


Fig no. 8 - Texture

Intrepratation:

78 people are fully satisfied with our product and 22 members have responses below 10.

3. Flavour

Rating range (1-10)	No. of responses
Below 10	19
10	81

Table no. 8 – flavour

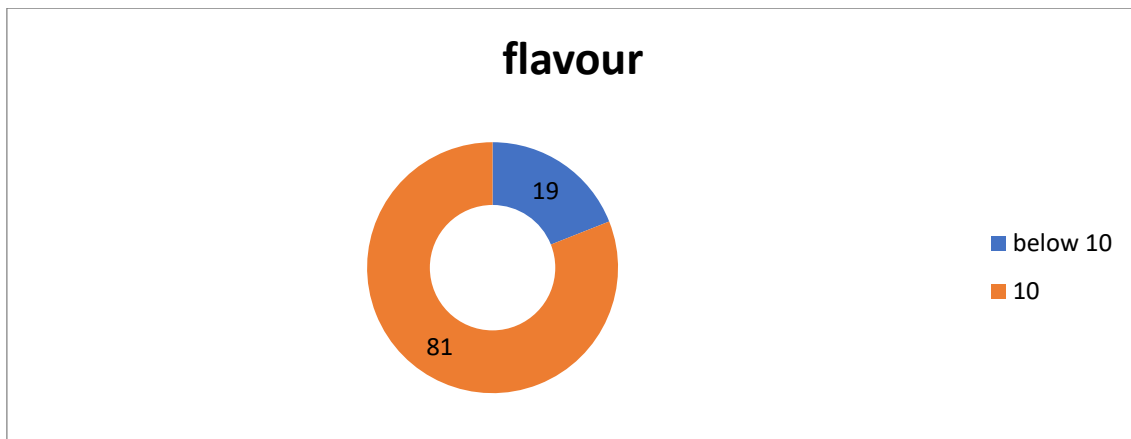


Fig no. 9 - Flavour

Intrepratation:

81 people are fully satisfied with our product and 19 members have responses below 10.

4. Aroma

Rating range (1-10)	No. of responses
Below 10	26
10	74

Table no. 9 - Aroma

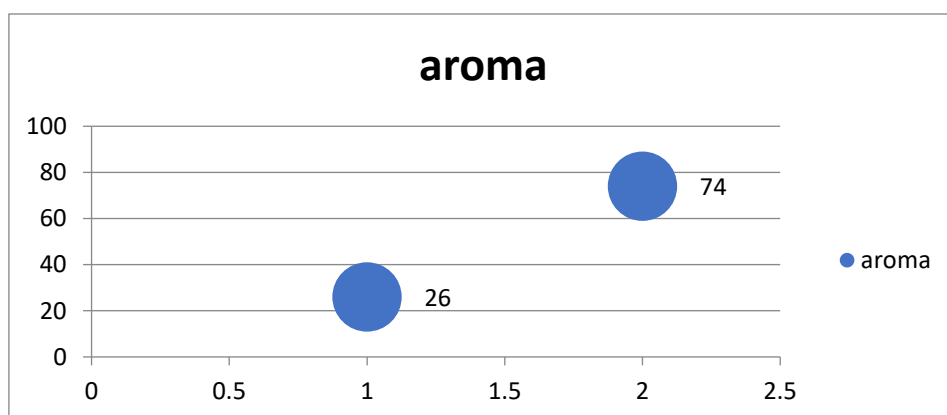


Fig no. 10 - Aroma

Intrepretation:

74 people are fully satisfied with our product and 26 members have responses below 10.

5. Mouth feel:

Rating range (1-10)	No. of responses
Below 10	18
10	82

Table no. 10 - Mouth feel

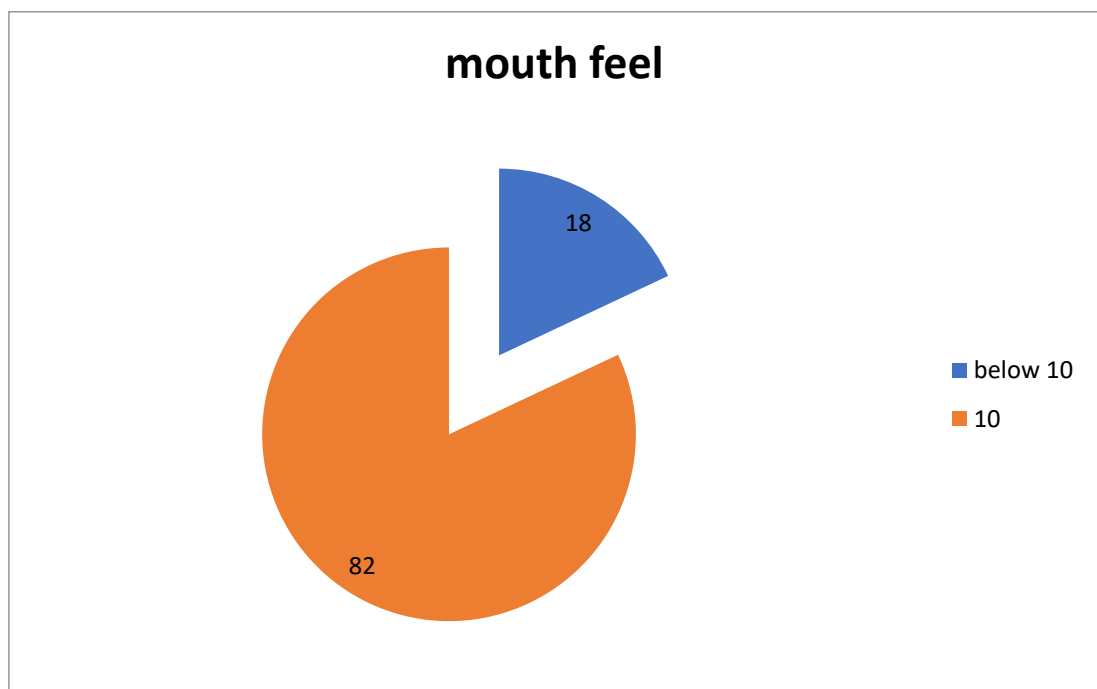


Fig No. 11 – Mouth Feel

Intrepretation:

82 people are fully satisfied with our product and 18 members have responses below 10

6. Overall acceptability:

Rating range (1-10)	No. of responses
Below 10	25
10	75

Table no. 11 - Overall acceptability

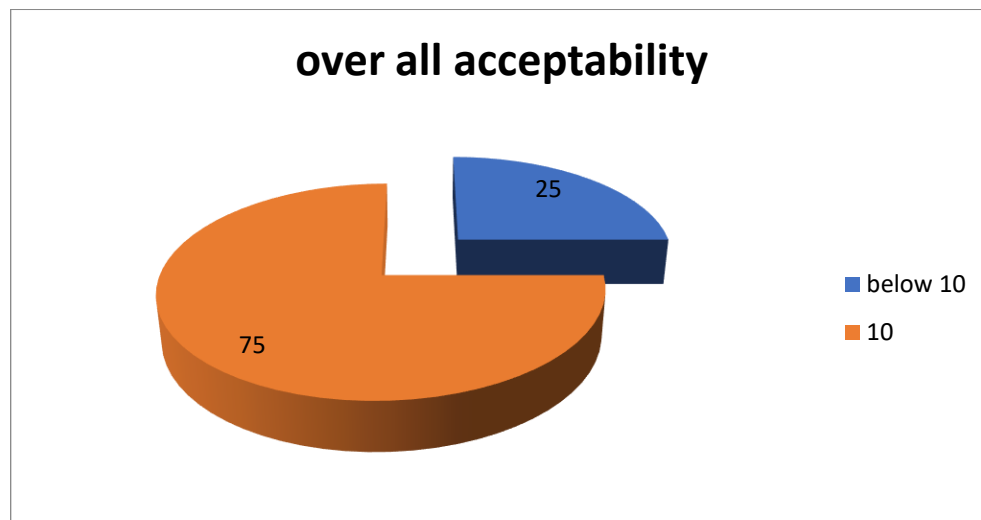


Fig No. 12 – Overall Acceptability

Intrepratation:

75 people are fully satisfied with our product and 25 members have responses below 10.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 SEPERATION OF COMPONENTS IN THIN LAYER CHROMATOGRAPHY



Fig No. 13 – VIEW OF SEPERATED PHYTOCHEMICAL PIGMENTS

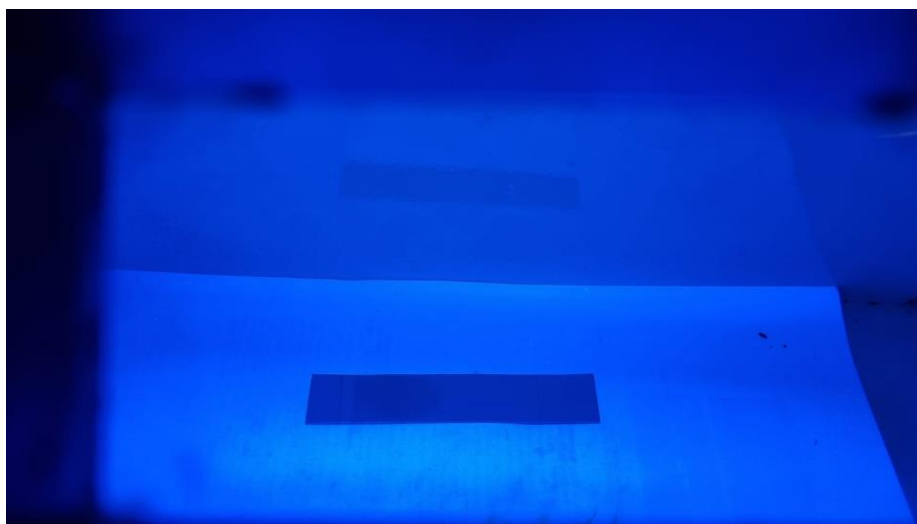


FIG NO. 14 - TLC VIEWED UNDER UV LIGHT

The presence of phytochemical as not been determined since the pigments separated are very light and colours are not properly visible.

5.2 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

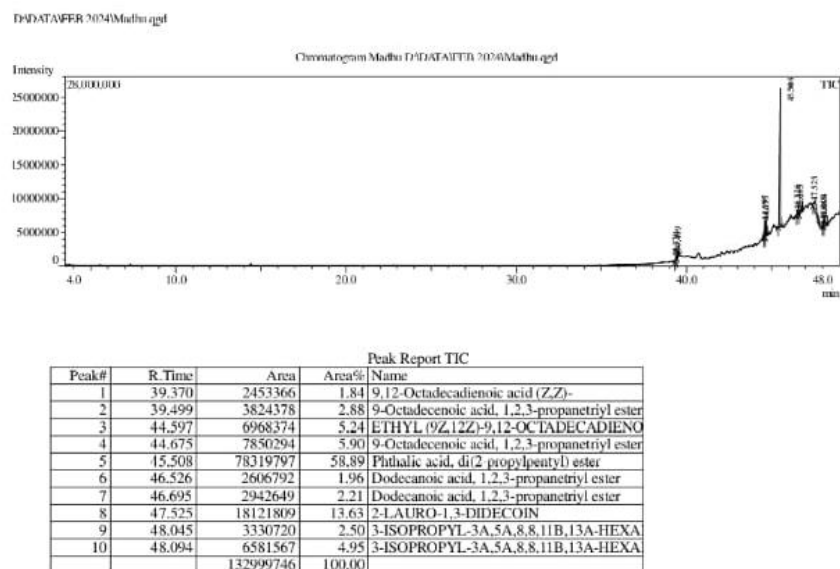
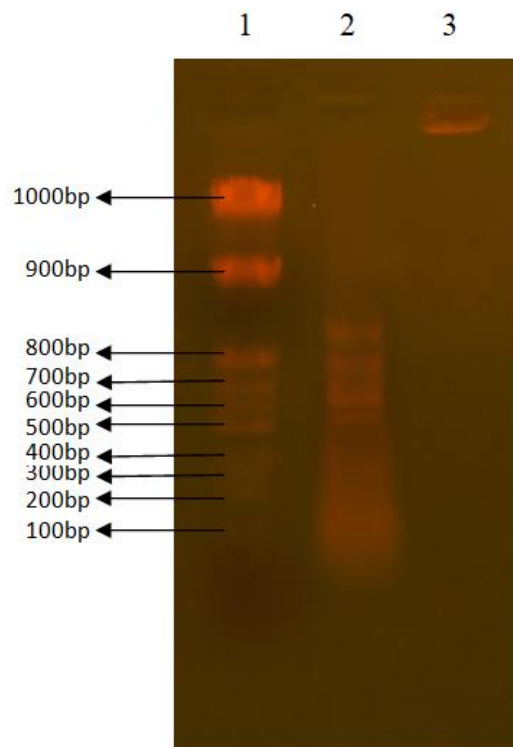


Fig No.15-Gc/Ms

5.3 DNA FRAGMENTATION

RESULT:

DNA fragmentation of MET sample in MCF7 cells



Lane 1: Marker
Lane 2: MET Sample
Lane 3: Control

Fig No. 16 – Fragmented Dna

Interpretation: The control DNA is diffused and no clear fragments were seen. The sample DNA shows fragments

5.4 ANTI CANCER ACTIVITY

Anticancer effect of Sample MET on MCF-7 cell line

S.No	Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
1	1000	0.272	28.07
2	500	0.354	36.53
3	250	0.436	44.99
4	125	0.511	52.73
5	62.5	0.582	60.06
6	31.2	0.667	68.83
7	15.6	0.738	76.16
8	7.8	0.817	84.31
9	Cell control	0.969	100

Table No. 12 – Anti Cancer Concentrations

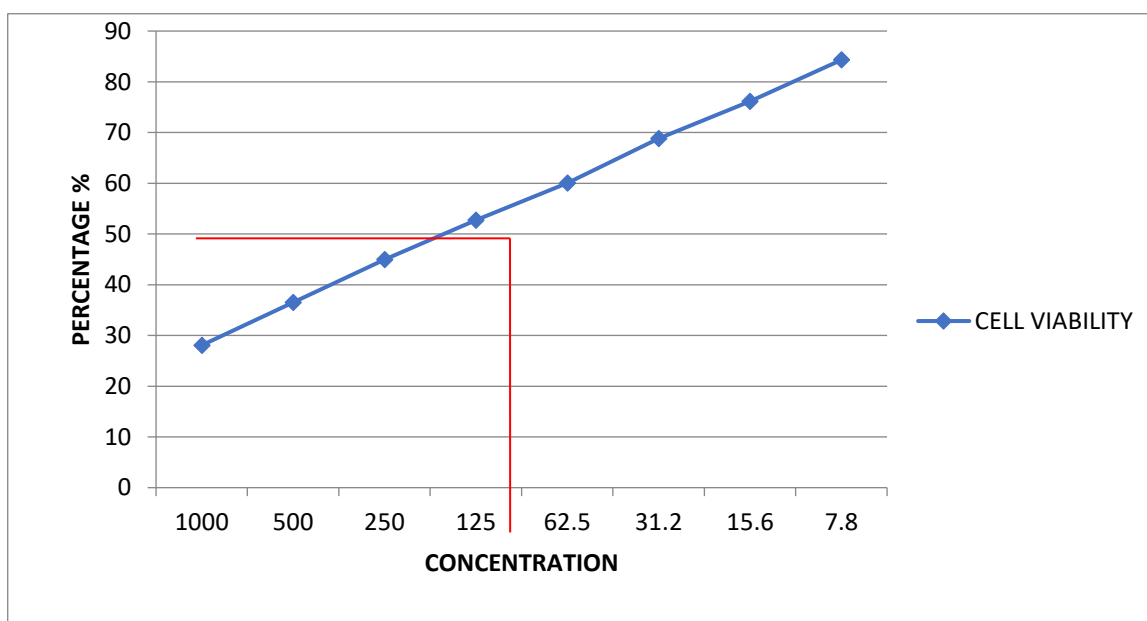


Fig No. 17 – Anti Cancer Graph

5.5 MCF 7 cells

Sample MET

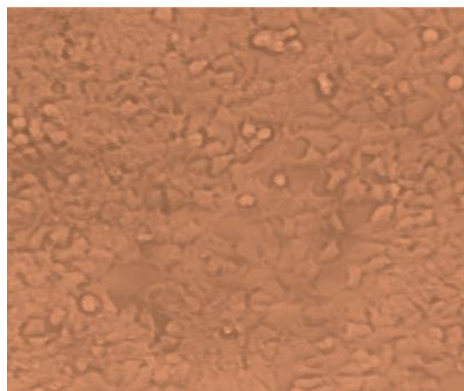


Fig No. 18 – MCF 7 Cells

125 $\mu\text{g/ml}$

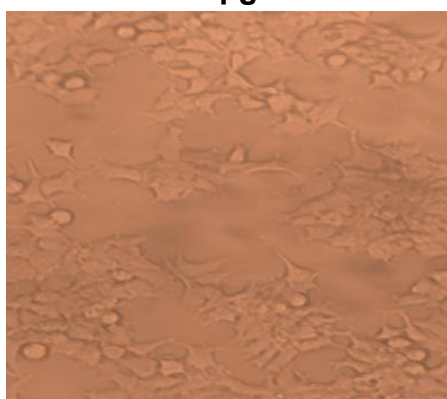
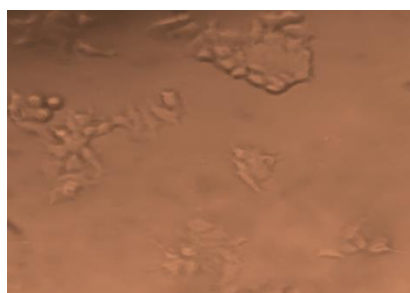
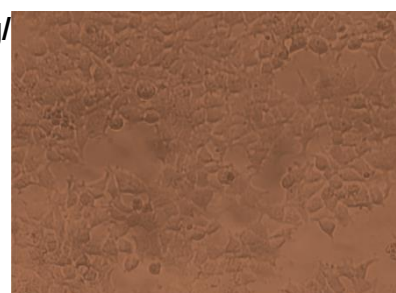


Fig No. 19 – MCF 7 Cells



7.8 $\mu\text{g/ml}$

1000 $\mu\text{g/ml}$



5.5 TOTAL PHENOLIC CONTENT

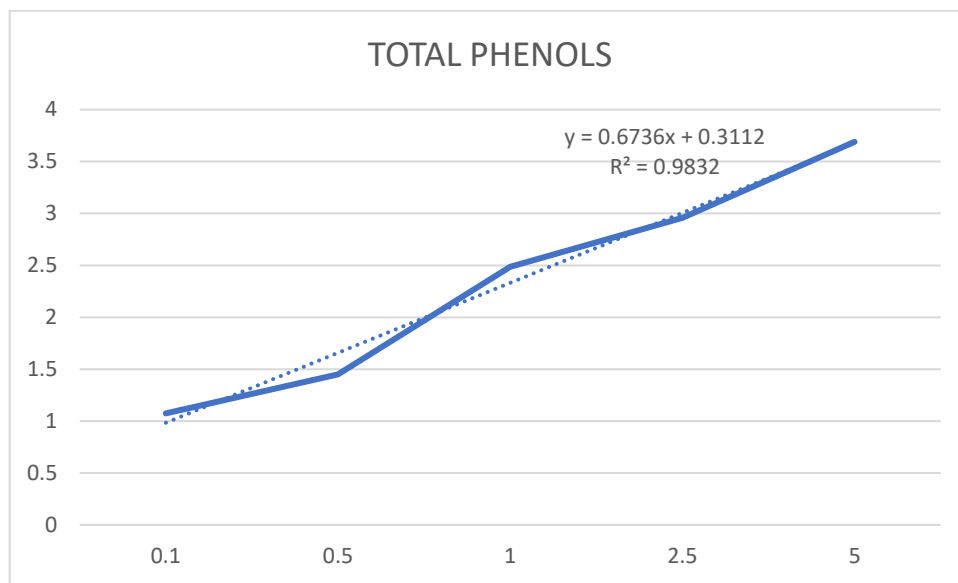


Fig No. 22 Phenolic Component Graph

$$Y = 0.6736x + 0.3112$$

$$0.084 = 0.6736x + 0.3112$$

$$= 0.6736x + 0.3112 - 0.084$$

$$X = \frac{0.2272}{0.6736}$$

$$= 0.337 \text{ MG/G}$$

5.6 NUTRITIVE ANALYSIS OF TAMARILLO JAM

Test Results:

The above sample tested as received, and results are as follows:

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1	Energy (By Calculation)	FAO Method	kcal/100g	333
2	Carbohydrates (By difference)	CTL/SOP/FOOD/262 - 2014	g/100g	80.0
3	Total Fat	AOAC 20 th Edn.2016, 920.39	g/100g	BDL(DL:0.1)
4	Protein (Nx 6.25)	AOAC 20 th Edn.2016, 986.25	g/100g	3.16
5	Total Sugars	FSSAI Manual 2015- Beverages, Sugar and confectioneries	g/100g	32.9
6	Dietary fibre	AOAC 20 th Edn.2016, 985.29	g/100g	BDL(DL:1.0)
7	Sodium as Na	AOAC 20 th Edn.2016, 969.23	mg/100g	16.8
8	Iron as Fe	AOAC 20 th Edn.2016, 999.11	mg/100g	1.75

Note : BDL - Below Detection Limit; DL - Detection Limit;

The energy value for 100g of our jam is 333kcal, carbohydrate is 80g, 3.16 g of protein, 32.9 g of total sugars, 16.8g of sodium and 1.75 of iron. Our products is fat free and contains palm sugar healthier and better option of jam to consume has is has no preservatives and artificial colours or chemical.

CHAPTER 6

SUMMARY AND CONCLUSION

The preparation of tamarillo jam involved several formulations using different sugars and de-seeding methods to improve texture and taste. The sensory evaluation of the jam indicated high satisfaction among consumers in terms of appearance, texture, flavor, aroma, mouthfeel, and overall acceptability.

Additionally, various analytical techniques such as Thin Layer Chromatography (TLC), Gas Chromatography-Mass Spectrometry (GC-MS), DNA fragmentation analysis, quantification of total phenolic content, and anti-cancer activity assessment (MTT assay) were employed to study different aspects of the jam, including its chemical composition, potential health benefits, and anti-cancer properties. Overall, the jam formulation and evaluation process demonstrated a comprehensive approach to product development and quality assessment

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STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF *DYNARIA QUERCIFOLIA* PORRIDGE MIX

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Clinical Nutrition & Dietetics

By

POOMUHI.D.S (41736012)

SWATHIKA.V (41736016)



**DEPARTMENT OF CLINICAL NUTRITION & DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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April 2024



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BONAFIDE CERTIFICATE

This is to certify that this project report is the Bonafide work of Swathika.V (41736016) and Poomuhi.D.S(41736012) who have done the project work entitled “**STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF DYNARIA QUERCIFOLIA PORRIDGE MIX**” under our supervision from October 2023 to April 2024.

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DECLARATION

We, Poomuhi.D.S (41736012) and Swathika.V (41736016) hereby declare that the project entitled **"STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF DYNARIA QUERCIFOLIA PORRIDGE MIX"** done by us under the guidance of **Ms. Roshni .M** at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

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We, Swathika. V (41736016) and Poomuhi.D.S (41736016) hereby declare that the project entitled **"STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF DYNARIA QUERCIFOLIA PORRIDGE MIX"** done by us under the guidance of **Ms. Roshni .M** at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

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ABSTRACT

Autoimmune diseases, such as arthritis is now recognized as a common non-communicable disease worldwide, affecting around 1-2% of people globally. In recent years, studies suggested that diet play a major role in disease risk and progression. Several treatment strategies have evolved over time to enhance the quality of life for rheumatoid arthritis patients, yet a considerable number remain unresponsive to existing medications. Diet plays a major role in disease risk and progression. Hence, the present study aims to develop and evaluate the nutritional analysis of *Dynaria quercifolia* porridge mix for the benefit of arthritis patients. *D. quercifolia* was grated, shade dried while the other ingredients such as millets, grains, and nuts were roasted. Following this, all the ingredients were mixed together and ground. The optimized levels of all the ingredients were added for the development of porridge mix based on colour, appearance and taste. The proximate analysis of the product revealed that it comprises of 407g/100g of energy, 77.4g/100g of carbohydrates, 4.37g/100g of total fat, 14.4g/100g of protein, 13.1g/100g of dietary fibre with hedonic rating, the maximum and minimum overall acceptability is 8.1 and 7.2 respectively. During the study, it was observed that *D. quercifolia* had a higher calcium level of 297mg/100gm. Thus, incorporation of *D. quercifolia* in the porridge mix and including it in daily dietary habits could effectively manage, prevent and reduce the occurrence of arthritis.

Keywords: Arthritis, *Dynaria quercifolia*, porridge, Autoimmune, calcium.

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LIST OF ABBREVIATIONS

RA	Rheumatoid arthritis
QOL	Quality of life
ASEAN	Association of Southeast Asian Nations
DQR	<i>Dynaria quercifolia</i> rhizome
DQ	<i>Dynaria quercifolia</i>
AD	Autoimmune disease
M	Milk
W	Water
CFU	Colony forming units
WHO	World Health Organisation
NSAIDs	Nonsteroidal anti-inflammatory drugs
ALP	Alkaline phosphatase
SLE	Systemic Lupus Erythematosus
DL	Detection limit
%	percentage
kcal	kilocalorie
g	gram
mg	milligram

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CHAPTER 1

INTRODUCTION

Autoimmune diseases result from the immune system attacking the body's own cells and tissues. The diverse nature of these conditions makes them affect various organs, and they often show a higher prevalence in women (Davidson A. *et al.*, 2019).

Infections and microbiota play pivotal roles in triggering autoimmune diseases. The presence of autoantibodies before clinical signs, during the preclinical autoimmunity phase, underscores the potential for early detection and intervention in at-risk populations to mitigate tissue damage (Sethi S. *et al.*, 2022).

The autoimmune diseases manifest as advanced conditions where the immune system reacts against the host's own normal constituents, involving both B-cell and T-cell reactivity. These disorders often target specific organ systems and show a higher prevalence among women, emphasizing the complex interplay of factors in their development (Beck L H. *et al.*, 2019).

The autoimmune diseases exhibit clinical diversity. They can either manifest with organ-specific patterns or present in a generalized manner, affecting various systems throughout the body. The distinction between systemic and organ-specific involvement adds complexity to their clinical presentation and management (Pollak T A. *et al.*, 2020).

The perception of autoimmune diseases has shifted from being considered rare to now being recognized as prominent non-communicable diseases worldwide. The understanding of autoimmunity has advanced substantially, encompassing insights from gross structural levels to intricate micro-molecular details, contributing to a more comprehensive knowledge base in this field (Sakaguchi S *et al.*, 2000).

While significant progress has been made in understanding autoimmunology and autoimmune diseases, there is acknowledgment that the complete understanding of the ideology and causative mechanisms remains elusive. The complex pathological process involved have been unraveled to a considerable extent, and ongoing clinical observations continue to refine our understanding (Stojanorich L *et al.*, 2010).

Autoimmune diseases pose challenges in terms of preemptive strategies, and current treatment approaches often focus on managing symptoms rather than preventing onset. Drawing insights from protocols in infection, oncology, metabolic diseases, and transplantation might help develop more effective management models for autoimmune conditions (Chandrashekar, 2019).

Advancements in medical understanding and treatment strategies have significantly improved the survival rates of SLE over the past two decades. The increase in reported 90% survival beyond 5 years is a positive development in managing this autoimmune disease (Urowitz M B *et al.*, 2019).

The evolving understanding of autoimmune diseases has spurred the development of a wealth of information on their mechanisms. This knowledge has been instrumental in the creation of newer drugs and biologicals, opening up promising avenues for more effective treatments (Mc Gonagle D *et al.*, 2019).

The current approach in AD can be broadly categorized into two categories: (1) symptomatic or replacement therapy (a conservative approach) and (2) the immunosuppressive or immune-modulation therapy (aggressive therapy). Currently more target-specific drugs are available which have reduced the toxicity of immune suppressant drugs on other collateral systems and produces a more profound immunosuppression effect (Feldmann M *et al.*, 2005).

The current approach of immunosuppression resembles the concept of transplant rejection, but the immunosuppression used is much lesser in AD than what is normally prescribed in transplant rejection management. The immune homeostasis principle suggests that AD can be caused by lymphopenia and resurgence of the autoreactive clones (Dalakas MC *et al.*, 2006).

The diversity of autoimmune diseases poses a significant challenge in the medical field. Clinicians must navigate through a multitude of signs and symptoms to reach an accurate diagnosis, often requiring distinct and varied management approaches.

The clinical complexity and heterogeneity of autoimmune diseases, a focused search for aberrant B and T cell auto-reactivity becomes essential. Identifying these specific mechanisms is crucial for understanding how autoimmune processes drive the pathogenesis of these conditions (Den braanker *et al.*, 2021).

The exploring auto-reactivity becomes paramount in understanding poorly-understood autoimmune conditions with diverse clinical presentations. Investigating auto-antigens serves as the initial step, providing a foundation to assess the involvement of B or T cell auto-reactivity in the pathogenesis of these diseases (E K Mirmira *et al.*, 2020).

Autoimmune diseases like rheumatoid arthritis involve the immune system and targets various components of the joints, leading to the inflammation, tissue damage, and pain.

The global prevalence of the disease is estimated around 1–2%, with a large variation among different populations. In developed nations, osteoarthritis is considered to be one of the 10 most common disabilities in older individuals, especially those who remain active in the workforce (Palmer KT *et al.*, 2015).

The word arthritis is derived from the Greek words arthron for “joint” and itis for “inflammation”. Today, the term is used for hundreds of different varieties of joint problems that have specific symptoms, such as pain, swelling, and stiffness. Arthritis refers to a range of musculoskeletal conditions where a person’s joints become inflamed, which may result in pain, stiffness, disability, and deformity. These symptoms can often have a significant impact on a person’s everyday functioning life. There are more than 100 types of arthritis (Watson, 2019).

In 2019, 18 million people worldwide were living with rheumatoid arthritis. About 70% of people living with rheumatoid arthritis are women, and 55% are older than 55 years. 13 million people with rheumatoid arthritis experience severity levels (moderate or severe) that could benefit from rehabilitation. While rheumatoid arthritis is a systemic autoimmune disease that affects multiple body systems, the joints of hands, wrists, feet, ankles, knees, shoulders and elbows are most often affected (Koller smith L *et al.*, 2022).

Rheumatoid arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that initially affects small joints, progressing to larger joints, and eventually the skin, eyes, heart, kidneys, and lungs with varying severity among patients. Often, the bone and cartilage of joints are destroyed, tendons and ligaments are weakened. The risk factors include age, gender, genetics, and environmental exposure (cigarette smoking, air pollutants, and occupational) (Lee JE *et al.*, 2017).

Furthermore, patients with RA generally complain of gastrointestinal tract problems particularly dyspepsia (bloating, postprandial fullness, nausea, early satiety, epigastric pain, and burning and belching), mucosal ulceration, and altered bowel habits (constipation/diarrhoea) (Wolfe F *et al.*, 2016).

Certainly, environmental factors such as smoking, certain infections, and exposure to pollutants are known to contribute to the development of rheumatoid arthritis. These factors can have an impact before clinical symptoms appear, highlighting the complex interplay between genetics and the environment in autoimmune diseases (Edwards CJ *et al.*, 2008).

Common symptoms of RA include morning stiffness of the affected joints for >30 min, fatigue, fever, weight loss, joints that are tender, swollen and warm, and rheumatoid nodules under the skin (Chaudhari K *et al.*, 2016).

The goals of treatment for RA are to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity (Staheli LT *et al.*, 1998).

The first line of treatment for RA includes disease-modifying antirheumatic drugs (DMARDs) that suppress disease activity and reduce joint damages. There are many other treatments like medications (NSAIDs: Nonsteroidal anti-inflammatory drugs; helps to reduce the pain and inflammation. Corticosteroids: provide relief from inflammation), Physical therapy (Exercises to improve joint mobility, strength, and flexibility, Lifestyle modifications (maintaining a healthy weight, avoiding stress on joints and other Alternative therapies (some people find relief from arthritis through massage therapy, dietary supplements) which can cure the arthritis including the diet part (Rubbert roth *et al.*, 2019)

Although a number of studies suggested associations between dietary habits, mainly regarding fruit, vegetables or meat intake and the disease's development, the results are still inconclusive (Pedersen M *et al.*, 2006).

Diet and nutrients have received considerable attention as potential environmental factors influencing the development and the course of the disease. Although a number of studies suggested associations between dietary habits, mainly regarding fruit, vegetables or meat intake and the disease's development, the results are still inconclusive (Grant W.B *et al.*, 2000).

In recent years, an increasing number of studies have investigated the role of diet and nutrition as potential tools for RA prevention and management (Philippou E *et al.*, 2018).

Dietary habits could represent both disease risk and protective factors based on the properties of specific food, dietary choices, or needs. Dietary habits show pro-inflammatory effects, e.g., red meat, salt, excessive caloric intake, or, on the contrary, radical consumption, oily, fatty fish, fruit, and others (Oliviero F *et al.*, 2015)

Excessive consumption of red meat and a high total protein supply have been associated with an increased risk of inflammatory polyarthritis (Pattison D.J *et al.*, 2004).

RA patients presented a lower intake of fish, potatoes, mushrooms and organ meats; mushrooms, citrus fruits and dairy products consumption showed a protective effect on RA, while potatoes and other fruits' consumption was associated with an increased risk (Wang Y *et al.*, 2016).

A high dietary sodium (salt) intake, common in Western countries, has been associated with an increased risk of RA (Salgado E *et al.*, 2015).

RA and other chronic diseases, including cardiovascular disease (CVD), type 2 diabetes mellitus. Indeed, C-reactive protein (CRP), a marker of inflammation, predicts future development of hypertension and diabetes mellitus better than body mass index.

Nutrients can modulate the inflammatory status of humans and consequently the pro- or anti-inflammatory properties of specific foods and components have emerged in nutrition sciences.

Millions of people suffer from painful and swollen joints associated with arthritis. In the past, many doctors reported dietary changes for an arthritis patient would not help them. However, this conclusion was based on older research with diets that included dairy products, oil, poultry, or meat. New research shows that foods may be a more frequent contributor to arthritis than is commonly recognized (Panush RS *et al.*, 1983).

For years, people have suspected that foods are an important factor in the development of rheumatoid arthritis. At present, 69% of patients with OA take some

form of dietary supplements for their condition. The *Spinacia oleracea* vegetable can mitigate OA effects by increasing bone volume to tissue volume, which results in a decrease of the trabecular pattern factor by more than 200% (Basedow M *et al.*, 2014).

Common forms of arthritis include osteoarthritis, caused by wear and tear on the joints; rheumatoid arthritis, an autoimmune disease in which the body attacks its own joints; and gout, caused by a buildup of uric acid in the body. Regardless of the type of arthritis you have, cutting back or eliminating foods that can aggravate inflammation may help alleviate the pain and swelling.

When a subject has arthritis, their body is in an inflammatory state. What the subject eat may not only increase inflammation, but it may also set up for other chronic conditions such as obesity, heart disease, and diabetes.

A survey conducted in 2015 by D. Sobel for more than 1000 arthritis patients, concluded that red meat, sugar, fats, salt, caffeine, and nightshade plants (e.g., tomatoes, eggplant) were most commonly believed to worsen the condition (Sobel D *et al.*, 1989).

On the other hand, certain foods can help alleviate arthritis symptoms and promote joint health. Some of foods like Fatty fish (salmon, mackerel, sardines are rich in omega 3 fatty acids), which have anti-inflammatory properties and may help reduce joint pain. Berries high in antioxidants, which helps in neutralize free radicals' leafy greens support overall joint health whole grains foods like brown rice, quinoa, barley provide fiber and nutrients that may reduce inflammation etc.

Certain dietary approaches, like anti-inflammatory diets or those rich in omega-3 fatty acids, are being explored for their potential benefits in mitigating symptoms and supporting overall health (Hermann, 2017).

Fruits, vegetables and olive oil could decrease RA risk, providing several nutrients with antioxidant properties, such as tocopherols contained in olive oil acting as free radical scavengers (Serra-majem *et al.*, 2003).

However, the already mentioned Danish study demonstrated no association between RA risk and the intake of citrus fruits, vegetables, retinol, beta-carotene, vitamins A, E, C, D, zinc selenium, iron and meat (Di Giuseppe D *et al.*, 2014).

Not only natural ingredients also medicinal plants have a history of being utilized for treating arthritis and various other conditions. They possess therapeutic properties that can alleviate inflammation, reduce pain, and improve joint mobility in arthritis sufferers. Furthermore, these plants are employed for diverse purposes beyond arthritis treatment, such as addressing digestive issues, enhancing immune function, and promoting overall well-being through their natural healing compounds (Sofowora A *et al.*, p. 1993).

Likewise, *D. quercifolia* known as 'Mudavattukal' in Tamil has gained popularity in recent times and being used in different group of people to cure various kinds of health problems.

So far *Dynaria quercifolia* has been mostly used in treating stomach ache, peptic ulcers, dysentery, gastro-intestinal disorders; skin problems, cold, cough, jaundice, fever, urinary complaints, fracture of bones and hypertension.

Ethnomedicinal information shows that the rhizome of plant DQ was traditionally used for the treatment of body pain, joint pain, dyspepsia, diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin diseases. Plants from the *Drynaria* genus can be used topically as a hair tonic to promote hair development and to improve the condition of the hair. *D. quercifolia* as a whole plant can be used to treat Tuberculosis, rheumatoid fever, dyspepsia, and cough. The plant's tonic has an astringent effect on the bowels. It is utilised in traditional medicine by a variety of people to address variety of health issues, including urinary tract infection. The rhizome is popularly used as a traditional soup condiment and analgesics by the people of Eastern Ghats, Tamil Nadu, India (Brahmam OM *et al.*, 2002).

It has also been used in Bangladesh by traditional healers for the treatment of jaundice, hepatitis, diabetes, gonorrhoea and malaria (Sen A *et al.*, 2001).

In Southeast Asia, rhizome decoction of *D. quercifolia* is used in antipyretic preparation and as poultice on swellings (Dixit RD *et al.*, 1984).

Based on the above evidence, our current study is focuses on development and nutritional analysis of *D. quercifolia* porridge mix which not only manage but prevent arthritis.

CHAPTER 2

REVIEW OF LITERATURE

The word arthritis is derived from the Greek words arthron for “joint” and its “inflammation.” Today, the term is used for hundreds of different varieties of joint problems that have specific symptoms, such as pain, swelling, and stiffness. Arthritis refers to a variety of musculoskeletal situations wherein a person’s joints grow to be inflamed, which can also additionally bring about pain, stiffness, disability, and deformity. These symptoms can often have a significant impact on a person’s everyday functioning of life. There are more than 100 types of arthritis (Watson R. r., 2019).

They are rheumatoid arthritis, osteoarthritis, childhood arthritis, reactive arthritis, fibromyalgia arthritis, systemic lupus erythematosus arthritis, spondyloarthropathies arthritis, etc.

Among the different types of arthritis research, the largest share of papers (49.35%) was published in rheumatoid arthritis, followed by adjuvant arthritis (10.70%), tuberculosis arthritis (8.39%), osteoarthritis (7.96%), bacterial arthritis (7.83%), lupus arthritis (7.64%), juvenile arthritis (7.60%), psoriatic arthritis (3.85%), hand arthritis (2.84%), polyarthritis (2.81%), gout arthritis (2.74%) and septic arthritis (2.20%) during 2007-16 (Gupta B.M, 2017).

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder characterized by the destruction of joints and persistent synovitis; it affects nearly one percent of the adult population worldwide (Paulraj, 2001).

The chronic inflammation leads to the development of pannus, an aggressive inflammatory state where activated T lymphocytes, macrophages and B cells together with the cytokines that they produce, as well as active angiogenesis, play a major part in the progression of the joint destruction (Paran. *et al.*, 2001).

Rheumatic Arthritis is the inflammatory disease of the immune system and it targets first the synovium or lining of the joint, resulting in pain, stiffness, swelling, joint damage, and loss of function of the joints. Inflammation most often affects joints of the hands and feet and tends to be symmetrical (Grapevine, 2017).

Rheumatoid arthritis (RA) is a systemic, debilitating, chronic inflammatory autoimmune disorder affecting approximately 1% of the world population (Gibofsky A., 2012).

The disease severely impacts quality of lifestyles with improved morbidity and decreased lifestyles expectancy. With the rapidly expanding population with RA, the disease has put a lot of economic burden on the society (Aggarawal A. *et al.*, 2006).

The global prevalence of the disease is estimated around 1–2%, with a large variation among different populations. Genetic and environmental factors interact during RA pathogenesis, a multistep process that begins years before clinical onset of the disease (Alamanosy Y. *et al.*, 2005).

Arthritis affects 15% of Indian population which is about 180 million people. There are more than 100 rheumatic diseases that collectively affect more than millions of people worldwide (Mela *et al.*, 2017).

In developed nations, osteoarthritis is taken into consideration to be one of the 10 most common not un usual place disabilities in older individuals, particularly individuals who stay energetic withinside the workforce.

Of persons with arthritis, more than half (58.9%) had osteoarthritis (deterioration of cartilage inside a joint), 11.5% had rheumatoid arthritis (an autoimmune disease in which the body is attacked by bacteria or viruses), and 34.8% had an unspecified type of arthritis (Masud parner GM. *et al.*, 2015).

In 2019, about 528 million people worldwide were living with osteoarthritis and 18 million people with rheumatoid arthritis; About 73% of people living with osteoarthritis are older than 55 years, and 60% are female whereas 70% of people living with rheumatoid arthritis are women, and 55% are older than 55 years (L K. s., 2022).

Musculoskeletal disorders are considered the second largest cause of disability globally, second but close only to behavioural and mental disorders (Vos T. *et al.*, 2012).

Furthermore, patients with RA generally complain of gastrointestinal tract problems particularly dyspepsia (bloating, postprandial fullness, nausea, early satiety,

epigastric pain, and burning and belching), mucosal ulceration, and altered bowel habits (constipation/diarrhoea) (Wolfe F. *et al.*, 2016).

Certainly, environmental factors such as smoking, certain infections, and exposure to pollutants are known to contribute to the development of rheumatoid arthritis. These factors can have an impact before clinical symptoms appear, highlighting the complex interplay between genetics and the environment in autoimmune diseases (Edwards CJ. *et al.*, 2008).

Many terms are used [excellent of life (QOL), fitness status, fitness-associated QOL] to talk about the impact of diseases, including arthritis, on functioning and experience of well-being. At the broadest level, QOL refers now no longer most effective to fitness reputation however additionally to environmental and monetary factors (e.g. profits and academic attainment that may drastically have an effect on well-being. Thus, different conceptual frameworks guide how quality of life is defined and measured (Wan G. *et al.*, 1997).

RA has major diverse effects on patients' quality of life, affecting both mental and physical do mains of well-being (Uhlig T. *et al.*, 2007).

The goals of remedy for RA are to lessen joint infection and pain, maximize joint function, and save you joint destruction and deformity. Treatment regimens consist of combinations of pharmaceuticals, weight bearing exercise, educating patients about the disease, and rest. Treatments are commonly custom designed to a patient's wishes and rely on their average health. This consists of elements which includes sickness progression, the joints involved, age, usual health, occupation, compliance, and training approximately the sickness. (Staheli LT. *et al.*, 1998). The intention of RA remedy is to obtain clinical, structural, and purposeful remission states (Vancher heijde DM. *et al.*, 1996).

In current years, the usefulness of phytopharmaceuticals, consisting of alisol-B, resveratrol, and arctigenin, has been suggested withinside the location of bone metabolism as a revolutionary adjuvant therapy. Resveratrol indeed shows promise in promoting osteoblast differentiation of mesenchymal stem cells, which are crucial for bone formation and repair. Its potential benefits in bone health have been studied extensively. The form of movement those drug treatments have on infection and bone metabolism warrants in addition research (Komori T. *et al.*, 2020).

Also, Medicinal plants have a history of being utilized for treating arthritis and various other conditions. They possess therapeutic properties that can alleviate inflammation, reduce pain, and improve joint mobility in arthritis sufferers. Furthermore, these plants are employed for diverse purposes beyond arthritis treatment, such as addressing digestive issues, enhancing immune function, and promoting overall well-being through their natural healing compounds (Sofowora A. *et al.*, p. 1993).

A medicinal plant is defined as any substance that can be employed for therapeutic purposes or as a precursor for the production of pharmaceuticals. In the last few years, researchers are becoming highly interested in the usage of physiologically active chemicals found in nature of therapeutic value. Medicinal plants encompass numerous compounds that may be used to deal with variety of ailments. Flavonoids, alkaloids, tannins, and different phytoconstituents saponins, quinines, terpenoids, glycosides, and polyphenols are a number of the compounds located in plants. Many plants are yet to be researched for their potential benefits and medicinal significance (Sofowora A. *et al.*, p. 1993).

Cinnamomum cassia Presl, also known as cassia or cinnamon, is a tropical aromatic evergreen tree, commonly used in traditional Oriental medicine. More than a hundred and sixty chemical compounds were recognized from *C. cassia*. The main constituents are terpenoids, phenylpropanoids, and glycosides (Zhang C. *et al.*, 2019).

The main components of *C. cassia* have a wide range of pharmacological effects, including anti-inflammatory and analgesic activity (Yang YL. *et al.*, 2020).

The dry root of *Aconitum kusnezoffii Reichb.* (caowu) has been used to deal with RA and joint ache for decades thanks to its anti-inflammatory properties. Pharmacological research has proven that diterpenoid alkaloids, along with aconitine, mesaconitine, hypaconitine, neoline, talatizamine beiwutine, and deoxyaconitine, are liable for the primary bioactive outcomes of *A. kusnezoffii* (Sun H. *et al.*, 2013).

According to the report of the W.H.O, about 80% of the world's populations rely mainly on traditional therapies which involve the use of plant extracts or their active substance. The use of medicinal plants as a source for relief from illness had been

recognized by early civilization in China, India and the other country (Yatskieych G *et al.*, 2002).

In ancient texts, about 500 plants have been indicated in the treatment of arthritis; however, only a small number of plants have been evaluated scientifically. In ancient Indian medicine, several ferns were also used, and in particular by Unani physicians in India and Western Asia. Ferns play a vital position in folklore medicine (Mondal AK *et al.*, 2011).

Accordingly, the ferns of *D. quercifolia* rhizome is used by local tribes in the rain forests of Western Ghats of Maharashtra, India. The rhizome is ground into a paste and used to treat diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin disease (Nejad B S *et al.*, 2009).

However, the rhizomes had been utilized in Indian machine of medication as anodyne, anti-inflammatory and for the remedy of arthralgia and migraine (Warrier *et al.*, 1995). Also used in the treatment of tuberculosis, loss of appetite, cough, and fever (Bhattacharya, 1990).

2.1 DYNARIA QUERCIFOLIA

D. quercifolia commonly known as “Oak Leaf Fern” is a medicinal pteridophyte belonging to the family Polypodiaceae. *Dynaria quercifolia* (L.) (Drynariaceae) is known as ‘Mudavaatukkal’ in Tamil.

In Ayurvedic system of medicine, it is called ‘Ashwakatri’ use by different tribal people for the treatment of arthritis (Nayar KB *et al.*, 1959).



Fig 2.1: Leaves of *D. quercifolia*

D.quercifolia is a medicinal fern with a huge variety of makes use of in treating a number of ailments. It is a terrestrial fern that can be found in fissures, shelves, or in the soil among boulders. They also can be discovered as epiphytic on trees, open forests and rainforest trunks (Cumming P *et al.*, 2011).

In Bangladesh, *D.quercifolia* and its several plant parts has been used by the local inhabitants, folk and tribal medicinal practitioners to treat jaundice, hepatitis, Chest pain, diabetes, gonorrhoea, debility and malaria (Mollik A H *et al.*, 2009).The above figure 2.1 shows the leaves of *D.quercifolia*.

The ASEAN Centre for Biodiversity mentions in its “Checklist of Medicinal Plant in Southeast Asia” that rhizome decoction or drink of *Drynaria quercifolia* is used as an antipyretic preparation (Chattopadhyay *et al.*, 2005).

D.quercifolia is an important ethnomedicinal plant used in traditional medicinal system by different group of people to cure various kinds of health problems. DQ along with other combination of herbs is used in pain from traumatic injury and also effective for lower back and ligament injuries (Dagar JC *et al.*, 1987).

Ethnomedicinal information shows that the rhizome of plant DQR was traditionally used for the treatment of body pain, joint pain, dyspepsia, diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin diseases.

2.2 RHIZOME

The plant part utilized in peoples remedy of *D.quercifolia* are rhizomes. (L, 2016). Traditionally, the soup organized from the rhizome of *D.quercifolia* will be very well-known among tribes of Eastern Ghats, Tamil Nadu and the soup drink enables to get treatment from rheumatic complaints (Samuydurai P *et al.*, 2012).



Fig 2.2: Rhizomes of *D.quercifolia*

The rhizomes, measuring 2cm in thickness, creep with a dense brown covering. Scales are 20-25mm long and 0.7-2.5mm broad and come in a variety of colours. The base of the rhizome is winged, but the lamina is not. Spores are 37.5-55 microns in length and 22.5-37.5 microns broad (Hooker W J *et al.*, 1862). The figure 2.1 below are the rhizomes of *D.quercifolia*.

The rhizomes are bitter, astringent and tonic. Rhizome paste is applied on the forehead to relieve head ache. As per the reports of Dagar (Dagar J C *et al.*, 1987).

The rhizomes have antibacterial and constriction properties. By mixing with the plant *Asparagus racemosus* Willd. (Liliaceae) then implemented on the top, it reasons coldness to the top and decreases alopecia (Hasan, 1993).

An herbal anti-pyretic agent with decreased or no toxicity is consequently essential. As rhizome of *D.quercifolia* is an old medicament used in ailments that caused fever (Bhattacharya *et al.*, 1990).

2.3 D.QUERCIFOLIA IN BONE HEALTH

D.quercifolia aids in the healing and strengthening of damaged bones. It promotes bone density and protects against osteoporosis and aids in the recovery of fractured bones ligaments (Brahmam O M *et al.*, 2002).

D.quercifolia has been shown in multiple studies to stimulate and osteoblasts to produce more healthy bone tissue (Wong K C *et al.*, 2012).

Anti-osteoporotic effects of extracts of *D.quercifolia* have been shown in female mammals following the loss of ovaries (Ma Z *et al.*, 1999).

An animal study done by one group of researchers revealed that the extract of *D.quercifolia* suppressed bone resorption and promoted bone (Chen L L *et al.*, 2004).

In 2011, scientists from School of Medicine in Hangzhou observed a significant decrease in the number of bone dissolving cells (Osteoclasts) in the *D.quercifolia* treated group as compared to the control animals. Their study has shown positive effects on improving alveolar bone remodelling and the study concluded that *D.quercifolia* could be an alternative medicine for periodontal therapy based on its

ability to reduce alveolar bone resorption while supporting the bone-building effects of osteoblasts (Ding P H *et al.*, 2011).

Naringin, one of the active ingredients in *D.quercifolia*, stimulates protein synthesis and metabolism of human periodontal ligament cells and naringin, also can promote proliferation of human periodontal ligament cells by enhancing ALP activity which is an important osteoblast function in bone building (Jiang J Q *et al.*, 2009).

The study done by Chinese researchers reported that total flavonoid content of *D.quercifolia* rhizomes has the ability to promote osteogenic differentiation of bone marrow cells into bone-building osteoblast cells at different concentrations of exposure to glucose (Shu X C. *et al.*, 2010).

2.4 OTHER PROPERTIES OF *D.QUERCIFOLIA*

The antifungal interest of the ethanolic extract of rhizome of *D.quercifolia* on dermatophytic species was first reported by Batool in 2009.

The methanol extracts of rhizome of *D.quercifolia* have effective against gram positive bacteria (*Bacillus subtilis*) and some gram-negative bacteria (Nath K. *et al.*, 2016).

The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases and still in the regular practice of the local people of Kolli hills by consuming it as a soup for treating various ailments (Samydurai P. T. *et al.*, 2012).

So far, the market has exclusively offered *D.quercifolia* in form of a soup powder only. Being a functional ingredient, it has gained a demand in market in various other forms. Considering the above facts and need in the market our current study aims on the development and nutritional analysis of *D.quercifolia* porridge mix which not only aids in management but also in the prevention of arthritis.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

The aim of the project is to develop and analyse the nutrients of *D.quercifolia* porridge mix.

3.2 OBJECTIVES

1. To examine the nutritional analysis of *D.quercifolia*.
2. To examine the macronutrients of *D.quercifolia* porridge mix.
3. To examine the micronutrients of *D.quercifolia* porridge mix.
4. To analyse the sensory quality of *D.quercifolia* porridge mix.
5. To determine the shelf life of *D.quercifolia* porridge mix.

CHAPTER 4

MATERIALS AND METHODS

4.1 Locale of the study

The major part of the present study has been carried out in the Department of Clinical Nutrition and Dietetics at the Sathyabama Institute of Science and Technology. The nutritional analysis and shelf life was performed in the nearest lab.

4.2 Procurement of raw materials

Dynaria quercifolia was purchased from the markets of Yercaud and the other ingredients for the preparation of the porridge were purchased from the local markets of Chennai.

4.3 Development of *D.quercifolia* porridge mix

D.quercifolia, the rhizome was thoroughly washed under running tap water. The fur portion of the rhizome was removed. The flesh part was isolated, grated and subsequently shade-dried. Additionally, a variety of grains including finger millet, pearl millet, kodo millet, red rice, maize, white corn, black gram, whole wheat, barley, sago, green gram, badam and cashew were dry roasted. The ingredients were then combined and ground into a fine mixture.

The figure 4.1 and 4.2 summarizes the steps in preparation and the pictorial representation of preparation of *D.quercifolia* porridge mix.

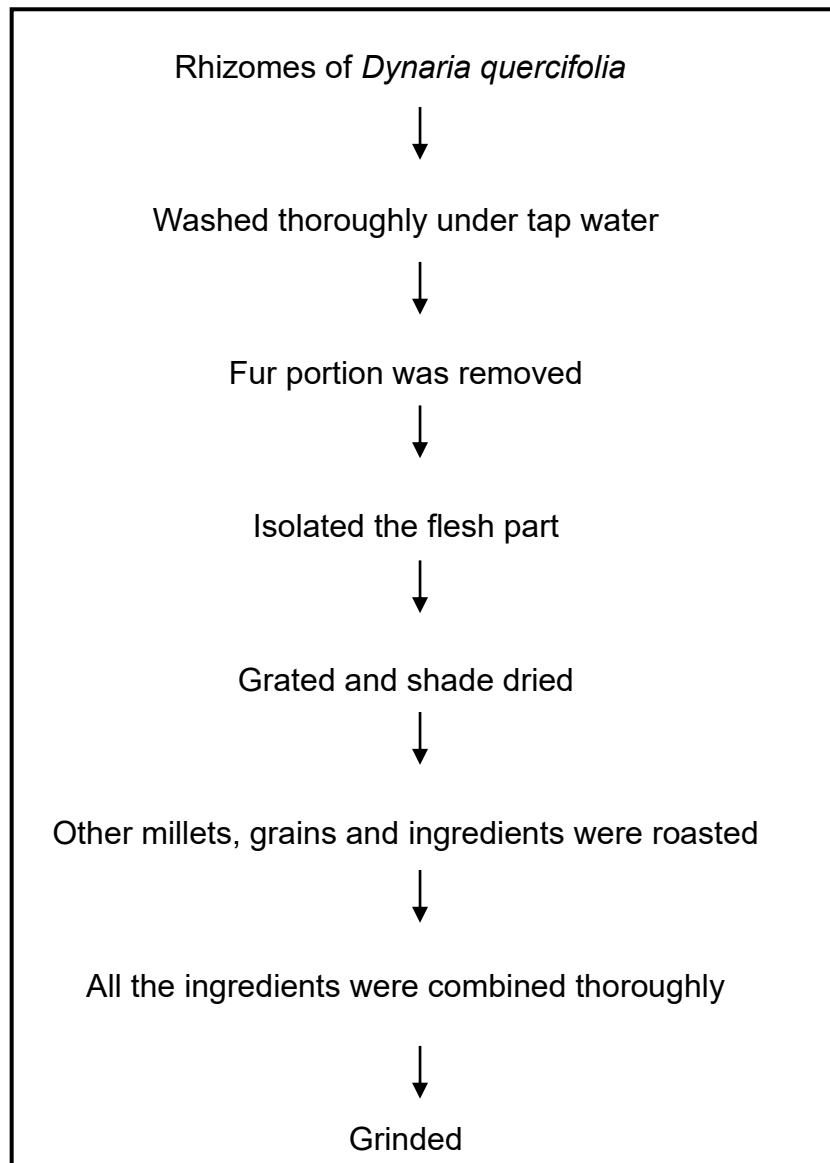


Fig 4.1: Steps in preparation of *D. quercifolia* porridge mix



Fig 4.2: Flow chart of pictorial representation in preparation of *Dynaria quercifolia* porridge mix

4.4 Nutritional analysis of *D.quercifolia*

4.4.1 Energy

Energy is calculated by the FAO method.

It is calculated by the following formula:

$$\text{Energy(kcal/100g)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

4.4.2 Total Carbohydrates

The total carbohydrate content in the porridge powder was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fibre, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate(g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat(g)} + \text{Protein(g)} + \text{Fibre(g)}]$$

4.4.3 Total fat

The total fat was determined by the method of AOAC 20th Edn.2016,920.39

Procedure:

Weigh accurately about 2g of sample in a fat extraction tube; add 2.0ml of alcohol to prevent lumping on the addition of acid, and shake to moisten all particles. Add 10ml HCl (25+11), mix well and set tube 30 to 40 mins in water bath at 70 °C and 80 °C, shaking frequently. Cool to room temperature and add alcohol. Add 25ml diethyl ether to the flask and shake for 1.0 mins. Then add 25ml petroleum ether to the flask and shake 1min. Decant ether layer into the pre-weighed dry flask through anhydrous sodium sulphate. Then, Decant ether layer into a pre-weighed dry flask through anhydrous sodium sulphate. Then add 25ml of 1:1 diethyl ether, and petroleum ether to the flask and shake for 1.0min. Ether layer can be evaporated under a water bath and keep the flask into the oven for further evaporation of ether residue. Remove the flask from the oven and place in desiccators to cool to room temperature and record the weight of the flask plus fat.

Calculation:

% of Fat = (Weight of flask + fat) – Empty weight of the flask × 100 / Sample weight

4.4.4 Protein

The protein($N \times 6.25$) was determined by the method of AOAC 20th Edn.2016, 986.25

Procedure:

Weigh suitable test portion of sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g CuSO₄ and 25mL or more, if necessary, of Conc.H₂SO₄ to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H₂O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

%N (w/w) = (TV_s-TV_b) × Normality of standard HCl × 1.4007 / Sample weight

where, TV_b = Titer value of blank; TV_s = Titer value of sample

4.4.5 Dietary fibre

The dietary fibre was determined by the method of AOAC 20th Edn.2016,985.29

Procedure:

Weigh duplicate 1g test portion, accurate to 0.1mg into 400ml tall-form beakers. Add 0.1ml tetramethyl solution. Cover the beaker with Al foil and place in boiling water bath for 15mins. Add 5mg protease. Incubate 30 mins at 60°C with continuous agitation and cool down. Add 10ml 0.325M HCl solution. Measure pH and dropwise and add acid if necessary. Add 280ml 95% ethyl alcohol preheated to 60°C. Wash residue successfully with three 20ml, portions of 78%ethyl alcohol and two 10ml portions of acetone. Time for filtration and washing will vary from 0.1h to 6h. Dry crucible containing residue overnight in 70°C vacuum oven. Cool in desiccator and weigh to nearest 0.1mg.

Calculations:

Determination of blank:

B= blank, mg= weight residue- $P_B - A_B$

where,

weight residue = average of residue weights(mg) for duplicate blank determination;
and

$P_B - A_B$ = weights(mg)of protein and ash

Calculate TDF as follows:

$TDF (\%) = [(weight\ residue - P - A - B) / weight\ test\ portion] * 100$

where,

weight residue = average of weights (mg) for duplicate blank determination

P and A = weights(mg) of protein and ash;

weight test portion = average of two test portion weights(mg) takes.

4.4.6 Total sugars

The total sugars were determined by the method in FSSAI Manual (2015).

Procedure:

Weigh accurately about 5 g sample, transfer to a 200 ml volumetric flask dissolve in warm water, dilute to about 150 ml. In case solution is not clear, add 5 ml of Carrez 1 solution followed by 5 ml of Carrez 2 solution. Make up to 200 ml. Filter through a dry filter paper. Titrate the solution obtained as such to determine % Reducing sugars.

Preliminary Titration:

Pipet 5 ml each of Fehling A and B into 250 ml conical flask. Mix and add about 10 ml water and a few boiling chips or glass beads. Dispense solution. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Continue the addition of solution dropwise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

Final Titration:

Pipet 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration duplicate and take the average.

Calculations:

Reducing sugars (%) = Dilution x Factor of Fehling (in gm) / Weight of sample x
Titre value x100

4.4.7 Sodium and potassium

The sodium and potassium were determined by the method of AOAC 20th
Edn.2016, 969.23

Procedure:

Weigh 4g test portion into the crucible, and char on electric hot plate or over low fame. Place on cold furnace and bring to 525°C and 2h to white ash. Add 15ml dil. HNO₃ (1-4) to the crucible, breaking up ash with a stirring rod if necessary. Filter through acid-washed quantitative paper into a 100ml volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume.

For flame emission- Read directly

For flame absorption- Dilute for direct readout as follows: Place 1ml aliquot in 100ml volumetric flask and dilute to volume with H₂O for Na; place 1ml aliquot in 25ml volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2ml HNO₃ to 100ml with H₂O.

Calculation:

For flame emission photometers:

$$\text{Na or K (100g)} = 100 \times F$$

where,

$$E = (\text{percent T of unknown}) - (\text{percent T of blank})$$

$$E = (\text{percent T of standard lower concentration than test solution}) - (\text{percent T of blank})$$

$E = (\text{percent T of standard of higher concentration than test solution}) - (\text{percent T of blank})$

$C = \text{mg in Na or K/ml in standard of lower concentration than test solution}$

$C = \text{mg in Na or K/ml in standard of higher concentration than test solution}$

$F = \text{dilution factor}$

For flame absorption photometers: Convert percent absorption to absorbance.

$\text{Mg in Na or K /100g} = (\text{concentration unknown} \times F \times 100) / \text{g test portion.}$

4.4.8 Calcium

Calcium was determined by titration method (IS 5949:1990 (RA.2010))

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 per cent sodium hydroxide solution (4.6) using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass $= V_1 \times 0.04008 / M$

Where V_1 = Volume in ml of EDTX solution in titration; and

M = Mass in g of the sample in the solution for the test

4.4.9 Iron

Iron was determined by the method of AOAC 20th Edn.2016,999.11

Procedure:

Homogenize the product if necessary, using non-contaminating equipment. In a crucible, weigh 10-20g test portion to nearest 0.1g. Dry in a drying oven or water bath at 100°C. Place dish in the furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C /h to 450°C. Let dish stand for at least 8 hours or overnight. Wet ash with 1-3ml water and evaporate on water bath. Put crucible in furnace at no more than 200°C and raise temperature (50°C -100°C /h) to 450°C. Proceed with ashing at 450°C for 1-2 hours or longer. Repeat the procedure until the product is completely ashed. Add 5ml 6M HCl to crucible ensuring that all ash comes into contact with the acid. Evaporate acid on water bath and dissolve residue in 10-30ml, to the nearest 0.1ml of 0.1M HNO₃. Cover with watch glass and lid for 1-2 hours. Then, it is determined by atomic absorption spectrometry (AAS).

Calculations:

Calculate the detection limit (DL) for Fe:

DL= 3 x standard deviation of the mean of the blank determination (greater than or equal to 20)

Calculate the concentration, c, of the metal in the test sample according to the formula:

$$c = (a-b) \times V / m$$

where,

c = concentration in the test sample (mg/kg)

a = concentration in the test solutions (mg/L)

b = mean concentration in the blank solution (mg/L)

V = Volume of the test solution (mL)

m = weight of the test portion (g)

If (a-b) is lower than the DL, then (a-b) is substituted with DL for calculation of the limit of detection in the test portion.

4.5 Evaluation of macronutrients in *D. quercifolia* porridge mix

4.5.1 Total Ash

The total ash was determined by the method of AOAC 20th Edn.2016,925.51

Procedure:

Weigh accurately about 5g of sample in a pre-weighed silica crucible. Ignite over the burner and complete the ignition by keeping in a muffle furnace at $550 \pm 20^\circ\text{C}$ until carbon-free ash. Cool in desiccators and weigh. Ignite the crucible again in the muffle furnace for 30 minutes, cool, and weigh. Repeat the process until the difference between the successive weighing is less than 1mg. Use the ash for determination of minerals content.

Calculation:

The percentage of Total ash(w/w) = $100(M_2 - M) / M_1 - M$

Where,

M_2 = the lowest mass in g of the dish with ash

M_1 = mass in g of the empty dish, M = mass in g of the dish with the material taken for test

4.5.2 Moisture

The moisture was determined by the method of AOAC 20th Edn.2016,984.25

Procedure:

Thoroughly homogenize the test sample in the blender and weigh 10g in duplicate, using a balance accurate to 0.1mg. Use desiccated, tared weighing dishes, preferably made of Ni, Al stainless steel, or glass, 60-80mm diameter and 25mm deep, with well-fitting but easily removable lids. Place uncovered dish containing test portions with lid in convection drying oven at $103 \pm 2^\circ\text{C}$ for 16 hours. Replace the lid and transfer the desiccator to cool. When cool, weigh as quickly as possible to 0.1mg. Uncover and replace the dish and lid in the oven for an additional 2 hours. Replace the lid, cool in a desiccator and reweigh. Repeat 2 hours of drying,

desiccation and weighing steps until a decrease in mass between successive weightings does not exceed 0.5mg or until an increase in mass is recorded.

Calculations:

$$\% \text{ of Moisture(w/w)} = 100(M_1 - M_2) / M_1 - M$$

Where,

M₁ = mass in g of the dish with the material before drying

M₂ = mass in g of the dish with the material after drying, and M = mass in g of empty dish

4.5.3 Energy

Energy is calculated by the FAO method.

It is calculated by the following formula:

$$\text{Energy(kcal/100g)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

4.5.4 Carbohydrates

The total carbohydrate content in the porridge powder was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fibre, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat(g)} + \text{Protein(g)} + \text{Fibre(g)}]$$

4.5.5 Total fat

The total fat was determined by the method of AOAC 20th Edn.2016,920.39

Procedure:

Weigh accurately about 2g of sample in a fat extraction tube; add 2.0ml of alcohol to prevent lumping on addition of acid, and shake to moisten all particles. Add 10ml

HCl (25+11), mix well and set tube 30 to 40 mins in water bath at 70°C and 80°C, shaking frequently. Cool to room temperature and add alcohol. Add 25ml diethyl ether to the flask and shake for 1.0 mins. Then add 25ml petroleum ether to the flask and shake 1min. Decant ether layer into the pre-weighed dry flask through anhydrous sodium sulphate. Then, Decant ether layer into a pre-weighed dry flask through anhydrous sodium sulphate. Then add 25ml of 1:1 diethyl ether, and petroleum ether to the flask and shake for 1.0min. Ether layer can be evaporated under a water bath. Keep the flask into the oven for further evaporation of ether residue. Remove the flask from the oven and place in desiccators to cool to room temperature. Record the weight of the flask plus fat.

Calculation:

% of Fat = (Weight of flask + fat) – Empty weight of the flask ×100 / Sample weight

4.5.6 Protein

The protein(N×6.25) was determined by the method of AOAC 20th Edn.2016, 986.25

Procedure:

Weigh a suitable test portion of the sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g CuSO₄ and 25mL or more, if necessary, of Conc.H₂SO₄ to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H₂O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

%N (w/w) = (TV_s-TV_b) x Normality of standard HCl ×1.4007 / Sample weight

where,

TV_b = Titer value of blank ; TV_s = Titer value of sample

4.5.7 Dietary fibre

The dietary fibre was determined by the method of AOAC 20th Edn.2016,985.29

Procedure:

Weigh duplicate 1g test portion, accurate to 0.1mg into 400ml tall-form beakers. Add 0.1ml tetramethyl solution. Cover the beaker with Al foil and place in boiling water bath for 15mins. Add 5mg protease. Incubate 30 mins at 60°C with continuous agitation and cool down. Add 10ml 0.325M HCl solution. Measure pH and dropwise and add acid if necessary. Add 280ml 95% ethyl alcohol preheated to 60°C. Wash residue successfully with three 20ml, portions of 78%ethyl alcohol and two 10ml portions of acetone. Time for filtration and washing will vary from 0.1h to 6h. Dry crucible containing residue overnight in 70°C vacuum oven. Cool in desiccator and weigh to nearest 0.1mg.

Calculations:

Determination of blank:

B= blank, mg= weight residue- $P_B - A_B$

Where,

Weight residue = average of residue weights(mg) for duplicate blank determination; and

$P_B - A_B$ = weights(mg)of protein and ash

Calculate TDF as follows:

$TDF (\%) = [(weight\ residue - P - A - B) / weight\ test\ portion] \times 100$

Where,

Weight residue = average of weights (mg) for duplicate blank determination

P and A = weights(mg) of protein and ash

Weight test portion = average of two test portion weights(mg) takes.

4.6 Evaluation of micronutrients in *D.quercifolia* porridge mix

4.6.1 Sodium and potassium

The sodium and potassium were determined by the method of AOAC 20th Edn.2016,969.23

Procedure:

Weigh 4g test portion into the crucible, and char on electric hot plate or over low flame. Place on cold furnace and bring to 525°C and 2h to white ash. Add 15ml dil. HNO₃ (1-4) to the crucible, breaking up ash with a stirring rod if necessary. Filter through acid-washed quantitative paper into a 100ml volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume.

For flame emission- Read directly

For flame absorption- Dilute for direct readout as follows: Place 1ml aliquot in 100ml volumetric flask and dilute to volume with H₂O for Na; place 1ml aliquot in 25ml volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2ml HNO₃ to 100ml with H₂O.

Calculation:**For flame emission photometers:**

Na or K (100g) = 100×F (in mg)

where,

E = (percent T of unknown) - (percent T of blank)

E = (percent T of standard lower concentration than test solution)- (percent T of blank)

E = (percent T of standard of higher concentration than test solution)- (percent T of blank)

C = mg in Na or K/ml in standard of lower concentration than test solution

C = mg in Na or K/ml in standard of higher concentration than test solution

F = dilution factor

For flame absorption photometers: Convert percent absorption to absorbance.

Mg in Na or K /100g = (concentration unknown ×F ×100) /g test portion.

4.6.2 Calcium

Calcium was determined by titration method (IS 5949:1990 (RA.2010))

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 percent sodium hydroxide solution (4.6) using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass = $V_1 \times 0.04008 \backslash M$

where,

V_1 = Volume in ml of EDTX solution in titration; and

M = Mass in g of the sample in the solution for the test

4.6.3 Iron and Zinc

Iron and zinc were determined by the method of AOAC 20th Edn.2016,999.11

Procedure:

Homogenize the product if necessary, using non-contaminating equipment. In a crucible, weigh 10-20g test portion to nearest 0.1g. Dry in a drying oven or water bath at 100°C. Place dish in the furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C /h to 450°C. Let dish stand for at least 8 hours or overnight. Wet ash with 1-3ml water and evaporate on water bath. Put crucible in furnace at no more than 200.C and raise temperature (50°C -100°C /h) to 450°C. Proceed with ashing at 450°C for 1-2 hours or longer. Repeat the procedure until the product is completely ashed. Add 5ml 6M HCl to crucible

ensuring that all ash comes into contact with the acid. Evaporate acid on water bath and dissolve residue in 10-30ml, to the nearest 0.1ml of 0.1M HNO₃. Cover with watch glass and lid for 1-2 hours. Then, it is determined by atomic absorption spectrometry (AAS).

Calculations:

Calculate the detection limit for Zn and Fe:

DL= 3 x standard deviation of the mean of the blank determination (greater than or equal to 20)

Calculate the concentration, c, of the metal in the test sample according to the formula:

$$c = (a-b) \times V / m$$

where, c = concentration in the test sample (mg/kg)

a = concentration in the test solutions (mg/L)

b = mean concentration in the blank solution (mg/L)

V = Volume of the test solution (mL)

m = weight of the test portion (g)

If (a-b) is lower than the DL, then (a-b) is substituted with DL for calculation of the limit of detection in the test portion.

4.7 Analysis of sensory quality of *D.quercifolia* porridge mix

The cooked *D.quercifolia* porridge was served hot and evaluated for sensory attributes (Appearance, consistency, aroma, mouthfeel, flavour and overall acceptability) through a panel of 10 subjects using a 9-point hedonic scale of scores from 1-dislike extremely to 9-like extremely.

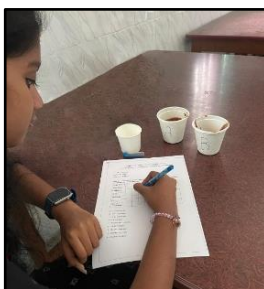


Fig 4.3: Sensory evaluation of the porridge

4.8 Determination of shelf life of *D.quercifolia* porridge mix

The chemical(moisture, acidity) and microbiological testing(Total pate count, Coliforms, Yeast and mould) was performed at the 12th, 24th, and 36th day of storage to determine the shelf life of *D.quercifolia* porridge mix.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Nutritional analysis of *Dynaria quercifolia*

Table 5.1: Nutritive content in *D.quercifolia* per 100g

S.No.	Nutrients	Results
1	Energy	357kcal
2	Carbohydrates	75.2g
3	Protein	7.01g
4	Total fat	3.13g
5	Dietary fibre	11.4g
6	Total sugars	13.1g
7	Sodium	246mg
8	Calcium	297mg
9	Iron	6.03mg

The data in Table 5.1 shows the nutritional analysis of *D.quercifolia* for different parameters. The nutritional analysis of the *D.quercifolia* porridge revealed that it comprises of 357g/100g of energy, 75.2g/100g of carbohydrates, 4.37g/100g of total fat, 7.01g/100g of protein, 11.4g/100g of dietary fibre, 13.1g of total sugars, 246mg of sodium, 297mg of calcium and iron of 6.03mg respectively.

The average daily requirement of a sedentary adult is 1885kcal, hence incorporating *D.quercifolia* in the diet gives 357kcal daily. The rhizome naturally has a low sugar content. There is a generous amount of calcium(297mg) which aids in reducing inflammation and also helps in the prevention of arthritis.

5.2 Evaluation of macronutrients in *D.quercifolia* porridge mix

Table 5.2: Macronutrients content in *D.quercifolia* porridge mix/100g

S.No.	Nutrients	Results
1	Moisture	4.59%
2	Total ash	2.83%
3	Energy	407kcal
4	Carbohydrates	77.4g
5	Protein	14.4g
6	Total fat	4.37g
7	Dietary fibre	13.1g

The macronutrients of *D.quercifolia* porridge mix is presented in Table 5.2 for different parameters. The macronutrient content in *D.quercifolia* porridge mix comprises of 4.59% of moisture, total ash content of 2.83%, energy of 407kcal, carbohydrates of 77.4g/100g, 77.4g/100g of carbohydrates, 14.4g of protein, 4.37g/100g of total fat, 13.1g/100g of dietary fibre.

The total ash content in the product is essential to analyse the amount of minerals present in it. The moisture content is a critical factor that directly impacts the physical and chemical properties of food. It is also useful for assessing the freshness, quality, and shelf life of food products. The moisture content in the porridge is 4.59%.

5.3 Evaluation of micronutrients in *D.quercifolia* porridge mix

Table 5.3: Micronutrients content in *D.quercifolia* porridge mix/100g

S.No.	Nutrients	Results
1	Sodium	16.4mg
2	Calcium	209mg
3	Iron	5.41mg
4	Potassium	757mg
5	Zinc	27.3mg

From the above table 5.3 the micronutrient content in the *D.quercifolia* porridge comprises of 16.4mg/100g of sodium, 209mg/100g of calcium, 5.41mg of iron, 757mg of potassium and 27.3mg/100g of zinc.

5.4 Analysis of sensory quality of *D.quercifolia* porridge mix

Table 5.4: Sensory evaluation of *D.quercifolia* porridge mix

Attributes	Quantity of DQ	Medium	Average scores
Appearance	25g	M	8.3
		W	7.2
	50g	M	7.8
		W	7.5
Consistency	25g	M	8.8
		W	8.3
	50g	M	8.1
		W	7.1
Aroma	25g	M	7.6
		W	7.2
	50g	M	6.2
		W	7.6
Mouthfeel	25g	M	8.1
		W	7.9
	50g	M	6.8
		W	7.5
Flavour	25g	M	8.8
		W	7.7
	50g	M	8.3
		W	7.4
Overall acceptability	25g	M	8.1
		W	7.6
	50g	M	7.2
		W	7.5

The table 5.4 shows the sensory evaluation of the porridge. The incorporation of 25g of *D.quercifolia* into porridge prepared with milk has been observed to yield superior taste profiles, as indicated by the average scores of overall acceptability. In assessing the sensory attributes of the porridge, the mean scores for appearance, consistency, aroma, mouthfeel, flavour, and overall acceptability were 8.3, 8.8, 7.6, 8.1, 8.8, and 8.1, respectively.

5.5 Determination of shelf life of *D.quercifolia* porridge mix

Table 5.5 : Determination of shelf life of *D.quercifolia* porridge mix (Chemical test)

S.No		1	2	3
Testing day from manufacturing		Day 12	Day 24	Day 36
Date of analysis		05.03.24	05.03.24	05.03.24
End date		11.03.24	11.03.24	11.03.24
Storage temperature		40°C	40°C	40°C
Storage		75%	75%	75%
Chemical test	Moisture (g/100g)	9.11	9.15	9.33
	Acidity (g/100g)	1.07	1.09	1.10

From the above table 5.5, the analysis of moisture and acidity in *D.quercifolia* porridge mix revealed an increase in both factors over consecutive days.

**Table 5.6: Determination of shelf life of *D.quercifolia* porridge mix
(Microbiological test)**

S.No		1	2	3
Testing day from manufacturing		Day 12	Day 24	Day 36
Date of analysis		05.03.24	05.03.24	05.03.24
End date		11.03.24	11.03.24	11.03.24
Storage temperature		40°C	40°C	40°C
Storage		75%	75%	75%
Microbiological test	Total plate count (CFU/g)	3,60,000	3,60,000	3,60,000
	Coliforms (CFU/g)	1,60,000	1,60,000	1,60,000
	Yeast and Mould (CFU/g)	1,20,000	1,20,000	1,20,000

The table 5.6 shows that there were no significant changes in the chemical and microbiological properties of *D. quercifolia* porridge mix upto 50 days of storage at 40°C ,75% storage humidity.

CHAPTER 6

SUMMARY AND CONCLUSION

Autoimmune diseases result from the immune system attacking the body's own cells and tissues. The autoimmune diseases exhibit clinical diversity. They can either manifest with organ-specific patterns or present in a generalized manner, affecting various systems throughout the body.

Autoimmune diseases like rheumatoid arthritis involve the immune system and target various components of the joints, leading to inflammation, tissue damage, and pain. The global prevalence of the disease is estimated at around 1–2%, with a large variation among different populations. In developed countries, osteoarthritis is recognized as one of the top 10 prevalent disabilities among older individuals, particularly those who continue to participate in the workforce. The objectives of rheumatoid arthritis treatment include minimizing joint inflammation and pain, optimizing joint functionality, and averting joint destruction and deformities.

Considerable focus has been placed on the role of diet and nutrients as potential environmental factors that may impact the development and progression of the disease. While several studies have suggested links between dietary habits, particularly regarding fruit, vegetable, or meat intake, and the development of the disease, the results remain inconclusive.

Hence, we have used an ingredient called *D.quercifolia* and formulated a porridge mix which not only helps in managing but also prevent arthritis.

The formulated porridge mix was evaluated for its nutritional quality, macronutrients and micronutrients, sensory analysis and shelf-life testing. The average daily requirement of a sedentary adult is 1885kcal, hence incorporating *D.quercifolia* in the diet gives 357kcal daily. The protein content in the porridge is notably low 7.01g/100g, hence it is safe to consume.

D.quercifolia has significant nutritional benefits. This porridge is suitable not only for arthritis patients but also for healthy individuals due to its various health benefits. Consuming a glass of porridge four times per week is recommended to assess the efficacy of the improvement in recovery. Taking all these factors into account, its

nutritional richness adds variety to patients diets and makes it a nutritious breakfast option for people of all ages, including those who are healthy.

Further it can be recommended that besides soup and porridge, other recipes such as biscuits, energetic bars can be formulated. The *D.quercifolia* porridge mix can be given to arthritis patients to determine the efficiency of the porridge in arthritis management and conducting a comparative study on Mudavattukal kilangu bought from various regions.

CHAPTER 7

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**FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH
PREBOITIC FOR IRON DEFICIENCY PATIENT**

PROJECT REPORT

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science in
CLINICAL NUTRITION AND DIETETICS

By

E.RUTH KEZIAH (41736014)

S.RAGAVI (41736013)



**DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade “A++” by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **E.RUTH KEZIAH (41736014)** who have done the project work as a team **E. RUTH KEZIAH (41736014)** AND **S.RAGAVI (41736013)** who carried out the project entitled "**FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH PREBOITIC FOR IRON DEFICIENCY PATIENT**" under our supervision from July 2023 to April 2024.

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DECLARATION

I am E.RUTH KEZIAH(41736014) hereby declare that the Project Report entitled "FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH PREBOITIC FOR IRON DEFICIENCY PATIENT "done by us under the guidance of Dr. (Mrs) GAYATHRI. P at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

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DECLARATION

We S. RAGAVI (41736013) and E.RUTH KEZIAH (41736014) hereby declare that the Project Report entitled **"FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH PREBOITIC FOR IRON DEFICIENCY PATIENT"** done by us under the guidance of Dr. (Mrs) GAYATHRI P at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

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ABSTRACT

The most common form of anemia in the world is Iron-deficiency anemia. Anemia is defined as the clinical state where the blood lacks healthy red blood cells and this result in decreased oxygen flow to different organs of the body. Iron deficiency anemia is occurred due to insufficient iron. Without adequate iron, the body cannot produce enough of hemoglobin which is a vital part of red blood cells and thus it causes impaired oxygen supply.

The aim of investigation to produce high quality, nutritional value, sensory properties biscuits from quinoa flour QF, flaxseed FS and wheat flour WF. The materials used in this investigation were QF, FS and WF as well as other ingredients used to produce biscuits.

Cookies were subjected to analysis as Energy, protein, carbohydrates, fat in testing for macronutrients as well as mineral analysis (potassium, calcium, sodium and iron) and evaluated for consumer acceptance by sensory analysis.

The proximate analysis of the product revealed that it comprises of 495g/100g of energy, 65.9g/100g of carbohydrates, 21.5g/100g of total fat, 9.54g/100g of protein, 6.81g/100g of dietary fiber, iron 3.74g/100g with hedonic rating the maximum and minimum overall acceptability is 4.3 and 4.5 respectively.

During the study, it was observed that Quinoa millet biscuit had a IRON level of 3.74mg/100gm.

Keywords: quinoa flour, flaxseed, wheat flour.

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CHAPTER -1

INTRODUCTION

Anemia is characterized by a clinical state wherein the blood lacks sufficient healthy red blood cells, leading to decreased oxygen flow to various organs of the body. Iron deficiency anemia occurs due to insufficient iron levels. Inadequate iron hampers the production of hemoglobin, a crucial component of red blood cells, resulting in impaired oxygen supply. Consequently, individuals may experience fatigue and shortness of breath. Iron deficiency can disrupt biological functions such as respiration, energy production, DNA synthesis, and cell proliferation.

Iron-deficiency anemia is the most prevalent form of anemia globally. Iron deficiency and iron deficiency anemia are among the most prevalent medical conditions and global health challenges encountered in everyday clinical practice. Iron deficiency remains a leading cause of anemia worldwide, significantly impacting the lives of young children and premenopausal women in both developed and low-income countries. Enhancing the diagnosis and treatment of this condition is imperative. (Prolay Paul *et al.*, 2021).

Iron deficiency and anemia of chronic disorders commonly coexist and are observed in elderly patients and those with chronic kidney disease. (susan.f clark., 2018) In adults, iron-deficiency anemia can lead to a wide array of adverse outcomes, such as diminished work or exercise capacity, impaired thermoregulation, compromised immune function, gastrointestinal disturbances, and neurocognitive impairment.

Globally, approximately 1.62 billion people are affected by anemia, accounting for around 24.8% of the population. The highest prevalence, at 47.4%, is observed among preschool-aged children, while the lowest occurrence is found in men, at 12.7%. Among all population groups, non-pregnant women are the most affected, with an estimated 468.4 million individuals affected.

One gram of iron is stored in the liver, with 0.4 grams in myoglobin and cytochromes. A small amount, approximately 3 mg, circulates bound to plasma

transferrin. Men and non-menstruating women typically lose around 1 mg of body iron per day, while menstruating women may lose an additional 1 mg daily on average. Full-term babies are born with 180 mg of iron, but they need to double their red cell mass within 12 months (low birth weight infants need to more than double their red cell mass). Iron requirements increase significantly during adolescence due to the rise in blood volume and lean body mass, which is further compounded in females by the onset of menstruation and during pregnancy by increases in maternal red cell mass and fetal erythropoietin.

Dietary iron consists of haem iron from animal sources and non-haem iron from cereals and vegetables. Haem iron, bound to hemoglobin and myoglobin, is more efficiently absorbed than non-haem iron. Non-haem iron is absorbed by intestinal luminal cells through a specific transporter, the divalent metal transporter located on the apical membrane of intestinal enterocytes. It is then released into circulation, where it binds to transferrin. Erythroblasts express transferrin receptors that accept iron-transferrin complexes, undergo endocytosis, and incorporate iron into hemoglobin. While the precise mechanism of haem-iron absorption remains unclear, potential transporters have been identified. (West AR, 2008;)

Enhancing dietary diversity to boost micronutrient intake represents the most favorable and sustainable strategy for preventing micronutrient malnutrition, (DeMaeyer EM, 1989) despite the superior absorption of heme iron from animal sources compared to non-heme iron from plant sources. Whole cereals, pulses, and vegetables, especially green leafy vegetables (GLVs), play a significant role in providing dietary iron in Indian diets. (Park K, 2019)

Cookies constitute the largest segment of snack items in bakery products, appealing to individuals across all economic backgrounds and age groups due to their affordability, extended shelf life, ease of consumption, and nutritional value. Baked for easy digestion and convenient portability, cookies are favored as on-the-go snacks during travel. According to statistical data, cookies rank as the second most consumed baked food product at 17.3%, following bread. Widely embraced worldwide, cookies offer a rich source of nutritional components and are produced in various types, including sugar-free, protein-

enriched, wheat germ and multigrain varieties. While refined flour, sugar, and fat are the primary ingredients in cookie production, minor components like flavonoids and additives are also utilized. Although synthetic colorants pose no health risks, there's a growing trend in the food industry towards natural colors in response to consumer demand. Natural colors offer numerous health benefits, including a diverse array of vitamins, minerals, nutritional value, and antioxidant properties. (Sunita Mishra, 2020)

Millets, among the oldest foods known to humanity, are likely the earliest cereal grains cultivated for domestic use. These small-seeded grasses exhibit remarkable hardiness and thrive in arid regions as rain-fed crops, even in conditions of low soil fertility and moisture. Their unique attributes distinguish millets from other cereals, as they can withstand dry climates, thrive in poor soils, and require minimal external inputs for growth. With superior nutritional profiles compared to conventional cereals, millets serve as staple foods in many households. India stands as the world's largest producer of millets, underscoring their importance in global agriculture. Properly stored, whole millets can maintain their quality for two years or longer. Baked goods predominantly rely on cereal flours blended with various ingredients. However, due to its lack of gluten, millet protein is unsuitable as the sole component for baking products.

The term "multigrain" flour denotes that the bread comprises various types of grains, setting it apart from whole grain breads. These grains may encompass oats, barley, cornmeal, wheat, millet, flax, and others. Nevertheless, there isn't a definitive list of grains required for a product labeled as "multigrain." Moreover, only a minimum of two different grains is necessary for a product to qualify as "multigrain" flour. Furthermore, the term doesn't specify how the grains should be processed. Hence, "multigrain" doesn't always equate to "whole grain." (Dhirendra Kumar Dinkar *et al*, July-2020)

Quinoa, scientifically known as *Chenopodium quinoa*, is classified as both a pseudo-cereal and pseudo-oil seeds crop, belonging to the family "Chenopodiaceae". Cultivated for many years in Andean regions, it is a dicot

plant reaching heights of 1-3m from the soil. Quinoa thrives in various soil types due to its extensive root system, extending up to 30cm. Remarkably, it can produce grains even when sown at altitudes of up to 4500 meters, boasting superior nutritional value compared to other cereal crops. Typically, quinoa exhibits cylindrical stems with a diameter of approximately 3.5cm, with variations in color among different varieties. Its leaves resemble those of a goose foot. The seeds of quinoa are round and flat, with a diameter ranging from 1.5 to 4.0 mm, and weigh about 1 g per 350 seeds. (Daraz. O *et al*, 2020)

The color of quinoa seeds varies from white to grey and black. Worldwide, there are nearly 250 varieties of quinoa, classified based on plant morphology, phenology, and physical appearance, with some consideration given to tissue chemical composition. (Mujica A., 1994)

Flax (*Linum usitatissimum*; Linaceae), commonly known as linseed, is primarily cultivated for its seeds and fiber. Its dark brown seeds are utilized in the food industry, often added to cookies bread and cakes. With an omega-3 fatty acid content of around 60%, flaxseed stands as one of the richest vegetarian sources of this compound, also containing omega-6 fatty acids. Incorporating flaxseed into one's diet provides appropriate levels of polyunsaturated fatty acids and plays a significant role in preventing and regulating cardiac and autoimmune diseases, as well as breast, prostate, and colon cancer. (Cunnane *et al*, 1993)

Cookies constitute the largest segment of snack items in bakery products, appealing to individuals across all economic backgrounds and age groups due to their affordability, extended shelf life, ease of consumption, and nutritional value. Baked for easy digestion and convenient portability, cookies are favored as on-the-go snacks during travel. According to statistical data, cookies rank as the second most consumed baked food product at 17.3%, following bread. Widely embraced worldwide, cookies offer a rich source of nutritional components and are produced in various types, including sugar-free, protein-enriched, wheat germ and multigrain varieties. While refined flour, sugar, and fat are the primary ingredients in cookie production, minor components like flavonoids and additives are also utilized. Although synthetic colorants pose no

health risks, there's a growing trend in the food industry towards natural colors in response to consumer demand. Natural colors offer numerous health benefits, including a diverse array of vitamins, minerals, nutritional value, and antioxidant properties. (Sunita Mishra, 2020)

Quinoa grains possess beneficial health attributes and can be utilized as a remedy for various diseases across all consumer groups. Individuals may suffer from conditions such as osteoporosis in women, anemia, obesity, and celiac disease. (Zevallos *et al.*, 2015)

Quinoa seeds have a long history of consumption, particularly in South America, where they are traditionally utilized. Similar to other cereals like rice, quinoa seeds are commonly used in soup preparations. Additionally, they serve as a breakfast cereal when puffed and are also processed into flour for various bakery products, including cookies, biscuits, breads and cakes. (Bhargava, 2006)

Functional foods encompass any substance or component within food that provides health benefits, aiding in the prevention and treatment of diseases. Key functional components of foods include fibers, polyunsaturated fatty acids, phytochemicals, active peptides, prebiotics, and probiotics. (Kucuk, 2002) While the human body synthesizes most saturated and unsaturated fatty acids, it does not produce essential ones such as omega-3 (linolenic acid) and omega-6 (linolenic acid). (Soccol *et al*, 2003)

Baking, a culinary method employing dry heat typically in an oven, or alternatively, in hot ashes or on hot stones, is primarily known for its application in producing bread but extends to various other food items. The gradual transfer of heat from the exterior to the interior transforms batters and doughs into finished goods with a crisp outer layer and a moist center. This technique can be combined with grilling to create a hybrid variation, utilizing both methods simultaneously or sequentially. Baking shares similarities with grilling, as the concept of the oven parallels that of a smoke pit. (Smita Kagwad *et al*, June 2019)

A functional food is characterized as a modified food or food ingredient that offers potential health benefits beyond its basic nutrients. Bakery products can be enriched with health-promoting active ingredients to create non-dairy-based functional foods. Ensuring the viability of these active components, such as probiotics, in the final products is essential to imparting beneficial effects on consumer health. However, the baking process presents a hurdle in maintaining the functionality of these heat-sensitive active ingredients due to the elevated temperatures involved. (Zhang, 2018)

The primary objective of defining marketing and tracing its evolution is to pinpoint the foundational field from which the study of consumer behavior emerged as a critical area of focus. Marketing is delineated as the process of identifying, foreseeing, and analyzing consumer needs and desires, with marketers striving to comprehend these needs and offer solutions in the form of goods or services. This definition underscores the central roles of marketers, organizations, and customers within the business landscape. In 1935, the American Marketing Association (AMA) provided a succinct definition of marketing as the systematic performance of business activities aimed at managing customer relationships, which emphasizes a comprehensive understanding of both business operations and customer dynamics.

Subsequently, in 1985, the AMA expanded this definition to include marketing's involvement in pricing, promoting, and distributing products to fulfill customer needs and desires, thus elucidating the fundamental principles of marketing theory and execution. It is elucidated that marketing operates at both managerial and societal levels, entailing the scientific selection of markets, the persuasion and retention of customer bases, and the facilitation of societal and consumer participation in value creation, provision, and exchange. Across these definitions, the pivotal role of the customer is highlighted, emphasizing the imperative nature of understanding consumer behavior for effective strategy formulation. (Maxwell Chufama, 2021,)

CHAPTER-2

REVIEW OF LITERATURE

Prolay Paul *et al.*, have reported that Anemia is characterized by a clinical state in which the blood lacks sufficient healthy red blood cells, leading to decreased oxygen delivery to various organs in the body. Iron deficiency anemia arises from insufficient iron levels, hindering the body's ability to produce an adequate amount of hemoglobin, a crucial component of red blood cells necessary for oxygen transport. This condition is more prevalent in women, particularly during menstruation and pregnancy, due to increased iron demands. Various factors, including physiological issues such as poor digestive function, contribute to iron deficiency. Insufficient iron leads to a deficiency in hemoglobin impairing oxygen delivery, as the bone marrow struggles to produce an ample supply.

Most cases of iron deficiency anemia are mild and easily treatable, untreated cases can lead to complications. Iron supplementation, typically taken on an empty stomach for optimal absorption, helps replenish iron levels in the body. However, if adverse effects occur, supplements can be taken after meals. Treatment often requires continued supplementation for several months, although these supplements may cause side effects such as constipation or black stools. Drug-induced iron deficiency anemia is a blood disorder triggered by medications that prompt the immune system to attack red blood cells prematurely, leading to their breakdown, a process known as hemolysis (Prolay Paul *et al.*, 08 august 2021)

Shashi Bhushan Kumar *et al.*, have reported that Iron deficiency anemia has reached epidemic proportions in developing countries and has become a major global public health concern, particularly affecting 0-5-year-old children and young women of childbearing age, especially during pregnancy. Iron deficiency can lead to life-threatening depletion of red blood cells, muscle function, and energy production, thereby causing weakness and impairing growth, motor skills, and cognitive performance. Iron deficiency anemia significantly impacts the well-being of the younger population and impedes the economic progress of developing nations like India. The etiology of Iron deficiency anemia involves an imbalance between iron intake/absorption/storage and iron utilization/loss.

Shashi Bhushan Kumar *et al.*, have reported that Despite numerous strategic programs aimed at increasing iron intake, simply improving iron intake alone has proven insufficient to effectively combat Iron deficiency anemia. Emerging critical risk factors for Iron deficiency anemia encompass various elements such as cultural dietary habits, infections, genetics, inflammatory conditions, metabolic disorders, dysbiosis, and socioeconomic factors. This review explores various Iron deficiency anemia mitigation initiatives in India and their associated limitations. The multifactorial nature of Iron deficiency anemia pathogenesis offers new avenues for enhancing mitigation programs and alleviating Iron deficiency anemia in India and globally (Shashi Bhushan Kumar *et al.*, 20 July 2022).

Shekhara Naik R *et al.*, reported that The role of flaxseed as a functional food continues to gain momentum, particularly in the cardiovascular health, owing to its rich content of alpha-linolenic acid, lignans, and soluble fiber. Flaxseed contains secoisolariciresinol diglucoside, a lignin derivative, which metabolizes into mammalian lignans, influencing early risk markers of mammary and colonic carcinogenesis in animal models.

Regular consumption of flaxseed products can impact serum cholesterol levels, glucose absorption, inflammation markers, and omega-3 fatty acid levels. Flaxseed exhibits significant antioxidant and anti-inflammatory properties in both experimental and human studies, with supplementation showing potential benefits in cardiovascular risk, certain cancers, and metabolic disorders. Numerous studies underscore the health-promoting and disease-preventing potential of raw flaxseed and its baked products, positioning it as a promising nutraceutical with protective and therapeutic properties (Shekhara Naik R *et al.*, 08 July 2020)

Charu Katare *et al.*, have reported that The surge in recognition of flax as a functional food continues, with recent attention focused on its potential benefits for cardiovascular health due to its rich content of alpha-linolenic acid, lignans, and soluble fiber. The potential of flax seed as a nutraceutical and its role as a protective and therapeutic medicinal food are underscored in this review (Charu Katare *et al.*, 2012)

Nihir Soni *et al.*, have reported that The study conducted aimed to determine

the most suitable blend of pulses flour for partially replacing refined wheat flour in cookies to enhance their protein content. Soybean, Moth bean, and Chickpea were identified as potential protein sources with sufficient biological value based on existing literature. Different replacement levels (10, 15, 20, and 25 per cent) of these flours were tested in the study. Cookies were then developed using these blends and subjected to analysis including physical, chemical, textural, and sensory evaluations. Following the analysis, cookies containing 20% Chickpea and 80% refined wheat flour were selected, resulting in an increase in protein content from 2.9% to 6.8% (Nihir Soni *et al.*, 2018)

PP Salunke *et al.*, have reported that The present research aimed to investigate the potential use of barnyard millet, an underutilized yet highly nutrient-rich grain, in cookies. Initial experiments were conducted to determine the optimal blend of barnyard millet flour with maida and wheat flour for producing high-quality cookies. Three formulations were selected: 50% maida and 50% wheat flour, 30% maida and 70% barnyard millet, and 20% wheat flour and 80% barnyard millet flour. These formulations were then packed in LDPE and stored at ambient temperature for 90 days to assess their storage stability.

The chemical composition of the fresh cookies varied across formulations. For MWF50, the moisture content was 4.0%, protein content was 11.55%, crude fat was 26.30%, crude fiber was 1.10%, carbohydrates were 71.65%, calcium was 35.50 mg/100 g, phosphorus was 238 mg/100g, and iron was 3.80 mg/100 g. For MBF70, the moisture content was 3.10%, protein content was 7.64%, crude fat was 26.81%, crude fiber was 6.95%, carbohydrates were 68.02%, calcium was 20.90 mg/100 g, phosphorus was 232 mg/100g, and iron was 4.31 mg/100 g. For WBF80, the moisture content was 3.0%, protein content was 7.38%, crude fat was 27.10%, crude fiber was 8.22%, carbohydrates were 66.28%, calcium was 30.60 mg/100 g, phosphorus was 295 mg/100g, and iron was 4.98 mg/100 g.

Sensory evaluation of the cookies was conducted monthly for three months during storage. Overall acceptability scores were affected by storage duration. The results showed a decrease in overall acceptability scores for the control (from 7.35 to 7.10), MWF50 (from 7.50 to 7.15), MBF70 (from 8.45 to 8.00), and WBF80 (from 8.63 to 8.15) formulations in LDPE over 90 days of storage. However, the storage study

demonstrated that cookies incorporating maida, wheat, and barnyard millet flour could be stored for up to three months in LDPE with minimal losses in sensory, nutritional, and textural characteristics. There were no significant differences in protein, crude fiber, calcium, and iron content with the advancement of the storage period over three months. The cookies remained acceptable for consumption even after three months of storage at ambient temperature (PP Salunke *et al.*, 30 June 2019)

Viktoria Angeli *et al.*, have reported that Quinoa (*Chenopodium quinoa* Willd.) is indigenous to the Andean region and has garnered global interest due to its exceptional nutritional value, boasting higher protein content compared to other cereals and a superior distribution of essential amino acids. Its versatility extends to serving as an alternative to milk proteins. Moreover, quinoa is rich in essential fatty acids, minerals, vitamins, dietary fibers, and carbohydrates with advantageous hypoglycemic effects, all while being gluten-free. The resilience of the quinoa plant against cold, salt, and drought has earned it the moniker "golden grain," driving its increasing production and product diversification, particularly highlighted during its designation as the international year of quinoa in 2013. This review comprehensively explores the nutritional and biological attributes of quinoa cultivated worldwide over the past two decades, delving into the evolution of traditional processing methods and products, their integration into modern food processing, and the potential utilization of quinoa by-products across various industrial sectors. Additionally, it addresses socio-economic and environmental challenges associated with quinoa's production, consumption, and marketization, advocating for a holistic approach to promoting quinoa's cultivation and consumption (Viktoria Angeli *et al.*, 19 February 2020)

Mariane Lutz *et al.*, reported that The resurgence of interest in quinoa and its reintroduction into the diet is motivated by the current epidemiological landscape, characterized by diseases with risk factors that can be mitigated through a balanced and nutritious diet, with quinoa being a prominent component due to its status as a "superfood." Additionally, it has been designated as one of the crops prioritized by the Food and Agriculture Organization (FAO) to enhance food security (Mariane Lutz *et al.*, 2017)

Vandana et al., reported that The raw and processed quinoa grains has been examined. Biscuits were formulated using varying ratios of refined wheat flour and quinoa flour, namely 100:0, 75:25, 50:50, 25:75, and 0:100. These biscuits were then evaluated for their physical, textural, sensory, and nutritional qualities. The physical characteristics of the biscuits indicated an increase in product thickness, weight, and density with the incorporation of quinoa flour, while diameter, spread ratio, and volume decreased. Changes in textural characteristics were also noted, with hardness values increasing as the proportion of quinoa flour in the recipe increased. Sensory evaluation results revealed that biscuits containing up to 75 percent quinoa flour were acceptable to panelists. Furthermore, the nutrient composition of the biscuits demonstrated that protein, fat, fiber, and energy content increased as the level of quinoa (Vandana *et al.*, 2020)

Dorna Davani-Davari *et al.*, reported that Prebiotics, a category of nutrients broken down by gut microbiota, have garnered increasing interest due to their potential impact on overall human health. By nourishing intestinal microbiota, they generate short-chain fatty acids that enter the bloodstream, influencing not just gastrointestinal health but also distant organs. Fructo-oligosaccharides and galacto-oligosaccharides stand out as pivotal prebiotic groups with significant health benefits. Given their natural occurrence in low quantities in foods, scientists are exploring methods to produce prebiotics on an industrial scale. With their demonstrated safety and advantages in production and storage over probiotics, prebiotics emerge as compelling candidates for enhancing human health either as substitutes for or in conjunction with probiotics. This review explores various facets of prebiotics, shedding light on their indispensable role in human well-being (Dorna Davani-Davari *et al.*, 09 march 2019)

Thaisa Menezes Alves Moro *et al.*, reported that Burdock roots, rich in fructooligosaccharides and phenolic compounds, are underutilized as a food ingredient. This study aimed to produce burdock root flour (BRF) through conventional drying and assess its viability as a prebiotic ingredient in cookies. The cookies were made by substituting refined wheat flour with BRF at 0% (C0), 5% (C1), and 10% (C2) concentrations in a blend with whole wheat flour. BRF contained $40.16 \text{ g} \times 100 \text{ g}^{-1}$ of total dietary fiber, $181.74 \text{ mg} \times 100 \text{ g}^{-1}$ of chlorogenic acid, and $6.19 \text{ g} \times 100 \text{ g}^{-1}$ of total fructooligosaccharides (FOS), demonstrating in vitro

prebiotic activity. The cookies C1 and C2 contained 2.2% and 4.94% (on a dry basis) of BRF, respectively, and exhibited similar ($p > .05$) physicochemical and technological properties, except for the hardness of C1 (24.83 N), which was higher ($p < .05$) than C0 and C2. Incorporating BRF significantly increased ($p < .05$) FOS content in cookies from 42.38 (C0) to 366.68 mg $\times$ 100 g⁻¹ (C2). Cookies with 4.94% BRF showed a significant positive effect ($p < .05$) on prebiotic activity. Burdock roots present a promising alternative to introduce prebiotic oligosaccharides into bakery products, catering to consumers seeking functional foods with health benefits (Thaïsa Menezes Alves Moro *et al.*, 2018)

Sargun Kaur *et al.*, reported that The aim of this chapter is to deepen our understanding of consumer behavior and the factors shaping purchasing decisions, while also shedding light on diverse schools of thought that have influenced the evolution of consumer research. By exploring various dimensions found in the literature, we can effectively characterize and distinguish between different viewpoints on consumer research. This sets the stage for driving transformative shifts in future consumer research paradigms (Sargun Kaur *et al.*, 146-153)

Rosna Tadju *et al.*, reported that The study aimed to assess the sensory attributes of biscuit products fortified with a blend of sorghum flour and corn bran flour. Analyzing the data involved employing Analysis of Variance (ANOVA) to compare three treatment combinations: P1 (62% sorghum flour + 38% corn bran flour), P2 (72% sorghum flour + 28% corn bran flour), and P3 (83% sorghum flour + 17% corn bran flour). The sensory characteristics evaluated by the panelists encompassed texture, color, aroma, and taste. The findings revealed that the P3 treatment, featuring 83% sorghum flour and 17% corn bran flour, emerged as the preferred choice across all sensory parameters, including texture, color, aroma, and taste (Rosna Tadju *et al.*, 2021)

Khaled M *et al.*, reported that This work aimed at determining the maximum acceptable level of wheat germ that can be added to one of homemade biscuits in Jordan, studying some chemical and sensory properties of this product, moreover, studying the effect of storage time after two months at room temperature on the sensory parameters of this product. The results showed that the addition of wheat germ in biscuit making at level of 20% (w/w) enhanced the nutritional value by

increasing protein and total mineral content, as well as decreasing fat, total carbohydrates and total calories. The acceptability of sensory characteristics was improved by this fortification immediately after preparation and during storage (*Khaled M et al., 2017*)

Gamal Saad Elhadidy et al., reported that The investigation aimed to create high-quality biscuits with enhanced nutritional value, physical attributes, and sensory properties by incorporating quinoa flour (QF), naked barley flour (NBF), carrot powder (CP), and wheat flour (WF). The materials utilized included QF, NBF, CP, WF, and additional ingredients for biscuit production. Results revealed that formulations containing QF, NBF, and CP exhibited higher levels of ether extract, ash, and crude fiber compared to those using WF alone. Additionally, minerals such as potassium (K), calcium (Ca), phosphorus (P), and iron (Fe) were found in higher concentrations in NBF, QF, and CP compared to WF. Blends of naked barley, quinoa, and CP were also identified as rich sources of essential amino acids. Furthermore, biscuits formulated with NBF, QF, and CP (B2, B3, B4, and B5) demonstrated higher biological value (BV) or computed protein efficiency ratio (C-PER) compared to the control (WF). Through a carefully implemented physical process involving a well-blended combination of supplements, the study successfully produced biscuits with outstanding sensory properties, including taste, colour, aroma, and overall acceptability (*Gamal Saad Elhadidy et al., july 2020*)

CHAPTER-3

AIM AND OBJECTIVE

3.1 AIM

To prepare a quinoa millet biscuit incorporated with prebiotics for iron deficiency patient

3.2 OBJECTIVE

- To prepare the quinoa millet biscuit.
- To process and evaluate the nutritional analysis of the biscuit.
- To assess Microbiological testing of the biscuit (total plate count, yeast and mould, *E.coli*, Coliforms & *Salmonella*)
- Sensory evaluation of biscuit(view, smell, taste, touch & hear)
- Packing and Labelling

CHAPTER-4

MATERIALS AND METHODS

MATERIAL

4.1.1 COLLECTION OF RAW MATERIAL:

Flax seed and Quinoa millet was purchased from a local market in Chennai city. The Bakery ingredients such as wheat flour, brown sugar, butter, baking soda, baking powder, vanilla essence and cardamom, salt were procured from local grocery shop in Chennai city.

4.1.2 PREPARATION OF COOKIES:

The biscuits were prepared after the flour preparation, followed by standard formulation, with the addition of three different levels of wheat flour, and quinoa flour. Dry ingredients like (flax seed flour, quinoa flour, wheat flour, baking powder) were mixed and sieved twice for uniform mixing of leavening agents to the flour. A weighted amount of salt was added in the flour mixture.

A weighted amount of unsalted butter was taken in a bowl and beaten until it melts. Sugar was added and beat continuously for creaming. Flour mix was added in smaller amounts into the cream and uniformly mixed. The dough was rolled and then biscuits were cut into small round shape using a biscuit cutter. Biscuits were kept in an electric oven for 15 to 20 minutes at 180°C for uniform baking.

4.1.3 PREPARATION OF QUINOA FLOUR:

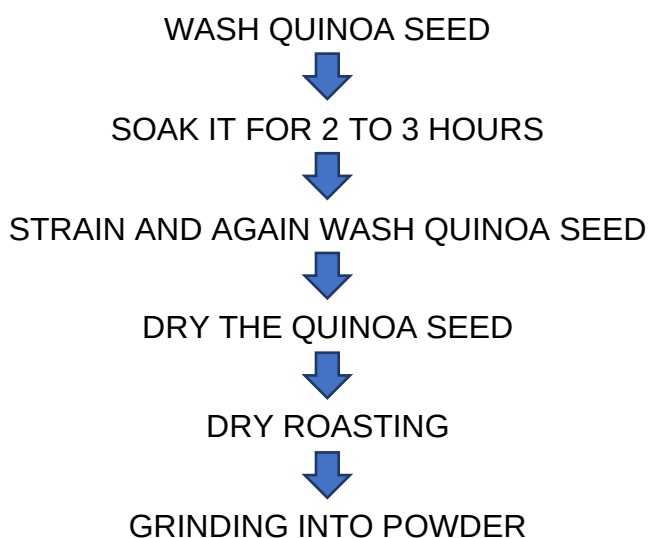


FIG 4.1: Flow diagram for preparation of Quinoa flour

4.1.4 PREPARATION OF QUINOA MILLET BISCUIT:

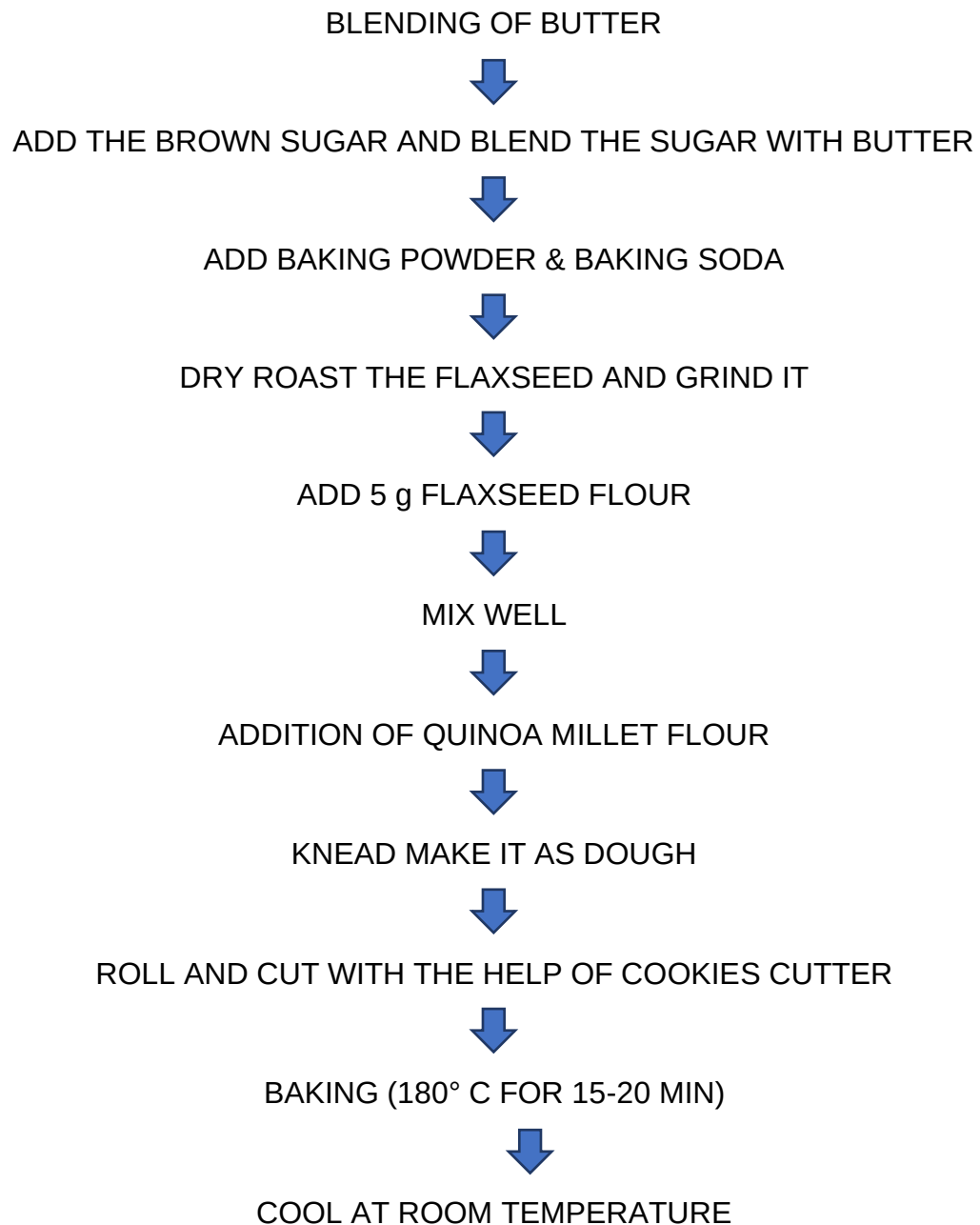


FIG4. 2: Flow diagram for preparation of Quinoa flour cookies

4.1.4 PREPARATION OF QUINOA MILET BISCUIT



Fig 4.3 DRYING

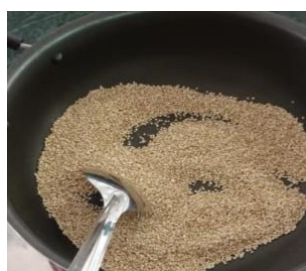


Fig 4.4 DRY ROASTING



Fig 4.5 GRINDING



Fig 4.6 ADDING
BAKING POWDER &
BAKING SODA



Fig 4.7 ADDING BROWN
SUGAR



Fig 4.8 BLENDING
BUTTER

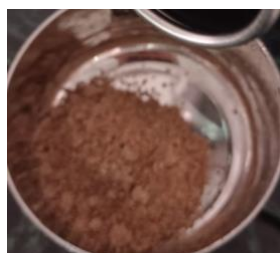


Fig 4.9 ADDING
FLAXSEED FLOUR



Fig 4.10 KNEAD



Fig 4.11 ROLL AND CUT



Fig 4.12 QUINOA MILLET BISCUIT

4.1.5 STANDARD FORMULATION OF COOKIES:

INGREDIENT	WEIGHT IN GM
Quinoa flour	120
Flax seed	5
Wheat flour	30
Butter	50
Brown sugar	50
Baking powder	0.1
Baking soda	0.1
Vanilla essence	5 Drops
Salt	0.1
Cardamom	1

4.2 METHODOLOGY

4.2.1 NUTRITIONAL EVALUATION ANALYSIS:

ENERGY

The caloric value was calculated in kilo-calories per 100g (kcal/100g) by multiplying the crude fat, protein and carbohydrate values by At water factors of 37, 17 and 17 respectively. This method is represented by the following formula.

$$\text{Energy (KCAL/100g)} = (\text{Carbohydrate} \times 4) + (\text{protein} \times 4) + (\text{fat} \times 9)$$

TOTAL CARBOHYDRATES

The total carbohydrate content in the quinoa millet biscuit was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fiber, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat (g)} + \text{Protein(g)} + \text{Fiber(g)}]$$

FAT

Use method A or C for feed ingredients and mixed feeds other than baked or expended, dried milk products, containing urea or mixed feeds that have at least 20% of crude fat derived from baked and expended or dried milk products.

- A. **Indirect methods:** determine moisture as in 934.01 or 920.36 then extract dried substance as in C And dried again. Report loss in weight as ether extract.

Direct methods:

- B. **Reagents:** Anhydrous ether: wash commercial ether with two or three portions H_2O , and solid NaOH or KOH, and let stand until most of H_2O is abstracted from the ether. Decant into Dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until H_2 evolution ceases. Keep ether, thus dehydrated, over metallic Na in lossely stoppered bottles.
- C. **Determination:**(Large amounts H_2O – soluble components such as carbohydrates, urea, latucacid, glycerol and others may interfere with extraction of fat if present, extract 2 g test portions on small paper in funnel with five 20 mL portion H_2O prior to drying for ether extraction).

Extract ca 2 g test portions dried as in 934 with anhydrous ether. Extraction period may vary from 4 h at condensation rate of 5 – 6 drops /s to 16 h at 2-3 drops/ s. Dry extract 30 min at 100°C, cool and weigh.

PROTEIN

Weigh suitable test portion of sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g $CuSO_4$ and 25mL or more, if necessary, of Conc. H_2SO_4 to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H_2O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

$$\%N (w/w) = (TVs-TVb) \times \text{Normality of standard HCl} \times 1.4007 / \text{Sample weight}$$

where, TVb = Titer value of blank; TVs = Titer value of sample

FIBER**Principle**

Duplicate test portion of dried foods, fat extracted if containing > 10% fat, are gelatinized with termally (heat stable α - amylase), and then enzymatically digested with protease amyloglucosidase to remove protein and starch. (When analyzing mixed diets, always extract fat prior to determining total dietary fiber.) Four volumes of ethyl alcohol are added to precipitate soluble dietary fiber. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying residue is weighed. One duplicate is analyzed for protein, and other is Incinerated at 525°C and ash is determined.

CALCULATION

Total dietary fiber = weight residue -weight (protein + ash)

Apparatus

- a) Fritted crucible- Porosity No. 2 (Pyrex No. 32940, coarse, ASTM 40-60 μ m; or Corning number and soak 529°C 36060 Buchner, fritted disk, Pyrex 60ML ASTM 40-60 μ m) Clean thoroughly heat 1 h at 525 °C and soak and then rinse in H₂O. Add ca 0.5 g Celite to air-dried crucibles and dry at 130°C to constant weight ($\geq$ 1h). Cool and store in desiccator until used.
- b) Vacuum source- Vacuum pump or aspirator equipped with in-line double vacuum flask to prevent contamination in case of H₂O
- c) Vacuum oven-70°C. Alternatively, 105°C air oven can be used.
- d) Desiccator
- e) Muffle furnace
- (f) Water baths – (1) Boiling (2) Constant temperature- Adjustable to 60°C, with either multi station shaker or multi station magnetic stirrer to provide constant agitation of digestion flasks during enzymatic hydrolysis.
- (g) Beakers. Tall-form, 400 or 600 ml
- (h) Balance. Analytical, readability to 0.1 mg.

(i) pH meter Standardized with pH 7 and pH 4 buffers.

Reagents

(a) 95% Ethanol-v/v Technical grade.

(b) 78% Ethanol - Place 207 mL H₂O into 1 L volumetric flask. Dilute to volume with 95% ethyl alcohol. Mix and dilute to volume again with 95% ethyl alcohol if necessary. Mix One volume HO mixed with four volumes 95% ethyl alcohol will also give 78% ethyl alcohol final concentration.

(c) Acetone.

(d) Phosphate buffer. -0.08 M. pH 6.0. Dissolve 1.400 g sodium phosphate dibasic, anhydrous (Na₂HPO₄) (or 1.753 g dihydrate) and 9.68 g sodium phosphate monobasic monohydrate (NaH₂PO₂H₂O) (or 10.94 g dihydrate) in ca 700 mL H₂O. Dilute to 1L with H₂O. Check pH with pH meter.

(e) α -amylase (heat stable). Termamyl.

(1) Store in refrigerator. Based on Nelson/Somogyi reducing sugar with soluble starck as substrate-10 000+1000 units/mL. (1 unit is defined as the amount of enzyme required to release 1 μ mole reducing sugar equivalents/ min at pH 6.5 and 40°C).

(2) Based on Ceralpha method using p-nitrophenyl-maltosaccharide as substrate in the presence of α thermostable α -glucosidase- 3000 + 300 Ceralpha units/ml. (1 unit of enzyme is required to release 1 μ mole p-nitrophenyl/min at pH 6.5 and 40°C)

(f) Protease-Keep refrigerated

(1) Casein assay- 300-400 Units/mL. [1 protease unit is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μ mole tyrosine equivalents/min from soluble casein at pH 8.0 and 40°C]; 7-15 units/mg (1 unit will hydrolyze casein to produce color equivalent to 1.0 μ mole tyrosine/min at pH 7.5 and 37°C). Color by Folin-Ciocalteureagent (2) Azo-casein assay-300-400 Units/ml. 1 unit endo-peptidase activity defined as the of enzyme required to hydrolyze (and solubilize in TCA) 1 μ mole tyrosine from soluble casein at pH 8.0 and 40°C]

(g) Amyloglucosidase-Keep refrigerated. (1) Strach / glucose oxidase -peroxidase 2000-3300 Units /mL (1 unit enzyme activity is defined as the amount of enzyme required to release 1 μ mole glucose/ min at Ph 4.5 and 40°C (p-nitrophenyl method- 130-200 Units/mL. [1 unit enzyme (PNP unit) is the amount of enzyme, which in the presence of excess levels of B-glucosidase, will release 1 μ mole p-nitrophenyl from

p-nitrophenyl β -maltosidase/min at 40°C)

The only enzyme which has been found to be significantly contaminated with interfering activities is amyloglucosidase. Thermostable α -amylase and protease from commercial sources have been found to be generally free of interfering enzymes. Low levels of β -glucanase have been detected in protease preparations, at levels well below that which would interfere with total dietary fiber analysis. The major contaminant in amyloglucosidase endo-cellulase and resulted in oats, endo-depolymerization of mixed-linkage β -glucan from barley and oats, with resultant underestimation of this dietary fiber component. The contamination of amyloglucosidase with endo- cellulase (β -glucanase) can be easily detected.

Alternatively, there are kits containing all three enzymes (pretested) available from a number of companies.

(h) Sodium hydroxide solution -0.275 M. Dissolve 11.00 g NaOH ACS in ca 700 mL H₂O in 1 L volumetric flask. Dilute to volume with H₂O.

(i) Hydrochloric acid solution - 0.325 M. Dilute stock solution of known titer, e.g., 325 mL 1 M HCl, to 1 L with H.O.

(j) Celite Acid-washed.

D. Enzyme Purity

To ensure absence of undesirable enzymatic activity in enzymes used in this procedure, run materials listed in through entire procedure each time lot of enzymes is changed, or at maximum interval of 6 months to ensure that enzymes have not degraded.

E. Test Portion Preparation

Determine total dietary fiber on dried test sample. Homogenize test sample and dry overnight in 70°C vacuum oven, cool in desiccator, and dry-mill test sample to 0.3-0.5 mm mesh. If test sample cannot be heated, freeze-dry before milling. If high fat content (>10%) prevents proper milling, defat with petroleum ether (3 times with 25 mL portions/g test sample) before milling.

Record loss of weight due to fat removal and make appropriate correction to final % dietary fiber found in determination. Store dry-milled test sample in capped jar in desiccator until analysis is carried out.

F. Determination

Run blank through entire procedure along with test portions to measure

any contribution from reagents to residue.

Weigh duplicate 1 g test portions, accurate to 0.1 mg. into 400 mL tall-form beakers. Test portion weights should not differ >20 mg. Add 50 mL pH 6.0 phosphate buffer to each beaker. Check pH and adjust to pH 6.0 ± 0.2 if necessary. Add 0.1 mL Termamyl solution. Cover beaker with Al foil and place in boiling water bath 15 min. Shake gently at 5 min intervals. Increase incubation time when number of beakers in boiling water bath makes it difficult for beaker contents to reach internal temperature of 95-100°C. Use thermometer to indicate that 15 min at 95-100°C is attained. Total of 30 min in water bath should be sufficient.

Cool solutions to room temperature. Adjust to pH 7.5 ± 0.2 by adding 10 mL 0.275 M NaOH solution.

Add 5 mg protease. [Protease sticks to spatula, so it may be preferable to prepare enzyme solution (50 mg in 1 mL phosphate buffer) and pipet 0.1 mL to each sample just before use]

Cover beaker with Al foil. Incubate 30 min at 60°C with continuous agitation. Cool. Add 10 mL 0.325 M HCl solution. Measure pH and dropwise add acid if necessary. Final pH should be 4.0-4.6. Add 0.3 mL amyloglucosidase, cover with Al foil, and incubate 30 min at 60°C with continuous agitation. Add 280 mL 95% ethyl alcohol preheated to 60°C (measure volume before heating). Let precipitate form at room temperature for 60 min.

Weigh crucible containing Celite to nearest 0.1 mg, then wet and redistribute bed of Celite in crucible by using stream of 78% ethyl alcohol from wash bottle. Apply suction to draw Celite onto fritted glass as even mat. Maintain suction and quantitatively transfer precipitate from enzyme digest to crucible.

Wash residue successively with three 20 mL portions of 78% ethyl alcohol, two 10 mL portions of 95% ethyl alcohol, and two 10 mL portions of acetone. Gum may form with some products, trapping liquid. If so, break surface film with spatula to improve filtration. Time for filtration and washing will vary from 0.1 to 6 h, averaging

0.5 h per sample. Long filtration times can be avoided by careful intermittent suction throughout filtration.

Dry crucible containing residue overnight in 70°C vacuum oven or 105°C air oven. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine weight of residue. Analyze residue from 1 test portion of set of duplicates for protein by using a conversion factor, except in cases where N content in protein is known,

Incinerate second test portion of duplicate 5 h at 525°C, Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash.

G. Calculations

Determination of blank:

$$B = \text{blank, mg-weight residue} - P_B - A_B$$

where weight residue = average of residue weights (mg) for duplicate blank determinations; and P_B and A_B = weight (mg) of protein and ash, respectively, determined in first and second residues.

Calculate TDF as follows:

$$\text{TDF \%} = \left[\frac{\text{weight residue} - P - A - B}{\text{weight test portion}} \right] \times 100$$

SUGAR

where weight residue = average of weights (mg) for duplicate blank determinations, and P and A = weights (mg) of protein and ash respectively, in first and second test portion residues; and weight test portion = average of two test portion weights (mg)

PROCEDURE:

Weigh accurately about 5 g sample, transfer to a 200 ml volumetric flask dissolve in warm water, dilute to about 150 ml. In case solution is not clear, add 5 ml of Carrez 1 solution followed by 5 ml of Carrez 2 solution. Make up to 200 ml. Filter through a dry filter paper. Titrate the solution obtained as such to determine % Reducing sugars.

PRELIMINARY TITRATION:

Pipet 5 ml each of Fehling A and B into 250 ml conical flask. Mix and add about 10

ml water and a few boiling chips or glass beads. Dispense solution. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Continue the addition of solution dropwise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

FINAL TITRATION:

Pipet 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration duplicate and take the average.

CALCULATIONS:

Reducing sugars (%) = Dilution x Factor of Fehling (in gm) / Weight of sample x Titre value *100

CALCIUM

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 per cent sodium hydroxide solution using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass = $V_1 \times 0.04008 \backslash M$

Where V_1 = Volume in ml of EDTX solution un titration; and

M= Mass in g of the sample in the solution for the test

IRON

Applicable to determination of Zn, Cu, and Fe in a variety of foods by dry ashing

and flame atomic absorption spectrometry (FAAS), and Cd and Pb by dry ashing and graphite furnace atomic Absorption spectrometry (GFAAS) Method is capable of determining these elements at concentrations above approximately Pb (0.3), Cd (0.1), Zn (1), Cu (5), and Fe (4) mg/kg.)

A. Principle

Test portions are dried and then ashed at 450°C under a gradual increase ($\leq 50^{\circ}\text{C}/\text{h}$ temperature. 6 M HCl (1+1) is added, and the solution is evaporated dryness. The residue is dissolved in 0.1 M HNO_3 , and the analytes are determined by flame and graphite furnace procedures.

B. Procedures

(a) Pretreatment - Homogenize product if necessary, using noncontaminating equipment. Check for leaching metals if the apparatus consists of metal parts.

(b) Drying-In a crucible, weigh 10-20 g test portion to nearest 0.01 g. Dry in a drying oven, on a water-bath, or a hot plate at 100°C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace.

(e) Ashing Ashing in a programmable furnace Place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C/h to 450°C. Let dish stand for at least 8 h or overnight. Continue according to

(d) Ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in B(d)-(k)-See Figure 999.11. Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put IR lamp down at the cover Pre-ash product by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200-250°C and slowly raise

temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8 h or overnight. Take crucible out of furnace and let cool.

(d) **Solution.** Wet ash with 1-3 ml. water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50-100°C/h) to 450°C. Proceed with ashing at 450°C for 1-2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary varies depending on type of product. Add 5 ml. 6 M HCl, C(b), to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water-bath or hot plate. Dissolve residue in 10.0-30.0 ml., to the nearest 0.1 ml, of 0.1 M HNO₃, C(d). Swirl crucible with care so that all ash comes

SODIUM AND POTASSIUM:

A. Apparatus and Reagents

(a) Glassware - Borosilicate glassware and intact Vycor, Pt or Si crucible precleaned with dilute HNO₃, and rinsed in distilled water immediately before use.

(b) Distilled water-H₂O, free from Na and K; either double-distilled or deionized. Use for preparing standards and dilutions.

(c) Sodium standard solutions - Stock solution-1 mg Na/mL. Dry reagent grade NaCl 2 h at 110°C; cool in desiccator. Weigh 2.5421 g into 1 L. volumetric flask and dilute to volume with H₂O. Working solution for flame emission -0.01, 0.05, and 0.05 mg Na/mL. Pipet 1, 3, and 5 mL. Na stock solution into separate 100 mL. volumetric flasks add 7 mL K stock solution and 2 mL HNO₃, to each flask dilute to volume with H₂O. Working solutions for flame absorption, -0.00003, 0.0001, 0.0003, and 0.0005 mg Na/mL. Pipet 1 mL. Na stock solution into 100 mL volumetric flask and dilute to volume with H₂O. Pipet 0.3, 1.0, 3.0 and 5.0 mL diluted stock solution into separate 100 mL. volumetric flasks and dilute to volume with H₂O

(d) Potassium standard solution- Stock solution- mg K/mL. Dry and cool reagent grade KCl as in c. Weigh 1.9068 g into 1 L volumetric flask and dilute to volume with H₂O. Working solutions for flame emission-0.04, 0.07, and 0.10 mg K/mL. Pipet 4, 7, and 10 mL. stock solution into separate 100 mL. volumetric flasks, add 3 mL. Na stock

solution to each flask and dilute to volume with H₂O. (5) Working solutions for flame absorption-0.0001, 0.0005, 0.0007, and 0.0010 mg K/mL. Pipet 1 mL K stock solution into 100 mL volumetric flask and dilute to volume with H₂O. Pipet 1, 5, 7, and 10 mL diluted stock solution into separate 100 mL volumetric flasks and dilute to volume with H₂O.

B. WET ASHING

Weigh 1 g test portion into 50 mL Pyrex beaker.

Dry 110°C, cool, and weigh if percent solids is to be determined

(a) Test samples with unknown or known high oil content Add ca 10 mL petroleum ether, and warm on steam bath or low temperature hot plate until oil is extracted. Decant and repeat until test portion is defatted.

(b) Test samples with low oil content- Add 5 mL HNO₃ [if total Cl content is desired, add enough 0.1 M AgNO₃ to precipitate chlorides (3.0 mL)] to each beaker. Digest on steam bath or low hot plate until test portion dissolves, evaporate to dryness. Add 5 mL HNO₃, and take to dryness. Repeat. Add 2 mL HNO₃, and warm to dissolved.

(c) For flame emission-Transfer digest to 25 mL volumetric flask with hot water, wash down sides of beaker three times with hot water, and add washings to flask. Cool, and dilute to volume with H₂O. If particles are too finely dispersed to settle, centrifuge aliquot at 2000 rpm.

(d) For flame absorption. Transfer digest to 100 mL volumetric flask and proceed as above. Dilute for direct readout as follows: Place 1 mL aliquot in 25 mL volumetric flask and dilute to volume with H₂O for Na; place 2 mL aliquot in 10 mL volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2 mL HNO₃ to 100 mL with H₂O.

C. DRY ASHING

Weigh 4 g test portion into crucible, and char on electric hot plate or over low flame. Place in cold furnace and bring to 525°C. Ash 2 h to white ash. Cool, and weigh if total ash is desired.

Add 15 mL dilute HNO₃ (1+4) to crucible, breaking up ash with stirring rod if necessary. Filter through acid-washed quantitative paper into 100 mL volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume. Proceed as in (a) or (b).

(a) For flame emission- Read directly.

(b) For flame absorption. Dilute for direct readout as follows: Place 1 mL aliquot in 100 mL volumetric flask and dilute to volume with H₂O for place 1 mL aliquot in 25 mL volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2 mL HNO₃ to 100 mL with

D. DETERMINATION

Follow manufacturer's directions for type of instrument available. Dilute test solution if necessary to bring 7 readings within range of working standards. Read blank, standards, and test solutions at 589 nm for Na and 767 nm for K until results are reproducible; record percent T or percent absorption for each.

E. Calculations

For flame emission photometers not equipped with direct readout:

$$\text{mg Na or K/100g} = 100 \times F \left(\left[\frac{(E_x + E_1)}{(E - E_1)} \times (C_2 - C_1) \right] + C_1 \right) / \text{g test portion}$$

where (percent of unknown) - (percent T of blank) E_1 = percent T of standard of lower concentration than test solution - (percent T of blank) E_2 - (percent T of standard of higher concentration than test solution) - (percent T of blank); C_1 = mg Na or K/mL in standard of lower concentration than test solution C_2 = mg Na or K/mL in standard of higher concentration than test solutions F = dilution factor

For flame absorption photometers: Convert percent absorption to absorbance (A). Plot standard curve of A against concentration. Read unknown concentrations.

$$\text{mg Na or K/100g (concentration unknown} \times F \times 100) / \text{g test portion}$$

4.2.2 MICROBIOLOGICAL TEST

YEASTS AND MOULD

Micro-organisms which at 25°C form colonies in a selective medium

according to the method specified in this standard.

PRINCIPLE

Preparation of poured plates using a specified selective culture medium and a specified quantity of the test sample if the initial product is liquid, or of an initial suspension in the case of other products. Preparation of other plates, under the same conditions using decimal dilutions of the test sample or -of the initial suspension. Aerobic incubation of the plates at 25°C for 3,4 or 5 days. Calculation of the number of yeasts and moulds per gram or per milli litre of sample from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

PROCEDURE

INOCULATION AND INCUBATION

Take two sterile petri dishes and Transfer to each dish, by means of a sterile pipette add 1ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products. Take two further sterile petri dishes. Transfer, by means of another sterile pipette, 1 ml of the 10^{-1} dilution to each dish (liquid product), or 1 ml of the 10^{-2} dilution (other product). Repeat the procedure described above using further dilutions, if necessary. Pour about 15 ml of the yeast extract dextrose chloramphenicol-agar medium previously melted and maintained at $45\pm 1^{\circ}\text{C}$ a water-bath from a culture bottle into each petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min. Carefully mix the inoculum with the medium and allow the mixture to solidify, by leaving the petri dishes to stand on a cool -horizontal surface. Prepare a control plate, with 15 ml of the medium, to check its sterility. Invert the plates and place them in the incubator at $25 \pm 1^{\circ}\text{C}$.

PLATE COUNT

yeast and mould-forming countable colony, produced under the conditions specified in this International Standard

PRINCIPLE

- Two poured plates are prepared using a specified culture medium and a specified quantity of the test sample, if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products. Other

pairs of poured plates are prepared, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

- The plates are aerobically incubated at 30 °C for 72 h.
- The number of microorganisms per millilitre or per gram of sample is calculated from the number of colonies obtained on selected plates.

PROCEDURE

Test portion, initial suspension and dilutions See the relevant part of ISO 6887 and the specific International Standard dealing with the product concerned.

INOCULATION AND INCUBATION

- Take two sterile Petri dishes and Transfer to each dish, by means of a sterile pipette 1 ml of the test sample, if liquid, or 1 ml of the initial suspension in the case of other products (10–1 dilution).
- Take two other sterile Petri dishes Transfer to each dish, by means of another sterile pipette, 1 ml of the 10–1 dilution (liquid product) or 1 ml of the 10–2 dilution (other products).
- If necessary, repeat the procedure with the further dilutions, using a new sterile pipette for each decimal dilution.
- If appropriate and possible, select only the critical dilutions steps (at least two consecutive decimal dilutions) for the inoculation of the Petri dishes that will give colony counts of between 15 and 300 colonies per plate.
- Pour about 12 ml to 15 ml of the plate count agar at 44 °C to 47 °C into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10–1 dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 45 min.
- Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface. After complete solidification, and only in the case where it is suspected that the product under examination contains microorganisms whose colonies will overgrow the surface of the medium, pour about 4 ml of the overlay medium at 44 °C to 47 °C onto the surface of the inoculated medium.

- Allow to solidify as described above. 9.2.8 Invert the prepared dishes and place them in the incubator at $30\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $72\text{ h} \pm 3\text{ h}$. Do not stack the dishes more than six high. Stacks of dishes should be separated from one another and from the walls and top of the incubator.

Counting of colonies

- After the specified incubation period, count the colonies on the plates, using the colonycounting equipment if necessary. Examine the dishes under subdued light. It is important that pinpoint colonies should be included in the count, but it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies. Examine doubtful objects carefully, using higher magnification where required, in order to distinguish colonies from foreign matter.
- Spreading colonies shall be considered as single colonies. If less than one-quarter of the dish is overgrown by spreading, count the colonies on the unaffected part of the dish and calculate the corresponding number of the entire dish. If more than one-quarter is overgrown by spreading colonies, discard the count.

COLIFORMS:

Bacteria which, at the specified temperature (i.e. $30\text{ }^{\circ}\text{C}$, $35\text{ }^{\circ}\text{C}$ or $37\text{ }^{\circ}\text{C}$, as agreed) form characteristic colonies in crystal violet neutral red bile lactose agar under the test conditions specified in this International Standard.

PRINCIPLE

- Preparation of two poured plates, using a solid selective culture medium, and using a specified quantity of the test sample if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products.
- Preparation of other pairs of poured plates, under the same conditions, using decimal dilutions of the test sample or of the initial suspension. incubation of the plates at $30\text{ }^{\circ}\text{C}$, $35\text{ }^{\circ}\text{C}$ or $37\text{ }^{\circ}\text{C}$ (as agreed) for 24 h.
- Calculation of the number of coliforms per millilitre or per gram of sample from the number of characteristic colonies obtained in the plates.\

PROCEDURE

Test portion, Initial suspension and dilutions See ISO 6887 and the specific

International Standard appropriate to the product concerned.

INOCULATION AND INCUBATION

- Take two sterile Petri dishes and Using a sterile pipette (6.4), transfer to each dish 1 ml of the test sample, if the product is liquid, or 1 ml of the initial suspension In the case of other products.
 - Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1 ml of the first decimal dilution (10^{-2}) of the Initial suspension in the case of other products.
 - Repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.
 - Pour about 15 ml of the VRBL medium at $45^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min.
 - Carefully mix the inoculum with the medium and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface. Also prepare a control plate, with 15 ml of the medium for checking its sterility.
- 9.2.3 After complete solidification, pour about 4 ml of the VRBL medium at $45^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$, on to the surface of the inoculated medium. Allow to solidify as described above.
- Invert the prepared dishes and incubate them in the incubator set at 30°C , 35°C or 37°C (as agreed) for $24\text{ h} \pm 2\text{ h}$

ESCHERICHIA COLI

The typical *Escherichia coli* is aerobic, Gram-negative rod, motile, fermenting lactose with the production of gas and usually produces smooth, non-mucoid colonies on solid media. However, there are non-lactose fermenting strains of *Escherichia coli* and some strains produce mucoid colonies. The organism also exhibits the following characters:

- a. Hydrogen sulphide (H_2S) production when done in TSI medium: Negative
- b. Urease: Negative
- c. Indole: Positive

- d. Methyl red: Positive
- e. Voges-Proskauer test: Negative
- f. Simmon's citrate: Not utilized
- g. Sucrose: Acid and gas production variable
- h. Salicin: Acid and gas production variable, and
- i. Growth at 44°C in MacConkey broth medium: Positive with acid and gas.

PROCEDURE FOR ISOLATION

blend the sample in a sterile blender jar for 2 minutes or macerate--with sterile sand in a sterile mortar using approximately 200 ml of diluting fluid per 25 g of the sample. The diluting fluid for preparing the homogenate should be 1 percent peptone solution in water, sterilized at 120°C for 20 minutes and final pH adjusted 6.8 $\pm$ 0.1, or 3-4 percent of potassium dihydrogen phosphate (KH_2PO_4) in water, pH adjusted to and sterilized at 120°C for 20 minutes. Inoculate 1 ml of the blended or macerated sample into 10 ml of single strength MacConkey broth medium. If the number of organisms are assumed to be very small, inoculate 10 ml of double strength MacConkey broth medium Also streak loopfuls on to MacConkey agar medium , eosin methylene blue lactose agar , and if available Tergitol-7 agar Incubate all the inoculated media at 37°C overnight. If there is growth with fermentation of lactose in the MacConkey broth medium streak out a loopful on to each of the solid media and incubate at 37°C overnight.

SALMONELLA

Microorganisms which form typical colonies on solid selective media and which display the biochemical and serological characteristics described when tests are carried out in accordance with this International Standard

DETECTION OF SALMONELLA:

Determination of the presence or absence of these microorganisms, in a particular mass of product, when tests are carried out in accordance with this International Standard.

PRINCIPLE

The detection of Salmonella necessitates four successive stages

Salmonella may be present in small numbers and are often accompanied by considerably larger numbers of other members of Enterbacteriaceae or of other families. Therefore, selective enrichment is necessary; furthermore, pre-enrichment is often necessary to permit detection of injured Salmonella.

PROCEDURE

Test portion and initial suspension

- For preparation of the initial suspension, use as dilution fluid the pre-enrichment medium specified.
- In general, to prepare the initial suspension, add a 25 g test portion to 225 ml of pm-enrichment medium which is the ratio of test portion to pre-enrichment medium specified in this method.
- If the prescribed test portion is other than 25 g, use the necessary quantity of pre-enrichment medium to yield approximately a 1/10 dilution (mass to volume).

4.2.3 SENSORY EVALUATION OF COOKIES

Sensory evaluation of quinoa millet cookies was carried on 5point hedonic scale. The average scores of the 40 judges for different quality characteristics. appearance, smell, taste, touch, hear and overall acceptability were recorded.

CHAPTER-5

RESULT AND DISCUSSION

The experimental bakery products, developed in the present study using refined wheat, flax seed, multigrain quinoa millets, were characterized to ensure acceptability and edibility for both the general population and elderly individuals, incorporating various parameters nutritional analysis, microbial analysis and sensory evaluation for product development.

5.1 NUTRITIONAL EVALUATION OF QUINOA MILLET COOKIES:

S.NO	PARAMETERS	UNITS	RESULTS
1	Energy	KCAL/100g	495
2	Carbohydrate	g/100g	65.9
3	Total fat	g/100g	21.5
4	Protein	g/100g	9.54
5	Dietary fibre	g/100g	6.18
6	Total sugar	g/100g	21.9
7	Sodium as Na	mg/100g	204
8	Potassium as K	mg/100g	256
9	Calcium as Ca	mg/100g	102
10	Iron as Fe	mg/100g	3.74

TABLE5.1: NUTRITIONAL CONTENT OF THE QUINOA MILLET BISCUIT

The data table 1 provides a comprehensive overview of the nutritional composition of quinoa millet biscuits across various parameters. Analysis indicates that these biscuits contain per 100g 495 Kcal for energy, 65.9g for of carbohydrates, 21.5 g for total fat, 9.54 g per for protein, 6.18 g per for fiber, 21.9 g for total sugar, 204 mg for sodium, 256 mg for potassium, 102 mg 100 g for calcium, and 3.74 mg for iron. These values reflect the nutritional richness and potential health benefits of incorporating quinoa millet biscuits into one's diet. The quinoa millet biscuit, providing 3.74 mg of iron, helps alleviate anemia in patients while meeting the daily iron requirement of 10-20 mg in a balanced diet. Sedentary women or men with a

recommended dietary allowance (RDA) of 1645 kcal benefit from its 495 kcal energy contribution.

5.2 MICROBIOLOGICAL EVALUATION OF QUINOA MILLET COOKIES:

S.NO	PARAMETERS	UNITS	RESULTS
1	TOTAL PLATE COUNT	CFU/g	1200
2	YEAST& MOULD COUNT	CFU/g	<10
3	COLIFORM COUNT	CFU/g	<10
4	ESCHERICHIA COLI	CFU/g	<10
5	SALMONELLA SPP	Per 25 g	ABSENT

TABLE5.2: MICROBIOLOGICAL CONTENT OF THE QUINOA MILLET BISCUIT

This data table 2 presents a detailed microbiological analysis of quinoa millet biscuits. The analysis reveals that these biscuits have a total plant count of 1200 CFU/g, with yeast and mold counts below 10 CFU/g, and coliforms and *Escherichia coli* counts also below 10 CFU/g. Moreover, the absence of *Salmonella spp* per 25 g further underscores the safety and quality of these biscuits. This comprehensive overview assures consumers of the high standard of hygiene and microbiological safety maintained in the production of quinoa millet biscuits.

5.3 SENSORY EVALUATION OF BISCUIT:

S.NO	SENSORY	5 ☆	4☆	3☆	2 ☆	1 ☆	OVERALL RATING
1	Appearance	30	9		1		4.7
2	Smell	33	4		2	1	4.6
3	Taste	25	6	3	5	1	4.2
4	Touch	25	12	3			4.5
5	Hear	23	10	6	1		4.4

OVER ALL ACCEPTABILITY = 4.5

TABLE5.3: SENSORY EVALUATION CONTENT OF THE QUINOA MILLET

BISCUIT

A panel of 40 individuals from diverse age groups was assembled to conduct a sensorial evaluation of quinoa millet biscuits enriched with hemp flour. Using a 5-point Hedonic scale, panel members rated various attributes including appearance, flavor, smell, touch, and overall taste. While the biscuits received lower scores for appearance (4.7), smell (4.6), touch (4.5), and sound (4.4) due to the altered appearance caused by the addition of hemp flour, they garnered higher scores for flavor and taste (over acceptability 4.5). Consumers appreciated the distinctive notes of quinoa millet, contributing to a balanced sweet-aromatic-slightly sour taste profile. Despite the initial lower ratings, the product was easily accepted by consumers, as evidenced by the overall weighted average grade of 4.5. Additionally, it's noted that a simple modification, such as changing the shape to a circle, could potentially improve the overall acceptance of the product further.

PACKING AND LABELLING:





Fig 5.1 PACKING AND LABELLING

CHAPTER-6

SUMMARY AND CONCLUTION:

The study concluded that developing and standardizing baked product recipes using quinoa millets and flaxseed as substitutes for maida flour holds immense potential. Quinoa millets, rich in minerals like iron and calcium, along with other functional components, offer numerous health benefits and can help address various nutrition-related disorders such as deficiencies, diabetes, and obesity. Although quinoa millet-based products may have lower shelf stability, this issue can be significantly mitigated by pre-treating the grains before milling and storing them refrigerated, as suggested in the study.

Quinoa millet-based snacks could serve as healthier alternatives to popular but less nutritious junk foods currently dominating the market. Biscuits, being convenient carriers of nutrients, are particularly suitable for delivering the abundant health benefits of quinoa millet to people of all age groups. Moreover, these biscuits are not only economical and shelf-stable but also highly acceptable among consumers. The salted and sweet biscuits developed using 95% quinoa millet flour in the study are not only high in energy and protein density but also rich in essential minerals like iron, calcium, and phosphorus. Commercializing quinoa millet products can offer a healthy alternative for consumers with special dietary needs such as anemia, diabetes, celiac disease, pregnant and lactating mothers, children, adolescents, and individuals with dementia. Integrating these products into schemes like Integrated Child Development Services (ICDS) can help address protein-energy malnutrition and anemia prevalent in many developing countries.

Furthermore, the study found that baked products, when stored in airtight glass jars at room temperature, exhibit good storage stability, with quinoa millet biscuits having a maximum shelf life of one week.

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