PREPARATION OF HORSE GRAM (MACROTYLOMA UNIFLORUM) INCORPORATED FUNCTIONAL SMOOTHIE

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

Master of Science

In

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Supervisor		Submitted by
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Dear Sir,

I have great pleasure in forwarding the thesis entitled, ***"preparation of Horse Gram (Macrotyloma uniflorum) Incorporateed Functional Smoothie"*** submitted by **Ms. Priyanka Panda, I.D No.:20412FST008, Enrolment No.:433953** in partial fulfilment of the requirements for the degree of **Master of Science** in **Food Technology** from Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and placing on record that she has completed the requisite residential requirements as contained in the ordinance of the University.

I certify that the entire scheme of investigation, presented here in, was planned and carried out solely by the candidate under my supervision and guidance. To the best of my knowledge, the data presented in the thesis are genuine and original and no part of the work has been submitted for any other degree or distinction.

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By

Priyanka panda

Thesis submitted in the partial fulfilment of the requirement for the degree of

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FROM

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INSTITUTE OF AGRICULTURAL SCIENCES,

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Date: ……....

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ABBREVIATIONS

/	Per
@	At the rate of
°C	Degree Celsius
BIS	Bureau of Indian Standard
et al.,	(*Et albeit*) and others
Fig.	Figure
g	Gram (s)
mg	Milligrams
mL	Millilitre
No.	Number
pH	Negative logarithm of hydrogen ion concentration
rpm	Revolution per minute
FSSA	Food Safety and Standard Authority
IS	Indian Standards
ISI	Indian Standards Institute
SD	Standard Deviation
cfu	Colony Forming Unit
HGE	Horse Gram Extract
etc	etcetera (and other things)
i.e.	id est (that is)
Wt.	Weight
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total Plate count

CHAPTER-I

INTRODUCTION

In the 1980s, Japan pioneered the concept of "functional food." Functional foods are foods that include essential nutrients that go beyond supporting an individual's normal growth and development **(Jain *et al*. 2014).** White oats, probiotics, almonds, tomato products, yoghurt, sports and energy drinks, and fibres, fatty acids, vitamins and minerals in the form of capsules and tablets are all examples of functional foods and beverages **(The Economic Times, 2015)**. Recently, the functional food business has seen a significant increase in growth. According to Statista **(2020),** the global market for functional foods produced $247.89 billion in revenue in 2017, and is expected to reach $319.93 billion by 2022.

Smoothie is derived from the English word smooth, according to the dictionary (tender, creamy). Smoothies were first introduced in the 1990s and are now one of the fastest-growing segments of the beverage industry **(Mordor Intelligence, 2017)**. Smoothies are first appeared in the United States in 1960. Furthermore, in the year 2000, they resurfaced all over the world. Smoothie is a fantastic and convenient way of increasing daily consumption of fresh fruits and vegetables **(Rodriguez-Verastegui, *et al*. 2015)**. Smoothie is a type of non-alcoholic beverage generally made from a combination of fruits and vegetables that have been processed into pulp or puree after the seeds and peel have been removed. They have a little thicker consistency than a slushie **(Castillejo *et al*. 2015)**. Smoothies are classified into three types: fruit itself only, fruit and dairy product, and functional smoothie. Functional smoothies are

relatively new product on the market that typically include probiotics. Other ingredients such as yoghurt, milk, lentils, and cereals may be added in smoothies to enhance their useful functional characteristics.

The smoothie is normally contains three parts. The first element is the fruit and vegetable component, which is commonly in the form of puree, pulp, or concentrate, and the second half is the liquid component, generally dairy (milk, dahi, stirred yoghurt) and non-dairy (coconut milk, soy milk, water etc.) liquids are both acceptable . The third section contains additives or some stabilizing ingredients, which include condiments, spices, stevia, honey, and other natural flavorings and sweeteners. Artificial flavorings (chocolate, orange, vanilla, etc.) and artificial sweeteners (acesulfame, saccharin, etc.) are also utilized nowadays. Beside this pulses and/or cereals are added in present days for nutritional as well as functional achievement.

Crops can be classified into two groups: staple and underused. Underused/underutilized crops, also known as orphan, neglected, or little used crops, are generally wild or semi-domesticated crops that have adapted to their local surroundings. They have a high nutritional content and are an excellent source of macro and micronutrients. Jack bean, winged bean, faba bean, Bambara groundnut, Horse gram, Moth bean, Adzuki beans, Lima beans etc. are the categories of underutilized crops.

Macrotyloma uniflorum is known as *Dolichos biflorus L.* In Asian countries, particularly India, *M. uniflorum* seeds are consider as the poor man's pulse crop. It is also acknowledged as 'Horse gram' an unexplored legume crop grown mainly in Southeast Asia and tropical Africa with an excellent nutritional value **(Pal *et al.*, 2015)**

is a great source of protein, carbohydrates, dietary fibre, micronutrients, and antioxidant properties. Water & oil absorption capacity, bulk density, foam stability are the main functional properties of horse gram. The biologically active compounds are also present in horse gram such as nutritional (flavonoids and isoflavonoids) and anti-nutritional (phenolic acid, phytic acid, proteinase inhibitors). In terms of nutraceuticals, Horse gram is a true super food. Super foods are a special category of nutrient-dense foods that are strong in antioxidants and protein. **A. Mehra (2013)**. In recent years, the isolation and utilization of potential antioxidants from legumes such as Horse gram has received a lot of attention because it minimizes the chances of intestinal diseases, diabetes, coronary heart disease, and dental caries, etc. **(Mohgana *et al.*, 2017, Saroj *et al*, 2015).** Horse gram is also known to have other therapeutic effects in traditional knowledge systems, and it has been recommended in ayurveda medicine to treat renal stones, piles, oedema, and other conditions **(Mohar et al., 2013)**.

In view of the growing demand for food with functional properties, the current study emphasises the importance of applying new scientific knowledge to the exploration of underutilized crop; Horse gram as a source of functional and nutraceuticals compounds.

Various challenges occur in developing functional smoothie, such as, freely available anti nutritional factors (ANFs), shelf life, off-flavour, colour degradation, sedimentation. Therefore to overcome from these challenges several works has been done like, soaking and germination; which reduced trypsin inhibitor activity and flatulence-causing oligosaccharides, boosting protein digestibility and sensory characteristics **(Embaby 2010; Kajihausa *et al*., 2014).** Pasteurization; main goal is to

destroy thermolabile microorganisms including yeasts, moulds, and vegetative bacteria that cause food spoiling or food poisoning. **(Petruzzi *et al.*, 2017)**. Addition of flavoring ingredient (banana); during shelf-life storage degradation of natural flavour occurs therefore to overcome this problem natural flavoring agent added to maintain the real flavour of a food product **(Hoda *et al* 2015).**

Addition of coloring ingredient (black carrot); pigment in black carrot Anthocyanins has water-soluble nature which makes them a desirable natural colourant for a variety of food applications **(Sucheta *et al.*, 2020).** Addition of stabilizer; sedimentation and wheying off during storage period of dairy beverages might be degraded by using pectin as a form of stabilizing agent **(Khanniri *et al.*, 2019) (Irem Bige *et al.*, 2022) Guimaraes *et al.*, 2018) (Krongsin *et al.*, 2015).** Development of functional smoothie by incorporation of Horse gram extract undertaken for research. Research project conducted under following objectives.

- Preparation of Horse gram extract.
- Preparation of functional smoothie by using Horse gram extract.
- Shelf-life study of control and developed product.

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CHAPTER II

REVIEW OF LITERATURE

This chapter aims to acknowledge the current state of our expertise of value addition and functional food stuff i.e. smoothie made from fruits, vegetables, underutilized cereals and/or pulses and dairy sources. It comprises the preparation and storage of functionally incorporated an underutilized pulse i.e. Horse gram extract (HGE) into milk-based food product. The nutritional qualities of underutilized pulse and each other ingredient that has been used in the final product are also highlighted in this section. Scientific inquiry includes several formulations of functional smoothies, storage stability, and evaluation of the smoothie's microbiological quality has been discussed in this chapter.

2.1 Current scenario of Functional food in India and Worldwide

2.1.1 Marketing status of functional food

Recently, the functional food business has seen a significant increase in growth. According to statista (2020), the global market for functional foods produced $247.89 billion in revenue in 2017 and is expected to reach $319.93 billion by 2022. In various regions of the world, several factors have contributed to the rise in demand for functional foods. People nowadays are more concerned with foods that have a functional advantage to their health. **(Küster-Boluda *et al*, 2017; Ozen et al., 2012)**. People expect foods that promote health and as well as part of their regular diets **(Iwatani *et al*, 2019)**. Functional food may provide a preventative response towards

rising health costs. Functional food innovation has been aided by advancements in science and technology in the food industry **(Bigliardi *et al*, 2013).**

For Indian customers, functional food is a fairly new phenomenon. In India, the Food Safety and Standard Act **(FSSA) (2006)** is the sole point of reference for the regulation of functional foods. India's functional food market is one of the fastest expanding in the Asia-Pacific region **(The Economic Times, 2015)**. As a result, the market for functional foods with health advantages will remain strong for the next many decades. People's knowledge of the impact of nutrition on health has been expanding in India's functional food market, which is growing at a rapid pace as health as a value has percolated in the Indian society, which is witnessing dramatic demographic transformations **(Sharma *et al,* 2013).**

The development of functional foods necessitates extensive research and development **(Nazir *et al*., 2019).** Identification of bioactive constituents, product formulation, processing, assessment of physiological activity and preparation for the clinical test are all part of the R&D effort to meet regulatory requirements. These studies will provide scientific proof of food functionality **(Betoret *et al*., 2011)**. In case of functional foods, R&D is just as important as developing innovatively functional products to fulfil consumer's demand.

Functional foods are foods that include essential nutrients that go beyond supporting an individual's normal growth and development **(Jain *et al,* 2014)**. Functional foods include fresh or processed foods that contain components which have physiological improvement and/or illness risk reduction functions; these claims should be supported up by scientific data; and functional food should be consumed as part of

a normal daily schedule **(Amaliah *et al*., 2019).** White oats, probiotics, almonds, tomato products, yoghurt, sports and energy drinks, and fibres, fatty acids, vitamins and minerals in the form of capsules and tablets are all examples of functional foods and beverages **(The Economic Times, 2015).**

However, there is still no universally accepted definition of functional foods. Following table shows the evolution of functional foods.

2.1.2 Definition of functional food evolution:

Various definition of functional food from different organization/sectors are given in Table 2.1

Table 2.1 Definition of Functional Food

Organization/sector	**Definition**	**References**
10th International Functional Food Conference	Natural or processed foods containing known or unknown biologically active constituents that have been clinically demonstrated to provide a documented health benefit in the prevention, management, or treatment of chronic conditions.	**Martirosyan *et al,* (2015)**
17th International Conference organized by the US Department of Agriculture (USDA)	Natural or processed foods containing known or unknown bioactive substances that provide a clinically established verified health	**Martirosyan *et al,* (2015)**

and Agricultural Research Service (ARS)	benefit in effective non-toxic amounts for the prevention, management, or treatment of chronic conditions.	
Academy of Nutrition and Dietetics	Whole foods, as well as fortified, enriched, or enhanced foods, have the ability to improve health when consumed as part of a diversified diet on a regular basis at effective amounts based on sufficient standards of evidence.	**Wolfram (2017)**
British Nutrition Foundation	Spanning a wide range of products from foods produced for a specific functional ingredient to staple foods fortified with a nutrient that is rarely found.	**British Nutrition Foundation (2018)**

2.1.3 Classification of functional foods

The functional foods are classified in different categories which are mentioned in the following table 2.2

Table 2.2 Classification of Functional Food

Types	Description	Examples	References
Conventional food	complete, unprocessed foods with natural bioactive compounds that provide health benefits	Fruits and vegetables, fish, dairy products, and grains	**(Crowe and Francis, 2013)**
Modified foods	food items that have been enhanced, enriched, or fortified with functional food components food that has been enriched, fortified, or enhanced with functional food ingredients	Fruit juices fortification with calcium, beverages with plant extracts, iodized salt and folate enriched breads.	**(Crowe and Francis, 2013)**
Synthetic food ingredients	Functional components that have been generated in the lab	Inulin-type fructans	**(Functional Food Ingredients Market, 2018)**, **(Crowe and Francis, 2013)**

2.2 Smoothie

Smoothie is derived from the English word smooth, according to the dictionary (tender, creamy). Smoothie is a type of non-alcoholic beverage generally made from a combination of fruits and vegetables that have been processed into pulp or puree after the seeds and peel have been removed. Smoothies are first appeared in the United States in 1960. They have a little thicker consistency than a slushie **(Castillejo *et al*., 2015).** Smoothie is a fantastic and convenient way of increasing daily consumption of fresh fruits and vegetables **(Rodriguez-Verastegui, *et al.*, 2015**). Smoothies were first introduced in the 1990s and are now one of the fastest-growing segments of the beverage industry **(Mordor Intelligence, 2017).**

Non-thermally processed smoothies are only held for a shorter period of time due to the likelihood of increased microbial growth. The longer the storage period, the more likely the natural colour and total phenols will deteriorate **(Cano-Lamadrid, *et al*. 2018).**

2.2.1 Types of smoothie

Smoothies are classified into three types: fruit itself only, fruit and dairy product, and functional smoothie. Various types of functional smoothies developed by incorporation of different functional ingredients cited in following Table 2.3.

Table 2.3 Types of Smoothie

Sl no.	Type of smoothie	Formulation	Observation	References
1	Carrots Smoothie	water: carrot= 1:1	Browning index, PH, Titrable acidity, TSS, Phenylalanine Ammonia-Lyase, Total antioxidant capacity, TPC	**Formica-Oliveira *et al*., (2017)**
2	Pomegranate Smoothie	Pomegranate:- Figs, Quinces Puree, jujubes (60:40 & 60:40) + Rhubarb Juice (5%)	Hunter Lab, UPLC-PDA (Ultra-performance Liquid Chromatography-Photodiode Array), Antioxidant activity, Polymeric Procyanidins, Four-way Anova	**Cano-Lamadrid, *et al*. (2018)**
3	Fruit Smoothie	Banana pulp- 10% + Apple Juice- 33% + Whole apple-10% + Strawberry-14% + Orangejuice-33%	pH, Total Phenols and Flavonoids, Total Sugars, Acidity, Vitamin C Sensory, Browning Index Peroxidase enzyme activity, Polyphenol oxidase enzyme activity, Pectin methyl esterase enzyme activity, Antioxidant activity , Density, Viscosity, Total soluble and insoluble solids, Turbidity and Transmittance, Hunter Lab, , and Microbial	**Hurtado *et al*. (2015)**
4	Dehydrated Smoothie	Dehydrated Strawberries + Dehydrated Banana + Dehydrated egg white + Demerara	Sensory, Hunter Lab, Viscosity	**Guazi *et al*. (2019)**

		sugar (2%) + Cold whole milk / cold water		
5	Purple Smoothie	Purple grapes- 45% + Beetroot- 12% + Cucumber- 35% + Broccoli- 8%	Vitamin C, Sensory, TPC, Total antioxidant content, Anthocyanin, Microbial	**Gonzalez-Tejedor *et al.* (2017)**
6	Apple Smoothie	Phenolic extract of apple + citric acid + CMC	Apple Fibre, Total Dietary Fibre, N2 Physisorption, Total Extracted Polyphenol Content, FTIR, HPLC, Rheological measurement	**Sun-Waterhouse *et al*. 2013**
7	Fruit Smoothie	whole apple-29.5% + Banana-12% + Squeezed apple concentrates-29.5% + Orange-8% + Strawberry-21%	Polyphenols, HPLC-DAD (diode ray detector), Sensory, Dynamic Oscillatory measurement, Hunter Lab	**Keenan *et al.* (2011)**
8	Aloe gel- spiced ripened papaya Smoothie	Papaya pulp- 15% + Aloe gel- 10%, 20%, 30% + Sugar Syrup- 20% + Aniseed, Ginger and Pepper- 5% + Citric Acid- 0.1%	Total polyphenols, TSS, pH, Titrable acidity, Vitamin C, Total flavonoids, non-enzymatic browning, Total and reducing sugar, Hunter Colour Lab, Microbial test, Sensory acceptability	**Ramachandran & Nagarajan (2014)**
9	Kiwi Smoothie	Kiwi- 3kg + Sugar- 0%, 10%, 15%, 20%, 25%	Bioactive compounds, Total Ascorbic Acid, FTIR, TAC, Total Carotenoid, Total Chlorophyll, Fluorometric measurement	**Park *et al.* (2016)**

10	Dehydrated Smoothie	Dehydrated Banana + Dehydrated Strawberries + Dehydrated egg white + Demerara sugar (2%) + Cold water/cold whole milk	Sensory analysis, Hunter Lab, Viscosity,	**Guazi *et al.* (2019)**
11	Mixed Fruit with Coconut Milk Smoothie	Banana- 28g + Pineapple- 50g + Apples- 12g + Orange- 3g + Coconut milk- 7g (per 100g smoothie)	Shelf-life analysis, Microbiological testing, Sensory, Heat and Pulsed electric field and thermal pasteurization for preservation	**Walkling-Ribeiro, *et al.* (2010)**

2.2.2 Functional Smoothie

Functional smoothies are relatively new product on the market that typically include probiotics. Other ingredients such as yoghurt, milk, lentils, and cereals may be added in smoothies to enhance their useful functional characteristics.

The smoothie is normally contains three parts. The first element is the fruit and vegetable component, which is commonly in the form of puree, pulp, or concentrate, and the second half is the liquid component, generally dairy (milk, dahi, stirred yoghurt) and non-dairy (coconut milk, soy milk, water etc.) liquids are both acceptable . The third section contains additives or some stabilizing ingredients, which include condiments, spices, stevia, honey, and other natural flavorings and sweeteners. Artificial flavorings (chocolate, orange, vanilla, etc.) and artificial sweeteners

(acesulfame, saccharin, etc.) are also utilized nowadays. Beside this pulses and/or cereals are added in present days for nutritional as well as functional achievement.

2.2.3 Recent trends and new innovations of functional smoothies

Some recent trends and new Innovations made in the preparation of functional Smoothies are summarized in table 2.4:

Table 2.4 Recent trends and new innovations of functional smoothies

Sl no	Constituents	Formulations	Observations	Reference
1	Flour-Milk based smoothie	Cow milk: 3% Fat, 8.5% SNF, flour of Sprouted finger millet, Germinated chickpea flour, Germinated sorghum flour, Germinated green gram flour, flour went between 2-6%, Mango mash 10%, 15% and 20%, Sugar-9%, 10% and 11%	Effect of flour levels on sensory attributes, Effect of apple juice and mango pulp on sensory attributes, , Effect of sugar level on sensory attributes, Sensory evaluation	**Rani *et al.* (2016)**
2	Chikoo Chia Smoothie	Chikoo – 50g Chia seeds – 4g, 8g and 12g Milk- 125ml Curd- 25g Honey- 10g	Sensory analysis-Colour, Consistency, Taste, Overall acceptability	**Battalwar *et al.* (2015)**

3	Cereal-milk fruit smoothie	Psyllium husk- 2.5% Full Cream Milk-42.4% Yoghurt-11.3% Banana-26.2% Blueberry- 14.5% SMP- 3.0% Water	Comparative study on difference in dietary behavior	**McCartney *et al*. (2019)**
4	Detox Smoothie	Mixer of Green and red juice smoothies, Wellness shots, Nuts shakes and filling smoothie	Recipe Book consisting of various types of healthy smoothies.	**Maranik, E. (2015)**
5	Olive Leaf Extract fortified smoothie	Olive leaf extract with oleuropein content, Strawberry-Banana smoothie, Sodium cyclamate, sodium chloride, sucrose, modified starch citric acid	Ranking Test, Threshold Test	**Kranz, *et al*. (2010)**
6	Jamun Synbiotic Smoothie	Jamun juice, Skim milk, Yoghurt, Sugar, SMP	Proximate analysis, acidity, pH, Prebiotic effect for microencapsulation, antioxidant activity, viscosity, total viable count, , total phenolic compounds, ascorbic acid, Shelf	**Saranyambiga, *et al*. (2017)**

			life at refrigerated temperature, Sensory evaluation.	
7	Soy/Carrot Flavoured with Beetroot	Beetroot/Carrot pulp: water- 1:4, Soybean milk: water- 1:10	Proximate Analysis, TSS, Viscosity, pH, Sensory Evaluation,	**Banigo, *et al.* (2015)**
8	Pumpkin leaves fortified Smoothie	Pumpkin leaves (1.5%, 3% and 4.5%) Pineapple, Banana, Apple.	Proximate analysis, Total flavonoids, Total Phenolic, Antioxidants, Mineral content, Vitamin C, Sensory analysis.	**Aderinola (2018)**
9	Green coconut smoothie	Solid albumen of green coconut 20% Pineapple pulp, Acerola pulp, and coconut water in different ratios.	Titrable acidity, pH, Vitamin C content, Antioxidant capacity, TSS, Sensory acceptability, Total Phenolic Compounds.	**Teixeira *et al.* (2019)**

2.3 Status of underutilized crops in India

Crops can be classified into two groups: staple and underused. Underused/underutilized crops, also known as orphan, neglected, or little used crops, are generally wild or semi-domesticated crops that have adapted to their local surroundings. They have a high nutritional content and are an excellent source of macro and micronutrients. Jack bean, winged bean, faba bean, Bambara groundnut, Horse

gram, Moth bean, Adzuki beans, Lima beans etc. are the categories of underutilized crops.

Food grains legumes are the second most significant crop category after cereals, and have long been a component of a well-balanced human diet **(Bhadana *et al*., 2013; Singh *et al*, 2016; Kaur *et al*, 2018; Mishra *et al*., 2018)**. They are also the second most valuable plant material for human and animal nutrition.

Underutilized legumes play an important role in rural communities' diets, especially during drought, famine, and the dry season **(Magbagbeola *et al*., 2010)**. Jack bean, Winged bean, Faba bean, Bambara groundnut, Horse gram, Moth bean, Adzuki beans, Lima beans etc. are the categories of underutilized crops.

This underutilized crop has tremendous potential to help smallholder rural farming citizens by providing income, food, and nutritional security, as well as maintaining the genetic resources required to address current and future environmental concerns **(Kahane *et al*., 2013).**

Around 40 leguminous crops from the genera Acacia, Abrus, Atylosia, Alysicarpus, Bauhinia, Canavalia, Cassia, Dolichos, Entada, Erythrina, Indigofera, Lens, Mucuna, Parkia, Parkinsonia, Phaseolus, Pongamia, Prosopis, Sesbania, Tamarindus, Vicia, Vigna, and Xylia are commonly consumed as the source of food by around 550 tribal communities and around 67.76 million of total Indian population in all over the lndia. The majority of the underutilized food legumes are found as wild species in various agro-ecological regions of India. There are 192 wild legume germplasms, including 45 tribal pulses that have been found in various agro-climatic regions of India **(Palai *et al*., 2019)**

With this in consideration, the current analysis focuses primarily on the potential of underutilized legumes as a source of food, feed, and nuetraceutically valuable components to give baseline data for alleviating malnutrition-related problems and sustaining pulse necessities in India.

2.3.1 Important underutilized and neglected crop species identified for Asia Pacific region.

Some important neglected and underutilized crop are found from Asia Pacific region are listed below table 2.5

Table 2.5 Important underutilized and neglected crop species identified for Asia Pacific region.

Sl no.	Underused crops	Examples
1	Pulses	Winged bean *(Psophocarpus tetragonolobus)*, Moth bean *(Vigna aconitifolia*), Hyacinth bean (Lablab purpureus), Grass pea (*Lathyrus sativus*), Horse gram (*Macrotyloma uniflorum*); Velvet bean (*Mucuna spp.*), faba bean (*Vicia faba*), Adzuki bean (Vigna angularis), Sword bean (*Canavalia spp*.), Rice bean (*Vigna umbellata*), others *pilipasara, Vigna trilobata, Parkia roxburghii* (multipurpose)
2	Pseudo cereals	*Amaranthus spp*., Buckwheat (*Fagopyrum esculentum; F. tataricum*), *Chenopodium spp*.,
3	Small millets	Finger millet (*Eleusine coracana*), Pearl millet (*Pennisetum americanum*), *Digitaria spp*., Proso millet (*Panicum miliaceum*), Little millet (*Panicum sumaternese*), Kodo millet *(Paspalum scrobiculatum*), Foxtail millet

		(*Setaria italica*), *Brachiaria spp*., *Job's* (*Coix lachyrma-jobi*), Barnyard millet *(Echinochloa spp*.)
4	Oil plants	Safflower (*Carthamus tinctorius*), Sesame (*Sesamum indicum*), Colocynth (*Citrullus colocynthis*), Niger (*Guizotia abyssinicca*), Physic nut (*Jatropha curcas*),
5	Fruits and nuts	Jack fruit (*Artocarpus heterophyllus*), Mangosteen (*Garcinia mangostena*), Bread fruit (*A. allitis*), Carmbola (*Averrhoa carambola*), Durio (*Durian zibethinus*), Longan (*Dimocarpus longan*), pillinut (*Canarium ovatum*), Indian gooseberry (*Emblica officinalis*), Duku (*Lansium domesticum*), Litchi (*Litchi chinensis*), *Manilkara spp*., rambuttan (*Nephelium lappaceum*), Pistachio (*Pistachio vera*), Jamun (*Syzygium cumini*), Tamarind (*Tamarindus indic*a), Indian jujube/ber (*Ziziphus mauritiana*), Chinese jujube (*Ziziphus jujube*).
6	Fibres and pulp	Dhaincha (*Sesbania bispinosa*), Ramie (*Boehmeria nivea*), Kenaf (*Hibiscus cannabinus*), Flax (*Linnum usititissimum*, Sunn hemp (*Crotalaria juncea*).
7	Vegetables	Leafy amaranth (*Amaranthus spp*.), Cucubitaceae (*Benincasa, Luffa momordica, Trichosanthes spp*.) Aibika (*Abelmoschus manihot*), Brassica spp., Kangkong (*Ipomoea aquatica*)
8	Roots and tubers	Elephant foot yam (*Amorphophallus paeoniifolius*), taro (*Colocasia esculenta*), yams (*Dioscorea spp.),* Vigna vexellata
9	Others	Sago palm (*Metroxylon sago*), Bamboo, *Perrilla spp*

Because of their great nutritional value, many underused crops have received a lot of attention in recent years. Due to their nutritious benefits, these grains are now considered to as Nutri-cereals (Nutritious grains). Underutilized crop species that are

high in micronutrients can help to balance diets. From the perspectives of resource management, agricultural diversification, self-sufficiency, economic advantages, germplasm augmentation and conservation, and nutritional security, attention is being focused on under-utilized crops.

In this context, underutilized legumes offer a lot of potential for food security, nutritional needs, and agricultural development. Many known underutilized legumes (such as *Mucuna spp., Canavalia spp., Sesbania spp., Phaseolus spp.*, and others) have adequate amounts of protein, essential amino acids, polyunsaturated fatty acids (PUFAs), dietary fibre, essential minerals and vitamins, along with the beneficial bioactive compounds, comparable to other common legumes.

2.3.2 Nutritive value of some underutilized legume

Nutritive composition of various underutilized legumes are summarized in table 2.6.

Table 2.6. **Nutritive value of some underutilized legumes**

Sl no.	Legumes	Fat	Protein	Fibre	Carbohydrate
1	Winged bean	15-20	30-40	6-7	35-45
2	Lima bean	1-2	19-25	4-6	70-75
3	Horse gram	0.5	22	5.3	57.2
4	Kidney bean	0.8	24	25.0	60.0
5	Moth bean	1.6	23	5.0	62
6	Chickpea	6.0	19.0	17.0	61.0
7	Faba bean	1.53	26.12	25.0	58.59

8	Rice bean	0.9	20.9	4.0	60.7
9	Adzuki beans	0.5	20.0	13.0	6.0
10	Lathyrus pea	1	28	2	47
11	Bambara groundnut	6-8	20	3-6	60
12	Jack bean	-	29-30	7.34-9.9	50.77-54.28
13	Velvet bean	6.3-7.4	20.2-29.3	8.7-10.5	49.9-61.2

Adopted from Palai *et al*., (2019)

2.4 Horse gram: an underutilized crop

Horse gram (Macrotyloma uniflorum) is a minor or lesser known neglected unexploited food legume among the underutilized crops, found primarily in Southeast Asia and tropical Africa. It has a wide range of functional and nutritional qualities. on the other hand, the origin of cultivated species, is the southern section of India **(Kumar DS *et al*, 2013, Pal *et al*., 2015)**. The name "Macrotyloma" comes from the Greek words "makros" which means "large," "tylos" which means "knob," and "loma" which means "margin," and refers to the knobby appearance of the pods. Dolichos biflorus L. is the scientific name for Macrotyloma uniflorum. M. uniflorum seeds are considered the poor man's pulse crop in Asian countries, particularly India. It is also acknowledged as “Horse gram”. The crop produces roughly 0.65 million tonnes per year, accounting for about 5-10% of India's pulse production **(Kiranmai K *et al*., 2016)**

The Horse gram [Macrotyloma uniflorum (L.) Verdc] is vital to the nutritional security of rural, tribal, and underprivileged populations **(Tontisirin, 2014).** Horse gram is a very nutritious vegetable pulse crop with many ethno-medicinal properties. It's a good source of protein, carbohydrate, dietary fibre, molybdenum, iron, calcium, and other micronutrients, as well as bioactive phytochemicals **(Prasad *et al*., 2010, Bhokre *et al*., 2012).**

Horse gram has a variety of therapeutic properties, including; anti-diabetic **(Raj *et al*., 2016, Parthsarthi *et al*., 2013),** antioxidant **(Singh *et al*., 2012, Ravishankar *et al*., 2012)**. anticalcifying **(Atodariya *et al*., 2013)**, anti-hypercholesterolemic **(Sengupta *et al*., 2012, Kumar *et al*., 2013)**, analgesics (**Ashraf *et al*., 2018, Fatima *et al*., 2018)**. Horse gram in local language known as Kulattha (Sanskrit), Kurti-kalai (Bengali), Kollu (Tamil), Ullavallu (Telgu), Muthira (Malyalam), Gahot (Kumaon and Garhwal), and etymologically, Gahot means "which destroys stone in the earliest stage" **(Pati *et al* , 2013).**

Taxomical Description

Kingdom – Plantae	Family – Fabaceae
Class – Magnoliopsida (Dicotyledons)	Subfamily – Faboideae
Subclass – Rosidae	Tribe – Phaseoleae
Subtribe – Phaseolinae	Genus – Macrotyloma Verdc.
Order – fabales	**(Ramteke *et al*., 2016)**

Horse gram is a self-pollinated crop in the Fabaceae family. It is an annual herb with an erect and branched stem, alternate, petiolate, stipulate, trifoliolate leaves, an axillary inflorescence, bracteate, bisexual flowers, and papilionaceous corolla. Single yellow or greenish yellow flowers with a violet blot on the standard are produced. Pods measure 5-8cm long and 3-9mm wide, and contain 5-8 seeds. The cultivated annual form, Macrotyloma uniflorum, has larger pods. It is drought resistant but not water logging resistant. Horse gram is grown in drought-prone parts of South India during the late rainy season (August to October). This crop is harvested and farmed as a "Kharif" crop in India's arid regions **(Bhardwaj J et *al*. 2015).**

Distribution

It is one of the most important untapped food legume, growing essentially everywhere in the world, including temperate and sub-tropical regions such as East and Northeast Africa, Asian countries such as India, China, the Philippines, Bhutan, Pakistan, Sri Lanka, and Queensland, Australia **(Durga, 2012; Krishna, 2010).**

In Karnataka, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Maharashtra, Odisha, Bihar, Jharkhand, Uttar Pradesh, and the foothills of Uttaranchal and Himachal Pradesh, certain wild varieties of horse gramme were domesticated and became a staple of local agricultural crop.

2.4.1 Different varieties, distribution and cultivation of Horse Gram (*Macrotyloma uniflorum*)

Following table 2.7 describes varieties, distribution of horse gram all over the world.

Table 2.7 Different varieties, distribution and cultivation of horse gram (*Macrotyloma uniflorum*)

Categories	Distribution	Harvested areas
M. uniflorum (Lam.) Verdc. var. verrucosumVerdc.	Eastern and southern Africa at 550 m altitude	Thicket and Grassland
Macrotyloma uniflorum (Lam.) Verdc.	Wild species are distributed in Southeast Asia and southwest Africa (Namibia)	Widely harvested in tropical areas
M. uniflorum (Lam.) var. benadirianum (Chiov.) Verdc.	Found in East Africa at sea level on sand dunes	Somalia and Kenya
M. uniflorum var. stenocarpum (Brenan) Verdc.	Seen in India and Africa at higher altitude	Cultivated in parts of Australia and USA

Table Adopted from Chakravarty *et al*. (2019)

2.4.2 Nutritional properties

HG is a true super food in terms of nutraceuticals. Super foods are a type of nutrient-dense food with a high antioxidant and protein content **Mehra, A. (2013).**Horse gram has been identified as a possible protein and nutritional source **(Sreerama *et al*., 2012)**. It has a high nutritional value that is comparable to that of other regularly grown pulse crops in every way, and it is also a good source of iron, molybdenum, and calcium **(Prasad *et al*., 2010; Bhokre, 2012).** However, a variety of factors such as genotype, soil, fertilizer application, cultural techniques, weather and climatic factors, postharvest management and storage can all have an impact on

nutritional quality, either directly or indirectly. Horse gram seed has a low fat content and is high in protein, dietary fibre, minerals, and phytochemicals *(Sreerama* ***et al.*****, 2012).** It is still an underutilized food legume, consumed primarily by farmers in inaccessible places and low-income populations.

2.4.2.1 Carbohydrate

There are two types of carbohydrates in legumes: digestible and nondigestible sugars. Carbohydrates are rich and common in M. uniflorum seeds. Starch (partly digested), monosaccharide, oligosaccharide, and polysaccharide are among the carbohydrates accessible. Starches have a lesser digestibility than cereals because they are legumes. They have less carbohydrate (55-65%) and energy than cereals. M. uniflorum seeds contain a low-glycemic-index carbohydrate **(Prasad *et al.*, 2015).** Some carbohydrates, known as resistant starch, are fermented in the large intestine with the help of gut microorganisms because they are not digestible in the small intestine. The non-digestible carbohydrate content of horse gram is high, resulting in slow glucose release into the bloodstream, potentially positive effects in diabetic dietary management, and this resistant starch is recognised as a prebiotic among the new generation of dietary fibres **(Samanta *et al.*, 2011).** Starch is the most readily accessible carbohydrate, accounting for 22–45% of the total carbohydrate content of a Horse gram. Oligosaccharides and dietary fibre account for 1.8–18% and 4.3–25% of total calories, respectively **(Norazalina *et al.*, 2010).**

2.4.2.2 Protein and amino acid

Horse gram is a high-protein plant-based protein that is relatively affordable. Protein content varies between 18 and 28.5 percent **(Sander *et al.*, 2017)**. The cotyledon

portion of the seed has approximately 89 percent protein, whereas the seed coat and embryonic axe have 10 and 1% protein, respectively **(Muricken *et al.*, 2010).** Apart from glutelins (10–20%) and albumins (10–20%), globulin is the most abundant protein, accounting for 50–90% of total protein content. In Horse gram lysine is the most abundant amino acid (0.52 g nitrogen), **(Prasad *et al.*, 2010),** followed by arginine, histidine, valine, and leucine.

2.4.2.3 Minerals

The mean concentrations of macro minerals (Ca, K, Mg, P, and S) in horsegram accessions range from 1.3–14 mg per gram, whereas micro minerals (Cu, Fe, Mn, Ni, and Zn) range from 1.0–95.0 µg per gram dry weight **(Morris et al., 2013).**

The calcium and iron content of raw Horse gram seeds ranges from 244–312 mg and 5.89–7.44 mg per 100 g, with in-vitro bio-accessibility of 22.50–38.50 mg of calcium and 0.26–0.85 mg of iron per 100 g of seeds, respectively **(Khatun *et al.*, 2013).**

Potassium (762 mg/100 mg), calcium (287 mg/100 mg), zinc (3.4 mg/100 mg), magnesium (172 mg/100 mg), phosphorus (311 mg/100 mg), and iron (6.77 mg/100 mg are the primary minerals.

The ash content of Horse gram seeds significantly lower after germination, indicating mineral loss during the germination process and mineral decline after germination **(Minisola *et al.*, 2015)**. Phosphorus, potassium, magnesium, calcium, and iron concentrations were lowered to 256, 675, 136, 11.6, and 4.03 mg/100 mg, respectively.

2.4.2.4 Vitamins

Human growth necessitates only a modest amount of vitamin. They play an essential role in the human body's regular functioning. Even though they do not provide energy, they play an important part in energy utilisation. Thiamine (B1), riboflavin (B2), and niacin (B3) are three water-soluble vitamins found in horse gramme (B3). B1, B2, and B3 vitamin amounts in orse gram are 0.38, 0.19, and 1.42 mg/100 mg, respectively.The availability of vitamins in Horse gram is affected differently by processing and germination. While the vitamin content of Horse gram drops sharply after processing, after germination, vitamin B2 (from 0.1 to 0.19 mg/100 mg) and B3 (from 1.42 to 3.65 mg/100 mg) concentrations rise, but vitamin B1 (from 0.38 to 0.33 mg/100 mg) concentrations fall. The accessible concentration of vitamins is affected by several parameters such as the number of rinses, light sensitivity, and germination period **(Prasad *et al*., 2015).**

2.4.2.5 Fat

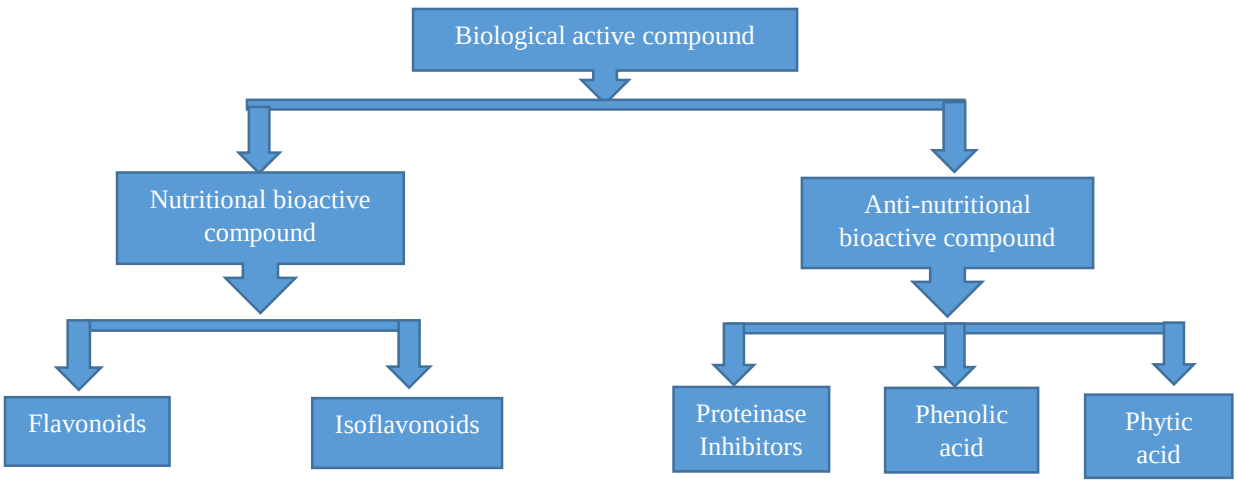
The amount of saturated fatty acids in M. uniflorum seeds is very low. Unprocessed seeds account for 72.49 %, whereas toasted seeds account for 71.99 %. Linoleic acid, an important fatty acid, is abundant in seeds. Linoleic acid is found in raw seeds in 45.58 percent and toasted seeds in 40.33 percent **(Morris *et al*., 2013).** Horse gram fat content ranges from 0.6 to 2.6 % **(Sreerama *et al*., 2010).** Dehulled horsegram seeds have a greater crude fat content (0.81–2.11%) than whole horsegram seeds (0.70–2.06 %). Horse gram seeds contain 27.5 percent saturated fatty acids (21.97 percent palmitic, 2.85 percent arachidic, 2.32 percent stearic acid, and 0.36 percent myristic), 72.49 percent unsaturated fatty acids (42.78 percent linoleic, 16.15 percent

oleic, and 13.56 percent linolenic acid), and linoleic acid is useful for the treatment of diabetes and cardiovascular diseases among unsaturated fatty acids **(Mishra *et al* 2011).**

2.4.3 Bioactive compounds

Horse gram includes a number of chemicals that can have biological effects in humans, including both positive and negative adverse effects. Nutritional bioactive molecules are the desirable ones, whereas anti-nutrients are the unwanted ones. Anti-nutritional factors such phenols, tannins, phytic acids, and flatulence-causing oligosaccharides are increasingly being examined at as potential antioxidants. Anti-nutrients have a multitude of health benefits, including a lower risk of intestinal illnesses (gallstones, diverticulosis, constipation, and colon cancer), coronary heart disease, dental caries prevention, and diabetes treatment. Saponins and another type of ant-nutrient molecule have been shown to have ant carcinogenic and hypocholesterolemic properties.

Fig 2.1 Bioactive compounds



2.4.3.1 Nutritional bioactive components

In legumes, flavonoids are significant nutritional bioactive phytochemicals. Horse gram is high in flavonoids and has antioxidant properties. In Horse gram, the main flavonoids are quercetin, kaempferol, and myricetin, whereas the main isoflavonoids are daidzein and genistein. The amount of flavonoid in different regions of the seed varies. The seed coat has more quercetin, kaempferol, and myricetin than the cotyledon and embryonic axe.

The concentrations of quercetin, kaempferol, and myricetin found were 129.5 mg, 117.2 mg, and 35.5 mg/100 g dry weight, respectively. Quercetin (113.4 mg/100 g), kaempferol (67.4 mg/100 g), and myricetin (32.9 mg/100 g) are moderately abundant in the embryonic axe part of horse gram seed. The embryonic axe, on the other hand, has more isoflavonoids than the seed coat and cotyledon section. Daidzein and genistein had concentrations of 22.2 and 44.7 mg/100 g, respectively **(Sree *et al*., 2014).**

The seed coat and cotyledon region are devoid of genistein. Flavonoids function as phytoalexins, which protect seeds from microbial infection and other environmental stresses. Isoflavonoids act as phytoestrogens, lowering the risk of coronary heart disease and, in particular, breast and prostate cancer. They also suppress the actions of tyrosine kinase and topoisomerases I and II, which decrease cell proliferation **(Sengupta *et al*., 2012).**

2.4.3.2 Anti-nutritional bioactive components

1. Proteinase Inhibitors

Protease is a catalytic enzyme that degrades proteins' peptide bonds. Its actions must be regulated because it has a negative effect at higher concentrations **(Mukesh *et al* 2011).** Protease inhibitors are a type of storage protein that binds to proteases and suppresses their activity. Apart from metallo and cysteine inhibitors, there are eight different forms of serine proteinase inhibitors documented in plants. Bowman-Birk inhibitors, which are serine proteinase inhibitors, are found in legume seeds. It is a single polypeptide with a molecular weight of 6–9 kDa that is heat and acid stable **(Mukesh *et al* 2011).**

Which forms dimers, trimers, and tetramers and are resistant to proteolytic enzymes. A four-headed inhibitor that inhibits trypsin and chymotrypsin was identified as a double-headed inhibitor with two active sites **(Rajagopal *et al*., 2018).** HGI-III is the name of the Bowman-Birk inhibitor found in Horse gram. It is a single polypeptide chain with 76 amino acids and seven inter-weaving disulphides, four of which are associated with trypsin and three with the chymotrypsin-binding domain, respectively **(Kumar *et al*., 2013) (Kumar *et al*., 2013).** It can withstand severe pH and high temperatures (up to 95°C). It has anticancer, anti-inflammatory **(Kumar *et al*., 2013**), and anti-diabetic properties, as well as activity against a variety of degenerative and autoimmune diseases. Horse gram is a functional food due to its trypsin inhibitory activity. Trypsin-inhibiting activity in Horse gram flour is 9246 TIU/g, which is substantially greater than in chickpea and cowpea flour **(Sreerama *et al*., 2010).**

However, once the seed is germinated, the activity decreases due to structural changes that occur during germination.

(Sreerama *et al*., 2010).

2. Phenolic Acid

Leguminous plants generate significant phytochemicals called phenolic compounds, which have antioxidant properties that have a vital role in regulating the taste, flavour, and colour of the pulse. They are produced in response to stress and serve to defend the plant against predators. They also provide a number of health benefits for humans. Legumes contain polyphenolic compounds in amounts ranging from 0.325 to 6.378 mgGAE/g **(Marathe *et al*., 2011).** They are considered antinutritional because they react with proteins, inactivating or reducing the solubility of enzymes including trypsin, lipase, and amylase.

The effects of phenolic components in legumes affect the rate of protein and carbohydrate digestion, as well as the availability of vitamins and minerals **(Sahoo *et al*., 2014).** The tanins and phenolics in Horse gram seeds are higher than in other legumes. Horse gram seeds contain 763.7–895.9 mg/100 g of free tannin and 1.67 g/100 g of phenolics, respectively. P-coumaric acid (8.95 mg) and p-hydroxyl benzoic acid (7.81 mg) are the two most common phenolics found in horse gram. The phenolic content of black Horse gram seeds is higher than brown seeds, and the seed coat contains more tannin and phenolics. The tannic and phenolic content of the seeds is reduced after dehulling and soaking. Phenolic compounds have antioxidant, free radical scavenging, and metal ion chelating properties, all of which serve to prevent disease and improve human health. Apoptosis, platelet aggregation, blood vessel dilation,

different enzymatic activity, carcinogen inactivation, and detoxification are all linked to phenolics. Antimutagenic, anti-inflammatory, antimicrobial **(Sundaram U *et al*., 2013)**, anticarcinogenic, antiproliferative, vasodilatory, and antiviral properties have been observed in phenolic compounds found in horse gram.

3. Phytic acid

Phytic acid, also known as inositol hexaphosphate, is a simple carbohydrate ring with six phosphate connected to each carbon (hexaphosphate) **(Neelam *et al*., 2014).** In legumes, it is a primary source of phosphorus, accounting for up to 85 % phosphorus content. It is found as phytate, phytin, and in free forms that are interchangeable and forms complexes with minerals, proteins, and starch.

Phytic acid levels are higher in the cotyledon section of the seed than in the embryonic ash and seed coat of Horse gram seed. Phytic acid has an antinutritive property in that it binds to minerals such as zinc, magnesium, iron, and calcium, reducing their bioavailability and inhibiting the proteolytic activity of specific enzymes. It does, however, have powerful antioxidant and anticarcinogenic properties, aids cell growth, and boosts the immune system **(Ravishankar *et al*., 2012).** The amount of phytic acid in seeds is reduced during processing and germination **(Khamgaonkar *et al*., 2013).**

2.4.4 Nutritional properties of Horse gram

Table 2.8 shows nutritional value of horse gram

Proximate analysis	**Raw horse gram**	**Germinated horse gram**
Fat	1.60±0.01	1.48±0.02
Protein	22.50±0.1	24.10±0.1
Carbohydrates	58.50±0.02	58.11±0.01
Crude fibre	3.17±0.2	3.09±0.1
Moisture	6.90±0.01	7.60±0.01
Ash	2.89±0.02	3.07±0.2

Table adapted from (Kachave *et al*., 2020)

2.4.5 Pharmacological properties

2.4.5.1 Antidiabetic effect

Horse gram contains slowly digestible starch, which is thought to have a low postprandial glucose reaction when consumed by people with diabetes. The anti-diabetic action of -amylase inhibitor isolated from Macrotyloma uniflorum seeds in diabetic mice induced with streptozotocin and nicotinamide. **(Lakxmi *et al*., 2011)**. Biochemical markers such as serum total cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels have been determined. M. uniflorum α-amylase inhibitor (MUAI) suppressed both mouse pancreatic and human salivary α-amylase, according to **Purwar *et al*. (2013)**. The diabetic mice treated with MUAI had lower serum glucose levels. In comparison to diabetes control mice, histological studies demonstrated little pathological alterations in the treated diabetic mice.

2.4.5.2 Diuretic activity

In albino rats, Ravishankar *et al*. investigated the diuretic impact of ethanol extract seed of M. uniflorum. After oral administration of extracts at doses of 200mg/kg and 400mg/kg, the urine volume, sodium, potassium, chloride, and bicarbonate contents were determined. In comparison to the control, Furosemide (5mg/kg), the diuretic impact of M. uniflorum extracts was considerable in experimental animals **(Ravishankar, et al., 2012).**

2.4.5.3 Anti-obesity activity

Antihypercholesterolemic effects were revealed in Horse gram leaf extracts. After 5 weeks of using Horse gram leaf extracts, researchers noted a decrease in total cholesterol, triglycerides, low-thickness lipoprotein, extremely low-thickness lipoprotein, and an increase in high-thickness lipoprotein. The ethanol concentration of Horse gram resulted in much higher faecal cholesterol excretion than the water concentrate, and water extract resulted in a significant reduction in body weight compared to ethanol extract **(Kumar, 2013**). In rodents (rats), the antihypercholesterolemic effect of Horse gram extract is investigated by examining its effects on food intake, serum glutamate oxaloacetate transaminase (SGOT), weight gain, serum lipid profile, and body fat **(Kumar , 2013).** The hot Extract of Dolichos biflorus (Horse gram) on Body Weight in Overweight or Obese Human Volunteers, according to **(Bhuvaneshwari *et al*. 2014)** Macrotyloma uniflorum was found to have anti-obesity properties.

2.4.5.4 Anti-oxidant activity

In-vitro antioxidant activity of ethanolic seed extracts of M. uniflorum was demonstrated by, **Renu Singh *et al*., 2012**. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione concentrations improved when M. uniflorum extract was given to rabbits with oxidative stress caused by a high-fat diet. **(Ravishankar, *et al*., 2012)**

2.4.5.5 Hepatoprotective activity

Parmar *et al*. (2012) revealed that M. uniflorum seeds have considerable hepatoprotective activities against D-Galctosamine and paracetamol-induced hepatotoxicity in rats.

2.4.5.6 Anti-helmintic activity

M. uniflorum seeds exhibit anthelmintic properties, making them useful for worm eradication. The anthelmintic activity of alcohol extracts of M. uniflorum seeds these extracts had significant anthelmintic activity against Pheretimaposthuma, comparable to the standard. albendazole **(Varicola *et al*., 2014).**

2.4.5.7 Anti-choliolithic activity

In mice, M. uniflorum seed had an anticholithogenic effect by reducing the development of lithogenic bile. The methanolic and acetone extracts (ME and AE) both reduced cholesterol hypersecretion into bile and boosting bile acid production. The AE had the greatest benefit, since it reduced gallbladder papillary growth and hepatic fatty degeneration. The antioxidant properties of polyphenol and tannin in AE may account its anticholithogenic activity. **(Bigoniya *et al*., 2014)**

2.4.5.8 Analgesics and anti-inflammatory effect

In vitro suppression of human secretory phospholipase A2 (sPLA2) by aqueous extracts of M. Uniflorum coat and pulp as a function of anti-inflammatory activity. The extract efficiently controlled indirect hemolytic activity and had equal potency in preventing PLA2-induced mouse paw edoema in vivo **(Giresha *et al*., 2015).**

2.4.5.9 Kidney Stone

Horse gram soup is known for its ability to reduce the risk of kidney stones. It was observed that Horse gram seed concentrates include at least two inhibitors of calcium phosphate crystallisation (one of the major ingredients of kidney stones) that are nonproteins, soluble in water, and heat stable **(Kaundal , 2019).** In this approach, a soup made from naturally collected Horse gram could be beneficial in the treatment of kidney stones.

2.4.6 Functional characteristics of Horse gram

The following table 2.9 reported the functional properties of horse gram.

Table 2.9 Functional characteristics of Horse gram

Horse gram	Chemical constituents	Functional properties
Seeds	I. Proteins and amino acids	I. Improve plasma density by maintaining the ratio and gives as affordable source of protein.
	II. Bioactive compounds	II. Exert ant-hepatotoxic and antioxidant activity.
	III. Vitamins	III. Helps in completing of growth.
	IV. Minerals	IV. Reduces blood pressure related problems.
	V. Isoflavones	V. Inhibits calcium oxalate crystallization, Potential against cold & throat infections, fever anti-lithic activity
	VI. Lipids	VI. Hypolipidemic activity.
	VII. Saccharides	VII. Lower the risk of diabetes and obesity by improving gut flora.
Seed coat	I. Phenolic acids	I. Antioxidant effect and reduces risk of coronary heart diseases and inflammation.
	II. Fiber content	II. Helps to regulating digestion.
	III. Calcium	III. strengthen the muscles and bone structure.
Dark colored Seeds	I. Higher phenolic acids	I. Reduces ferric ions

Table adopted from, Bhardwaj, et al., (2015)

2.4.7 Medicinal properties of horse gram

2.4.8 Horse gram have various medicinal values which are summarized in below table 2.10

Table 2.10 Medicinal properties of horse gram

Sl no.	Extracts	Medicinal activities	References
1	Ethanolic	Antihistaminic	**Suralkar AA, *et al*., 2013**
2	Methanolic	Analgesic and anti-inflammatory, Hepatoprotective larvicidal (□-amylase inhibitors)	**Giresha A S *et al*., 2015, Parmar H *et al*., 2012, Laxmi Gupta *et al*., 2011**
3	Acetone	Anticholilithiatic	**Bigoniya P *et al*., 2014**
4	Chloroform	Antioxidant	**Renu singh *et al*., 2012**
6	Alcohol	Antiobesity	**Sengupta K *et al*., 2012, Bharathi V *et al*., 2014**
7	Hydro-methanolic	nephrotoxicity management	**Sarmistha Saha *et al*., 2012**

2.4.9 Horse gram incorporated food and dairy products -

The inclusion of horse gram in various food products revealed that horse gram is high in protein, calcium, and dietary fibre; among the various procedures used, roasting was beneficial in lowering anti-nutritional elements **(Thirukkumar *et al*., 2014).** Horse gram was used to make bhakari, chapati, kharapara, shakkarpara, semolina burfi, salty biscuits, and cookies. In bhakari, chapati, semolina burfi, salty biscuits, and cookies, Horse gramm flours can be used up to 20%, whereas in kharapara

and shakkarpara it can be incorporated up to 15%. Kaulath, a fermented product (based on Horse gramme), helps patients with cardiovascular and diabetic issues by regulating salt and potassium levels and reducing -glucosidase activity **(Dwivedi *et al*., 2015).**

2.4.10 Some value added food products incorporated Horse gram

There are so many products are been developed by incorporating Horse gram mentioning table 2.11.

Table 2.11 some value added food products incorporated horse gram

Sl no.	**Product**	**Formulation**	**references**
1	Buns fortified with germinated horse gram	Maida 80% + germinated horse gram flour 20%, yeast, milk powder, sugar, calcium propionate, GMS, salt.	**Bhokre C *et al*., 2012**
2	Weaning food incorporated horse gram malt	Rice flour 60% + horse gram malt 20% + sugar 10% + carrot powder 5% + milk powder 5%	**S.K. Sadawarte *et al*., 2020**
3	Cookies incorporated horse gram flour	20gm horse gram flour + 80gm refined wheat flour + 50gm icing sugar + 50gm fat + 5gm milk powder + 2gm vanilla powder	**A.Swarnalatha *et al*., 2020**
4	Horse gram adai	60% roasted horse gram flour + 40% ragi flour, chopped onion, green chilli, cury leaves, salt	**A.Swarnalatha *et al*., 2020**

5	Horse gram fortified cow milk	Horse gram extract 40% + milk 60%	**G.M. Sivakumar *et al*., 2020**
6	Idli incorporated germinated horse gram powder	10gm horse gram powder + 90gm semolina + 25gm curd + 2gm eno + 20gm chopped vegetables, salt	**Niharika *et al*., 2016**
7	Khakra	10g horse gram powder + 90g wheat flour + 2g ajwain + 2gm turmeric + 1tsp oil, salt	**Niharika *et al*., 2016**
8	Cookies	Refined wheat flour, horse gram flour (30%, 40% & 50%), powder sugar, butter, baking powder	**M. Jayapriya *et al*., 2020**
9	Chapatti	Wheat flour, horse gram flour (30%, 40% & 50%), salt	**M. Jayapriya *et al*., 2020**

2.5 Black carrot

Carrots, which belong to the Apiaceae family, are a notable vegetable consumed for their edible fleshy roots. Carrots are divided into two groups based on their colouring compounds: the carotene group (Daucus carota ssp. Sativus) and the anthocyanin group (Daucus carota L. ssp. Sativus var. atrorubens Alef). **(Polat *et al*., 2022).**

Carrots (Daucus carota L.) have a high antioxidant content. These antioxidants could be lipophilic (carotenoids) or hydrophilic (phenolic compounds). Carrots come in a variety of colours all around the world. The presence of relevant pigments in the carrots is responsible for these colours. Lycopene is found in red carrots, and lutein is found in yellow carrots. Similarly anthocyanins are found in another important group

of carrots is purple or black-colored carrots. All of these pigments have been shown to have high free radical scavenging abilities. Black carrots is a sub-species having the scientific name Daucus carota L. ssp. Sativus var. atrorubens Alef, have an attractive purple colored vegetable **(Turkyilmaz *et al*. 2012).** Acylated cyaniding-3-(p-coumaroyl)-diglucoside-5-glucoside is the most significant anthocyanin identified in black carrots.

They are significant industrial crops grown mostly in Turkey and the Middle and Far East **(Elik, 2021).**

Anthocyanins are a class of polyphenolic pigments that are water soluble. They are commonly found in fruits and vegetables and give them a distinctive red to purple colour. Since anthocyanins are natural pigments, black carrots are now widely considered as a promising natural food colour source and a viable alternative to manufactured colours **(Ekici *et al*. 2015).** The unique profile of anthocyanin pigments in black carrots has piqued the interest of the scientific community. **(Olejnik *et al*. 2016**, **Padayachee *et al*. 2013** and **Kamiloglu *et al*. 2015).**

Anthocyanins have long been recognized as a dietary component with well-established nutraceutical effects, notably in terms of their ability to scavenge free radicals **(Kamiloglu *et al*. 2015)** anticancer activity **(Sevimli-Gur *et al*. 2013)** and anti-inflammatory properties.

The use of black carrot as a food of choice to battle physiological concerns in humans, such as long-term and acute hypoglycemia and hypolipidemic consequences, could be a wise and nutritionally sound decision. **(Akhtar *et al*. 2017).**

Anthocyanin, which is abundant in black carrot extract therefore, has a number of health benefits in addition to its colour feature. They are powerful antioxidants that may help to prevent heart diseases, certain tumorous cell cancers, diabetes, and obesity, as well as improve cognitive behaviour **(Alagarra *et al*., 2014, Ren *et al*., 2021).**

Apart from direct benefits, black carrots extract has antimicrobial effects against a variety of food-borne diseases, therefore enhances food storage quality by reducing microbial deterioration.

2.5.1 Biochemical composition

Black carrot comprises around 88 % moisture, 1 % protein, 0.14 % fat, 2.5 % fibre, and 8 % carbohydrate, according to Turkish food composition. Minerals such as calcium, phosphorus, sodium, potassium, magnesium, iron, and zinc are also abundant in carrots. **Gopalan *et al*. (2010)** found 80 mg calcium, 53 mg phosphorus, and 2.2 mg iron per 100 g.

2.5.2 Chemical parameters of fresh black carrot juice

The black carrot have following chemical characteristics reported in Table 2.12.

Table 2.12. Chemical parameters of fresh black carrot juice

Parameters	**Mean value**
Reducing sugar (g)	4.89±0.06
Total phenolic content (mg)	248.07±4.21
Vitamin C (mg)	1.68±0.04
Total carotenoids (mg)	1.93±0.10
Total acidity (g)	0.19±0.10
Total sugar (g)	7.95±0.04
Pectin (g)	1.03±0.06
Dry matter (g)	11.35±0.03
Anthocyanins (mg)	44.25±3.90

Table adopted from Zadernowski *et al*, 2010

2.6 Bananas

Bananas (Musa spp.) are perennial monocotyledons that belong to the Musaceae family. They are one of the high calories tropical fruits that grow more abundantly in tropical rain forest environments. Bananas are harvested in about 120 countries. The main producers are China, Brazil, Ecuador, Indonesia, Philippines, Costa Rica, Colombia, Thailand and Mexico. India is the world's leading and ranks first banana producer **(Anon, 2014)** and third in area among fruit crops. The major banana producing states are Karnataka, Gujarat, Andhra Pradesh and Assam, With a production of around 29725 thousand tonnes from an area of 803 thousand hectares

Bananas contains a very good level of health beneficiary antioxidants, carbs, dietary fibre, vitamins and minerals. Structurally banana fruit has a outer protective skin layer is composed of soft, edible and easily digestible flesh inside which is high in starch, which turns into simple sugars like fructose and sucrose when it ripens, but it is also a strong source of resistant starch that upon consuming instantly for replenishing energy and revitalizing the body. Thus, for these qualities, bananas are being used for the supplement diet in the treatment for underweight children. Potassium is abundant in fresh bananas. Potassium is a mineral component of cell and body fluids that serves to regulate heart rate and blood pressure.

2.6.1 Important functional ingredients

Banana is a highly nutritious sweet fruit and the flesh can vary in taste from starchy to sweet, and texture from firm to mushy is depending upon the harvest and ripeness,. The banana is a high-calorie tropical fruit. Fruit contains 89 calories per 100

grams. The fruit contains a significant quantity of soluble dietary fibre (2.6 g/100 g) that aids in normal bowel movements, as well as a carbohydrate content of 23%.

2.6.1.1 Antioxidants

In moderate levels, banana includes health-promoting flavonoid polyphenolic antioxidants such as lutein, zea-xanthin, and a-carotenes. These chemicals operate as scavengers for oxygen-derived free radicals and reactive oxygen species, which are involved in ageing and other disease processes.

2.6.1.2 Vitamins and minerals

Bananas are high in vitamin B6 (pyridoxine), which accounts for roughly 28% of the daily recommended allowance. Pyridoxine is a B-complex vitamin that has been shown to help with neuritis and anaemia. Furthermore, it aids in the reduction of homocysteine levels in the human body (one of the triggering factors in coronary artery disease (CHD)). The fruit also contains a minor amount of vitamin C (about 8.7 milligrammes per 100 g). Vitamin C-rich meals aid in the body's development of tolerance to infectious agents and the scavenging of damaging oxygen-free radicals.. Minerals including copper, magnesium, and manganese are abundant in fresh bananas. Magnesium is necessary for bone health and also helps to protect the cardiovascular. Manganese is essential as a cofactor for superoxide dismutase, an antioxidant enzyme. The synthesis of red blood cells necessitates the use of copper. The potassium content of a fresh banana is extremely high. Potassium is found in 358 mg per 100 g of fruit. Potassium is a mineral found in cells and body fluids that serves to regulate heart rate and blood pressure.

2.6.2 Proximate values of banana fruit

The chemical composition of ripe banana like fat, carbohydrate, dietary fibre, protein, minerals, vitamins, and fatty acids are mentioned in following table 2.13.

Table 2.13. Proximate parameters of Ripe Banana

sl no.	Chemical constituents	Value
1	Protein (g)	1.09
2	Carbohydrate (g)	22.57
3	Total dietary fibre (g)	2.6
4	Energy (kcal)	89
5	Moisture (g)	74.91
6	Total fat (g)	0.33
7	Ash (g)	1.1
9	Iron	0.26
10	Calcium	5
11	Potassium	358
12	Zinc	0.15
13	Phosphorus	22

Table adopted from (JOY *et al*., 2016)

2.7 Different types of Challenges during preparation of smoothie

Various challenges occur in developing functional smoothie, such as, freely available anti nutritional factors (ANFs), shelf life, off-flavour, colour degradation, sedimentation. Therefore to overcome from these challenges several works has been

done like, soaking, pasteurization, addition of flavoring ingredient (banana), addition of coloring ingredient (black carrot), finely grinding/mixing the smoothie.

Various types of challenges and overcome adopted during manufacturing of functional smoothie are summarized in table 2.14.

Table 2.14. **Challenges and overcome during development of smoothie**

Sl no.	**Challenges/factors**	**Overcome/improvinization**	**References**
1	Anti-nutritional	Soaking/germination	**(Maisont *etv al*, 2010), (Embaby 2010; Kajihausa *et al*. 2014)**
2	Self-life storage	Pasteurization/thermal heat treatment	**(Petruzzi, *et al*., 2017), (Marszałek, *et al*., 2017), (A ˘gçam., 2018).**
3	Color degradation during storage	Natural coloring agent anthocyanins (black carrot extract)	**(Sucheta & Yadav, 2020), (Petropoulos *et al*. 2019), (polat et al., 2022).**
4	Sedimentation /wheying off	Addition of stabilizer such as pectin.	**(Irem Bige *et al*., 2022), (Khanniri E *et al*., 2019), (Kiani H *et al*., 2010), (Guimaraes JT *et al*., 2018), (Krongsin J et al., 2015).**
5	Off-odor	Natural flavoring agent (banana)	**(Hoda H. M. Fadel *et al* 2015).**

2.7.1 Improvisation of different challenges

2.7.1.1 Soaking and germination

The soaking is initial step in water penetration is, which converts inactive tissue into living tissue. The metabolism of the grain is triggered in this step to prepare it for germination **(Maisont *et al,* 2010).** Several studies have found that soaking legumes for 12-18 hours is the most efficient way to minimise the level of phytic acid and proteolytic enzyme inhibitors, which are partially or completely solubilized in soaked water **(Embaby 2010; Kajihausa *et al*. 2014).**

Soaking and germination of grains and legumes significantly reduced trypsin inhibitor activity and flatulence-causing oligosaccharides (stachyose and raffinose), boosting protein digestibility and sensory characteristics. Germination is a low-cost, high-impact method for increasing nutrient availability, reducing anti-nutritional factors in legumes and cereal grains, and increasing the levels of some of the utilisable nutrients **(Maisont and *et al,* 2010).**

2.7.1.2 Pasteurization

Pasteurization's major goal is to destroy thermolabile microorganisms including yeasts, moulds, and vegetative bacteria that cause food spoiling or food poisoning. **(Petruzzi and colleagues, 2017)** Thermal pasteurisation has been successfully utilised to deactivate enzymes whose activities can produce oxidative alterations during processing and storage in addition to microbial inactivation **(Marszaek, *et al*., 2017).** As a result, thermally treated beverages have a longer shelf life in the refrigerated condition without reducing the quality. **(A ˘gçam., 2018).**

2.7.1.3 Natural coloring agent

In the food sector, anthocyanins derived from natural sources are frequently employed as a suitable substitute for synthetic colourants. Anthocyanins' water-soluble nature makes them a desirable natural colourant for a variety of food applications **(Sucheta *et al,* 2020).** The pigment in black carrot anthocyanins allows for a wide range of food applications, particularly in low-acid foods, beverages, and confectionaries **(Petropoulos *et al*. 2019)**. Furthermore, anthocyanins derived from black carrots are used to colour a variety of foods, including fruit juice concentrate, nectars, soft drinks, jellies, yoghurt, marmalade, and Turkish delight. **(Polat *et al*., 2022).**

2.7.1.4 Stabilizer

One of the common problems linked to beverages stuff is sedimentation of aggregated proteins and huge fruit particles **(Guimaraes *et al*., 2018**) and wheying off during storage period and this aggregation forms to phase separation of products **(Khanniri *et al*., 2019)** therefore the most preventative method to overcome this instability issues of dairy beverages includes usage of stabilizers such as pectin **(Krongsin *et al*., 2015).** It is needed to stabilize the dairy beverages without sacrificing protein content **(Irem Bige *et al*., 2022).** The high methoxy pectin are commonly used as a stabilizing agent to prevent phase separation in many acidified dairy beverages **(Kiani *et al.,* 2010)**

Off-flavour- flavour release of a particular food is a major factor determining consumer acceptance. **(Hoda**. ***et al*** **2015).** During food processing and storage the flavour of food products might be degraded so to maintain/enhance and remove of off-flavour some natural flavoring are now added in food and beverages industries

CHAPTER-III

MATERIAL AND METHOD

This chapter elaborates various materials used and experimental methodologies employed for development of the functional smoothie. Methodologies adopted for processing and analysis of raw materials, final product and monitoring its Physicochemical, sensory and microbiological quality during storage as well as determination of the nutritional value has been done. Shelf life study was evaluated by studying the changes in sensory quality, microbiological quality, pH and acidity. The experimental design and the proximate analysis are explained along with the procedures. The present investigation entitled "Preparation of horse gram extract incorporated functional smoothie" was carried out in Department of Dairy Science & Food Technology, Institute of Agriculture Science, Banaras Hindu University, Varanasi during year 2021-2022.

2.8 Materials:-

2.8.1 Collection of Cow milk

Fresh cow milk was collected from Dairy Farm of Department of Dairy Science and Food Technology, DFST, I, Ag. Scs. Banaras Hindu University, Varanasi.

2.8.2 Collection of Dahi

Dahi was prepared by using starter culture in the lab of DFST, BHU.

2.8.3 Horse gram

Horse gram seeds of good quality were purchased from a reliable local market, Lanka, Varanasi making sure that the seeds were free from foreign substances like soil particles, weed grains, pest-infected seeds, stones, insects, eggs, larvae, or their fragments. These seeds were soaked before grinding to extract.

2.8.4 Black carrot

Fresh black carrot (Daucus carota ssp. Sativus var. atrorubens) were procured from local vegetable market, Varanasi.

2.8.5 Banana

Fresh banana (Musa acuminata) were procured from local fruit market, Varanasi. Fruits were visually assessed for ripeness and any fruit found to be over or under ripened was discarded.

2.8.6 Sugar

Food grade crystalline sugar was procured from the local market.

2.9 Other ingredients and Stabilizers

2.9.1 Pectin

Pectin pure (Poly-D-Galacturonic Acid Methy Ester) was collected from Central Drug House (P) Ltd. 7/28 Vardaan House, Daryaganj, Delhi-110002.

2.9.2 Tri-sodium citrate

Tri-sodium citrate dehydrate was taken from Marck Life Science Private Limited, Godrej One, 8th Floor, Pirojshanagar, Vikhroli (East), Mumbai 400079.

2.10 Chemicals and reagents

All the chemicals used during study were of Analytical Grade (AR) and were procured from standard companies viz. HiMedia Laboratories Pvt. Ltd., Mumbai, India; Merck Specialties Pvt. Ltd., Mumbai, India; Fisher Scientic, Mumbai, India.

2.10.1 Reagents

The reagents required for analysis were freshly prepared each time adopting standard procedures.

2.10.2 Glassware

Glassware of Borosil make was used during throughout study. They were cleaned using laboratory soap solution, washed thoroughly with water and then rinsed with distilled water and dried before use.

2.10.3 Packaging material

Polypropylene bottles (200 ml) were used for the packaging of the four variants of smoothies. They were purchased from local market of Varanasi.

2.11 Experimental set-up

Instruments and equipment were used during the research work are; trays, knife, spatula, grinder, hand blender, electronic weighing balance, digital pH meter, laminar flow, digital spectrophotometer, viscometer, water bath, gerber centrifuge machine, centrifuge machine, muffle furnace, incubator, autoclave, micropipette etc.

Working feature of important instruments are described below.

2.11.1 Electronic weighing balance

(Metler Toledo, JB1603-C/Fact, Switzerland), top pan electronic weighing balance of high accuracy with having least count of about 0.01g was used for measure the weight of samples and chemicals.

2.11.2 Digital pH meter

The digital pH meter (Thermo Scientific, Sn B21899, and Singapore) was used to analyze the pH of the sample. At first the electrode of pH meter was dipped in standard buffer solution to standardize the pH then dipped into the sample and pH values were displayed on screen pH meter.

2.11.3 Autoclave

The autoclave (Tony SX-500, UK) made with a heavy metallic cylindrical sheet. It is a machine which convert water in to steam by increasing the pressure inside. The machine was operated maximum pressure of 20psi at 121˚C for 15min. At the top head of machine a pressure gauge positioned for measuring the pressure and a steam release valve was there. It was used for sterilization of glassware and prepared media for microbial testing.

2.11.4 Centrifuge machine

The centrifuge machine normally uses centrifugal force to separate the components from solid to liquid of a mixture sample. It was used to centrifuge the HGE and sample.

2.11.5 Laminar air flow

Laminar air flow is an enclosed cabinet (Labtech LCB 120IV, Daihan Pvt. Ltd, India) specially designed for microbioligal testing and prevent from contamination of biological samples.

2.11.6 Hot air oven

Hot air oven is an electrical device normally use dry heat to sterilize. It was used for moisture and TS analysis.

2.11.7 Muffle furnace-

Muffle furnace is a type of jacketed enclose chamber which is used to heat the materials at a very high temperature range from 1100˚C to 1800˚C. It is used to determined ash content of horse gram extract and developed product.

2.11.8 Spectrophotometer

Spectrophotometer was used to determine the optical density of a given sample. It had a wavelength range from 340-960nm

2.12 Methodology

2.12.1 Process for preparation of Horse gram extract

The horse gram extract was prepared by the procedure described by **Preeti verma *et al*., (2014)** as shown in fig.3.1

250 g of horse gram

Washed in tap water

Soaked in normal water (1:3) for 16 hours

Drain out the water

Ground in 150 ml of water for 5-7 minutes

Sieved through muslin cloth

Cooled at room temperature

Boiled for 10 minute

Horse gram extract (180 ml)

Fig. 3.1 Schematic diagram of steps in preparation of Horse gram extract

2.12.2 Process for preparation of Black Carrot juice

Black carrot juice was prepared by the following procedure shown in fig 3.2

Fresh black carrot

Sorting/grading

Washing in portable water

Peeling

Slicing/cutting

(to reduce surface area)

Pulping (mixer grinder)

Juice extraction

Filter through muslin cloth

Indirect pasteurization at 63 ˚C for 30 min

Cooling and storage

Fig. 3.2 Schematic diagram steps involves in preparation of black carrot juice

2.12.3 Preparation of functional smoothie

Using dairy product, underutilized pulse (horse gram), fruit, vegetables, sugar, and stabilizing agent such as pectin and tri-sodium citrate, an attempt was made to make a nutritious functional smoothie that contained, in addition to macronutrients, essential micronutrients, and dietary fibre. The following fig. 3.3 shows the steps of preparation of smoothie.

Cow milk 5%

Standardization of milk by using skim milk at 3.5% fat & 8.5% SNF

Pasteurization for 63˚C for 30 min

Cooling at room temperature

Addition of dahi (5%), black carrot juice (3%)

Addition of stabilizer; pectin (0.2%), tri-sodium citrate (0.04%), sugar (12%)

Addition of HGE (5-15%) & banana (15%)

Mixing through hand blender

Filling & Packaging (into PP)

Storage (at 7˚C)

Fig. 3.3 Schematic flow chart of processing functional smoothie

3.6 Analytical method

2.13 Proximate analysis

2.13.1 Ash analysis

The ash content of HGE and functional smoothie was estimated by the method as per the procedure outlined in IS: SP: 18 Part IV (1981). About 2 g of sample was weighed accurately in a previously weighed silica crucible. It was then charred on a heater and then the sample was kept into muffle furnace maintained at a temperature not more than 550±20°C for about 4 h until white ash was obtained. The crucibles were cooled and stored in a desiccator until the final weight was taken. The per cent ash was calculated based on following formula.

$$\boldsymbol{Ash\ (\%) = \frac{w1}{w} \times 100}$$

Where,

W = Weight of the sample (g)

W1= Weight of the residue after heating (g)

2.13.2 Analysis of moisture

The moisture content in the functional smoothie and HGE was determined by gravimetric method according to the procedure described in IS: SP: 18, Part IV (1981). About 10 g of sample was taken in clean, dry, and previously weighed moisture dish. The dish was placed in hot air oven maintained at 105ºC for 5 h. The sample was cooled in a desiccator and weighed. The process of heating, cooling and weighing was continued at half hour intervals till the loss of weight between successive weighing is less than one mg. The lowest weight was recorded

Moisture (% by weight) = $\frac{w1-w2}{w1-w} \times 100$

Where,

W= Wt. of the empty dish in g

W1= Wt. of dish with sample before drying in g

W2= Wt. of dried sample in g

2.13.3 Analysis of Total Solid (TS)

The determination of total solids of functional smoothie was determined as per formula by **Arora *et al* (1992).** Cleaned porcelain crucible was taken and dried in hot air oven at 105˚C for 1 hrs. Take 10 g of sample and weighed in an empty crucible. Crucible was put into hot air oven at 105 ± 1°C for 3-4 hrs. Take out the crucible from the oven and cool in desiccators and weighed. Again, placed the crucible for ½ hour in the hot air oven. Afterward, the crucible from oven was removed and cooled in desiccators, and weighed.

Total solids % = 100 – Moisture %

2.13.4 Protein analysis AOAC (2001.14) 2005

The protein content of HGE and functional smoothie was determined by taken about 10 g of sample weighed accurately and transferred into the 300ml of Kjeldahl flask. 2 g of digestion mixture (k_2SO_4:$cuso_4$ = 1:10) and 15–20 ml of concentrated sulphuric acid were added into the flask. After 5 hours the sample were digested over block digester until greenish. After cooling, Kjeldahl flask was washed with 6 times of distilled water and transferred into the volumetric flask, make upto 250 ml. In

distillation unit, take 10 ml sample from volumetric flask and 40 % NaOH solution transferred in distillation unit. The sample were steam distilled and the liberated ammonia was collected in Indicator. The distillation was continued until about 50 to 60 ml of distillate was collected. In the distillate was titrated against N/10 H_2SO_4 and color changes from green to purple color and record the volume of alkali used in the second iteration (v).

$$\textbf{Protein \%} = \frac{\mathbf{X \times V \times F}}{\boldsymbol{A \times W}} \times \mathbf{6.25} \times \mathbf{100}$$

Where, X – Volume of N/10 H2SO4 required for titration of sample distillate (ml)

V- Net volume of digested sample (ml)

F- 0.0014

A - Volume of aliquot taken for distillation (ml)

W - Weight of dried sample (g)

6.25 – factor for converting nitrogen into protein of feedstuffs assuming that average protein content.

2.13.5 Fat (AOAC 1998)

About 5gm of HGE was taken into a thimble. The beaker was placed in the soxhlet extractor. After that extractor was filled with petroleum ether. The temperature was set at 70 ℃ for ½ hour. After that raised the temperature up to 140℃ for 2 hours**.** The resultant ether was evaporated. Then beaker was taken out from the soxhlet extractor, immediately placed in hot air oven for 30 minute at 105˚C then beaker was kept into desiccator until it cools, weigh the beaker of sample to analyze fat percentage.

$$\textbf{Fat (\%)} = \frac{\textbf{W2} - \textbf{w1}}{\textbf{W}} \times \textbf{100}$$

Where, W2= Weight of beaker with fat (g)

W1= Weight of empty beaker (g)

W=Weight of initial sample (g) taken for testing

2.13.6 Fat analysis

The fat percentage of functional smoothie was determined by Gerber centrifugal method according to IS: 1224 part II (1977). About 10 ml of 90% concentrated sulphuric acid, 10.75 ml of sample, and 1ml of amyl alcohol was taken into a butyrometer. Rubber stopper was fixed into the butyrometer and then the content was mixed well and digested with occasional shaking until the sample was completely dissolved. The butyrometer was placed in a water bath maintained at 65˚C leave for 5min. Then the butyrometer was put into the Gerber centrifuge machine and centrifuged for 5 minutes at 6000 rpm speed. After then the butyrometer was taken out from the centrifuge machine, fat rising to the calibrated part of butyrometer was recorded the reading of fat percentage.

2.13.7 Fat analysis

The fat percentage of functional smoothie was determined by Gerber centrifugal method according to IS: 1224 part II (1977). About 10 ml of 90% concentrated sulphuric acid, 10.75 ml of sample, and 1ml of amyl alcohol was taken into a butyrometer. Rubber stopper was fixed into the butyrometer and then the content was mixed well and digested with occasional shaking until the sample was completely dissolved. The butyrometer was placed in a water bath maintained at 65˚C leave for

5min. Then the butyrometer was put into the Gerber centrifuge machine and centrifuged for 5 minutes at 6000 rpm speed. After then the butyrometer was taken out from the centrifuge machine, fat rising to the calibrated part of butyrometer was recorded the reading of fat percentage.

2.13.8 Analysis of carbohydrate

Total carbohydrate content was determined by difference method. The sum of moisture, fat, protein and ash contents (%) were subtracted from 100 to get carbohydrate content (%) using following formula.

% Total Carbohydrate by weight = 100 – (A+B+C+D) %

Where,

A = % by mass of moisture content

B = % by mass of total protein content

C = % by mass of fat content

D = % by mass of total ash content

2.13.9 pH analysis

The pH of smoothie was analyzed by using the digital pH meter. Digital pH meter was standardized at 27°C by using standard buffer solution. About 10 ml of functional smoothie was taken in a beaker, dipped the electrodes of pH meter into the sample. The pH values were displayed on screen of pH meter.

2.13.10Analysis of Anti-oxidant activity

Free radical scavenging activity (RSA) of HGE and smoothie was measured by using the method of Brand - **Williams's et al. (1995)**. 2 g of sample was taken in conical flask and added 25ml of methanol, then put the conical flask into the shaker machine leave them to dissolve for 2 hours, then centrifuged the sample at 6000 rpm for 10min at 27 ˚C. 2.5 ml of centrifuged supernatant was taken and mixed with 5ml of 2mM DPPH in methanol solution are vortexes. The reaction mixture was then left in the dark place for 30 min, after that the absorbance was measured at 517 nm. 80% methanol was used as a blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation.

$$\textbf{DPPH activity \%} = \frac{\textbf{Absorbance of blank} - \textbf{Absorbance of sample}}{\textbf{Absorbance of blank}} \times \mathbf{100}$$

2.14 Physical analysis

2.14.1 Viscosity

Viscosity of the finished product was estimated at 26°C by using 1-1 system and TL-7 spindle of Viscostar plus Viscometer.

2.14.2 Sedimentation

Sediment content in the functional smoothie was determined according to the method of **Lucey et al. (1999)**, with slight modifications. About 20 g of sample was taken in centrifuge tube and centrifuged at 6000 rpm for 20 min at 27ºC. The sediment content was calculated as percentage on weight basis.

$$\textbf{Sediment \%} = \frac{(w1 - w2)}{w1} \times \mathbf{100}$$

Where,

W1= Wt. of sample (in g)

W2= Wt. of whey (in g)

2.14.3 Whey syneresis

Whey syneresis of functional smoothie was determined by centrifugation method according to **Parnell-Clunies et al., (1986).** About 10 g of sample was taken for centrifugation at 6000 rpm for 15 min at 10°C. The supernatant was removed from centrifuge tube and measured the weight of suspended particle.

$$\textbf{Whey syneresis \% } = \frac{\textbf{Initial weight} - \textbf{Final weight}}{\textbf{Initial weight}} \times 100$$

2.15 Sensory evaluation

The smoothie was evaluated for its sensory characteristics by a trained and semi-trained panel of 10 judges using a 9-point Hedonic scale **(Shone et al., 1979)** at DFST, Banaras Hindu University. Format of Sensory score is attached in Annexer-1

2.16 Microbiological analysis

The optimized sample were determined for analysis of yeast and mould, coliform and TPC. Media and all the glassware were sterilized in an autoclave at 121˚C. All the steps of microbial testing was done under laminar air flow to avoid contamination. The dilution factor of sample was taken for yeast and mould 10^{-5} coliform 10^{-2} and for TPC 10^{-4}.

2.16.1 Yeast and Mould Count

Yeast and mould count was determined in functional smoothie by using PDA (Potato dextrose agar), suggested by **Hought et al., (1992)**. Plates were incubated at 30˚C for 3-5 days.

2.16.2 Coliform Count

The coliform counts of functional smoothie was determined by using Violet Red Bile Agar (VRBA) (Appendix IV) prepared as specified in the IS:1479 (part III, 1962), and plates were incubated at 37℃ for 48 hrs.

2.16.3 Total Plate Count (TPC)

The functional smoothie were analyzed for TPC by using plate count agar (PCA) media and plate were incubated at 37˚C for 48 hrs.

2.17 Shelf-life studies

The optimized products were stored in cleaned and sterilized 200 ml polypropylene (pp) bottles, at refrigerated temperature 7°C and room temperature 30°C. The products were analyzed for changes in sensory parameters (colour and appearance, flavour, consistency, sweetness and overall acceptability), acidity, pH, and microbial counts on 3 days intervals to determine the storage stability of the product.

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CHAPTER-IV

RESULT AND DISCUSSION

This chapter presents the results of current experiment on "Development of functional smoothie". The goal of this study is to develop an effective and healthy beverage by analyzing the nutritional benefits of an underutilized crop (Horse gram) in combination with dairy, fruit, and vegetable ingredients in smoothie, as well as to increase its acceptance. The evaluation of physicochemical features of raw materials and optimized product, sensory evaluation, microbiological analysis, and shelf life studies have all been made with serious efforts.

4.1 Chemical constituents of cow milk

The chemical properties of cow milk which was collected from the Dairy farm of Department of Dairy Science and Food Technology, DFST, BHU, Varanasi is described in the following table.4.1.

4.1 Chemical constituents of cow milk

Constituents	**Value**
Fat	3.19 ± 0.002
Protein	3.17 ± 0.001
SNF	9.91 ± 0.17
TS	13.18 ± 0.32
Ash	0.62 ± 0.002
Acidity	0.12 ± 0.007
pH	6.44 ± 0.002

Value are reported as **Mean ± SD (n=3)**

4.2 Chemical Characteristics Of Horse Gram Extract (HGM

Horse gram seeds of good quality were purchased from a reliable local market, Lanka, Varanasi making sure that the seeds were free from foreign substances like soil particles, stones, insects etc. These seeds were soaked before grinding to extract. The horse gram extract contains fat, protein, carbohydrates, ash, TS, antioxidant activity, and crude fibre at a level of 0.26 ± 0.14, 3.53 ± 0.13, 5.00 ± 0.95, 0.22 ± 0.03, 9.02 ± 0.91, 54.38 ± 1.59, and 0.72 ± 0.07, respectively. **Sivakumar *et al*. (2020)** states that horse gram extract contains fat 0.25%, fibre 0.80%, protein 3.64% and carbohydrate 3.19% respectively. The DPPH % of antioxidant activity in horse gram was identified as 52.68 ±2.24. (**Ojha *et al.*, 2020)**

Table 4.2 Proximate analysis of HGE

Constituents	**Level (Mean ± SD)**
Ash	0.22 ± 0.03
TS	9.02 ± 0.91
Protein	3.53 ± 0.13
Fat	0.26 ± 0.14
Carbohydrates	5.00 ± 0.95
Crude fibre	0.72 ± 0.07
Antioxidant activity	54.38 ± 1.59

Values are reported as **Mean ± SD** (n=3)

4.3 Optimization Of HGE Concentration For Preparation Of Functional Smoothie

The preparation of functional smoothie was conducted by addition of horse gram extract at the level of 5%, 10% and 15% along with milk, dahi (5%), banana (15%)

and black carrot juice (3%), sugar (12%), pectin (0.2%) and Tri-sodium citrate (0.04%) at a desired level. The prepared samples were subjected to sensory evaluation by using '9-point hedonic scale'. In which a panel of around 10 trained and semi trained judges was given their score to the individual samples. The sensory responses of the sample are described in the following table. 4.4

4.4 Sensory evaluation of prepared functional smoothie

Table 4.4 Sensory evaluation of prepared functional smoothie

Sample	**Flavour**	**Color and appearance**	**Body and texture**	**Sweetness**	**Overall acceptability**
Control	8.01 ± 0.18^{b}	7.64 ± 0.25^{b}	7.46 ± 0.21^{b}	8.05 ± 0.32^{a}	7.19 ± 0.26^{b}
S1	6.9 ± 0.41^{d}	6.98 ± 0.30^{d}	8.37 ± 0.60^{a}	7.10 ± 0.74^{b}	6.68 ± 0.48^{d}
S2	8.35 ± 0.41^{a}	7.77 ± 0.52^{a}	6.67 ± 0.44^{c}	6.51 ± 0.29^{c}	8.09 ± 0.20^{a}
S3	7.18 ± 0.96^{c}	7.11 ± 0.30^{c}	6.15 ± 1.4^{d}	5.92 ± 0.94^{d}	6.98 ± 0.13^{c}

Mean ± SD (Replication, n=3); a,b,c,d different superscript differ significantly ($p < 0.05$)

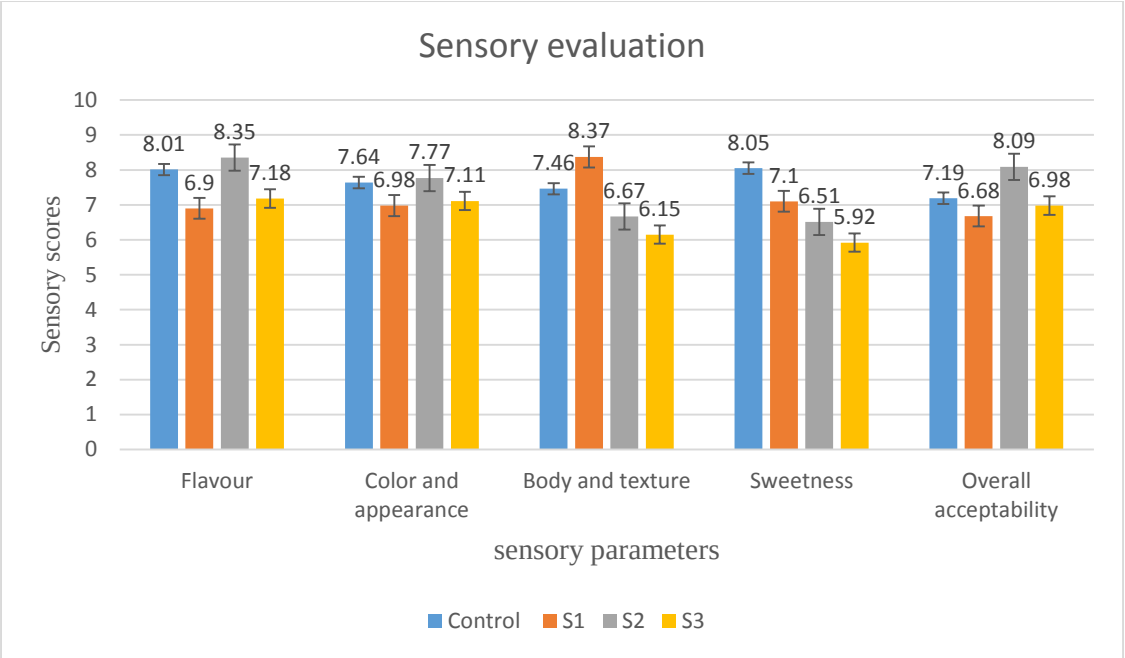

Fig 4.1 Sensory evaluation of prepared functional smoothie

4.4.1 FLAVOUR

The mean value of flavour of the functional smoothie for control C, S1, S2 and S3 were 8.01 ± 0.18, 6.9 ± 0.41, 8.35± 0.41, and 7.18± 0.96, respectively. The S2 and Control has scored highest value in comparison to other samples. The flavour score was found significant ($p < 0.05$) between the treatments. S2 was more liked by the panelists because of adequate taste and odor of HGE. S1 has negligible odor of HGE and S3 was more dis-liked due to highly smell of HGE. According to **Jothylingam *et al* (2013)** based on sensory evaluation 5% of aloevera extract with low calorie herbal flavoured milk was most acceptable.

4.4.2 COLOUR AND APPEARANCE

Mean score of colour and appearance in functional smoothie for C, S1, S2 and S3 were 7.64 ± 0.25, 6.98 ± 0.30, 7.77 ± 0.52, and 7.11 ± 0.30 respectively. The score

was highest for S2 and Control. No significant difference ($p > 0.05$) was found in the samples due to equal level of black carrot juice but S2 and Control was observed more liked by the judges. **Karaman *et al.* (2021)** investigated the colour of redness in the product of yoghurt with black carrot, yogurt with black carrot and pectin and yogurt with black carrot and gum arabica was due to the anthocyanin pigment present in black carrot.

4.4.3 BODY AND TEXTURE

In the case of body and texture of smoothie the mean value of C, S1, S2 and S3 were 7.46 ± 0.21, 8.37 ± 0.60, 6.67 ± 0.44 and 6.15 ± 1.47. The body and texture profile of smoothies' S1 sample shows highest score and S3 shows lowest value by the choice of panelists, because S3 has high amount (15%) of HGE. According to **(Sivakumar *et al*. 2020)** the higher % of horse gram extract in fortified milk was showed lower score value. An observation was done by (**Riberio *et al* 2014)** that a flavour formulation of papaya and mango contained about 62.7% of soy extract based drink was found to be as a good consistency. Therefore the smoothie samples shows slightly significant ($p < 0.05$) variations.

4.4.4 SWEETNESS

The mean value of sweetness of functional smoothies for C, S1, S2 and S3 were 8.05 ± 0.32, 7.10 ± 0.74, 6.51 ± 0.29 and 5.92 ± 0.94. The score value for control was higher and S3 was lower according to the choice of panelists. The samples were shows significantly increases ($p < 0.05$). The high and low sweetness of smoothies can be corrected by makeup the volume of sugar according to the taste.

4.4.5 OVERALL ACCEPTABILITY

The overall acceptability score of functional smoothie for C, S1, S2, and S3 were 7.19 ± 0.26, 6.68 ± 0.48, 8.09 ± 0.20 and 6.98 ± 0.13. The scores of functional smoothies showed significant variation ($p < 0.05$) among the all samples except the parameter of colour and appearance score in smoothie. The control and S2 functional smoothies of 10% HGE was chosen as a product of acceptability that was because in S2 the lower sweetness and better compatibility of other ingredients added in smoothie. Similar reason of acceptance was done by (**Adebayo-Oyetoro *et al*. 2016)** in evaluation of pawpaw juice milk blends. **(Balijeet *et al*. 2013)** suggested higher sugar level for whey based pineapple drinks due to high acidic nature of whey. The same type of selection was suggested by **Hassan *et al*. (2015)** for the fruit flavoured milk based beverages.

4.5 PHYSICOCHEMICAL CHARACTERISTICS OF SMOOTHIE

4.5.1 VISCOSITY

SAMPLE CODE	Viscosity (Cp at 20 rpm)	Whey Syneresis (ml/10g)	Sedimentation (ml/20g)
C	718 ± 274.58^{d} (20)	7.90 ± 3.12^{c}	10.33 ± 3.73^{d}
S1	740 ± 138.95^{c} (20)	9.02 ± 1.77^{a}	11.8 ± 5.99^{c}
S2	843.33 ± 199.77^{b} (20)	8.31 ± 0.85^{b}	13.64 ± 1.94^{b}
S3	880 ± 24.97^{a} (20)	6.19 ± 3.48^{d}	14.14 ± 2.54^{a}

Mean ± SD (n=3) a,b,c,d different superscript differ significantly ($p < 0.05$)

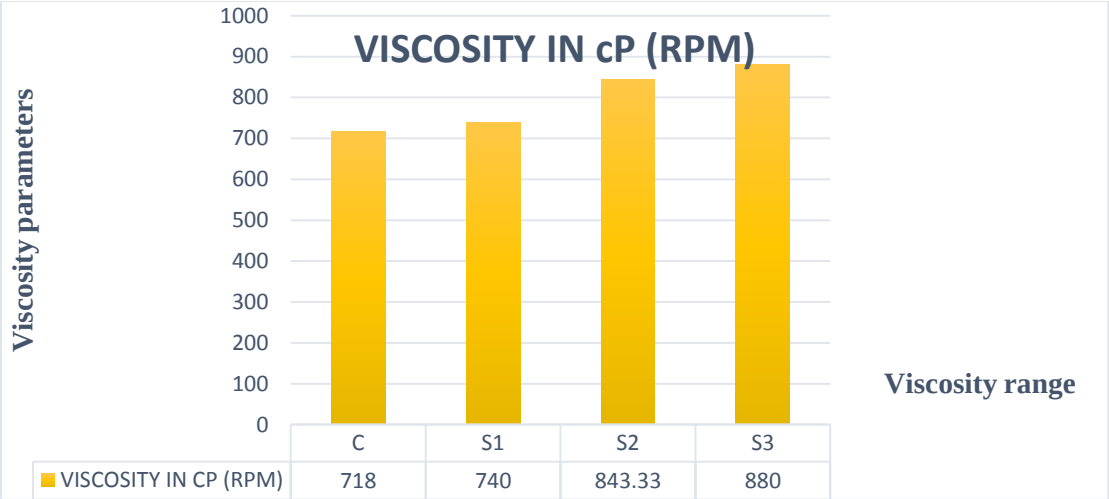

Fig. 4.2 VISCOSITY

4.5.1 VISCOSITY

The viscosity of functional smoothie for different concentrations of sample C, S1, S2 and S3 has been shown in table 4.5.1. At the range from 718 to 880 cP. Which shows highly non-significant in variation ($p > 0.05$). The score of S3 was observed maximum viscosity as it has contains high concentration (15%) of horse gram extract and the total solid as well as protein content was high and minimum score was observed in Control sample which has not been contain horse gram extract and total solid was less due to absence of HGE. **Kumar, S. (2012)** was investigate that the maximum viscosity was observed in 5.68% of green gram flour level and minimum viscosity was observed in 2.31% of green gram flour level.

4.5.2 WHEY SYNERISIS

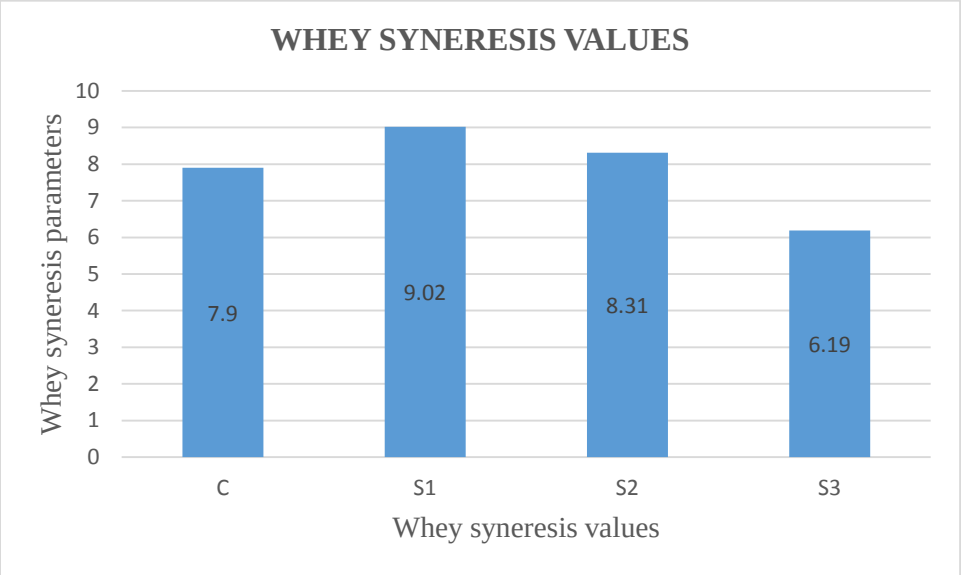

Fig 4.3 Whey syneresis

4.5.2 Whey syneresis

Whey syneresis of functional smoothie for the sample C, S1, S2 and S3 were 7.90 ± 3.12, 9.02 ± 1.77, 8.31 ± 0.85 and 6.19 ± 3.48 which shows non-significant ($P > 0.05$) difference between the smoothie samples. The table 4.5.1 shows highest value of whey syneresis in S1 due to lower concentration of HGE (5%) and lowest in S3 due to high concentration of HGE (15%) According to **Kumar, S. (2012)** highest concentration of green gram flour shows minimum value of whey syneresis and lowest concentration of green gram flour shows maximum level of whey syneresis.

4.5.3 Sedimentation

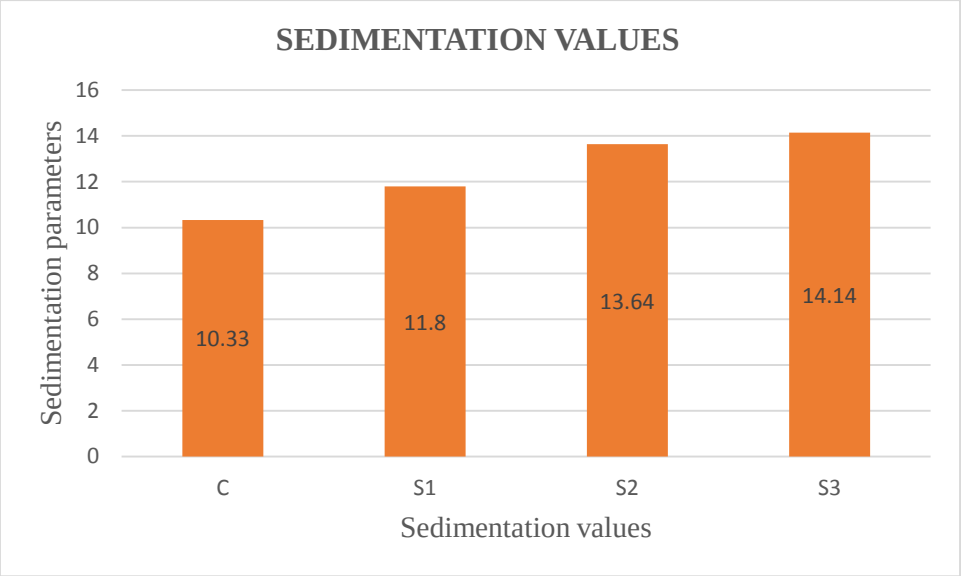

Fig 4.4 Sedimentation

4.5.3 Sedimentation

Sedimentation of functional smoothie shown in the table 4.5.1. For the sample C, S1, S2 and S3 were ranged from 10.33 to 14.14. There were highly non-significant ($p > 0.05$) variation found in all sample. The maximum value was found in S3 (14.14 ± 2.5) because it has more HGE concentration whereas the minimum value was found in Control sample (10.33 ± 3.73) due to the absence of HGE. According to **Kumar, S. (2012)** experiment the higher sedimentation was found in higher amount of green gram flour and lower sedimentation was found in lower amount of green gram flour of milk based smoothie. In fermented/cultured milk beverages, sedimentation is a significant barrier to product storage. Acidic milk products suffer from protein sedimentation due to low pH, which causes whey separation during storage. Additionally, products made from pulses and cereals cause more sedimentation because the corresponding particles

and milk solids are deposited on the sediment **Kumar, S. (2012)**. Therefore to overcome from this problem proper mixing of product is needed and always shake properly prior to use.

4.6 Proximate Analysis Of Prepared Functional Smoothie

The functional smoothie was subjected for various proximate analysis such as ash, TS, antioxidant activity, crude fibre, pH, acidity, Mentioned in the following Table 4.6.

Table 4.6 Proximate Analysis Of Prepared Functional Smoothie

Sample	Ash (%)	TS (%)	Antioxidant Activity (% inhibition)	Crude Fibre (%)	pH (%)	Acidity (%)
C	0.71 ± 0.10^{d}	18.33 ± 3.15^{c}	24.59 ± 3.49^{d}	0.76 ± 0.02^{c}	5.86 ± 0.005^{c}	0.39 ± 0.06^{b}
S1	0.74 ± 0.09^{c}	23.38 ± 0.20^{b}	28.18 ± 2.86^{c}	0.82 ± 0.03^{b}	5.87 ± 0.007^{c}	0.40 ± 0.009^{a}
S2	0.79 ± 0.06^{b}	23.66 ± 1.83^{a}	31.11 ± 4.17^{b}	0.86 ± 0.01^{a}	5.93 ± 0.03^{b}	0.41 ± 0.01^{a}
S3	0.82 ± 0.06^{a}	23.69 ± 0.53^{a}	38.20 ± 5.04^{a}	0.88 ± 0.01^{a}	5.98 ± 0.006^{a}	0.41 ± 0.02^{a}

Values are reported as **Mean ± SD (n=3) a,b,c,d different superscript differ significantly ($p < 0.05$)**

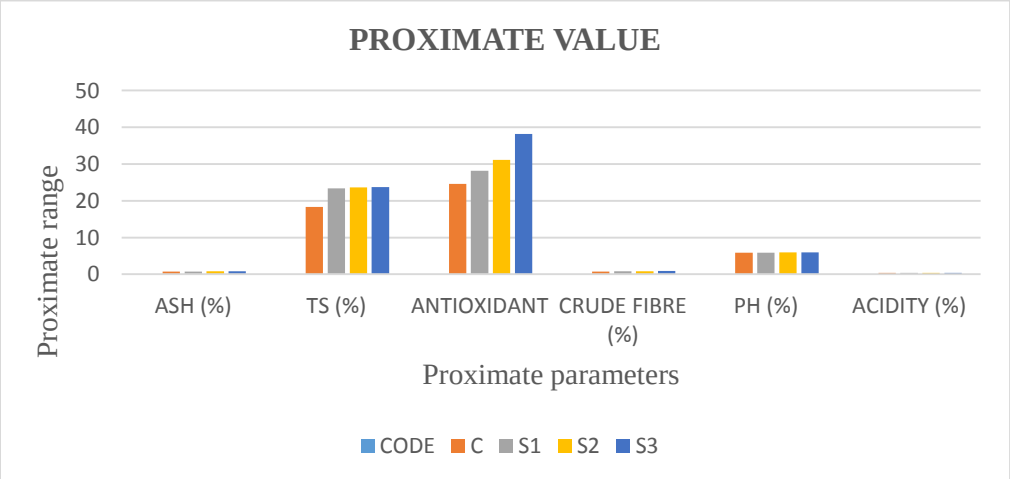

Fig 4.5 Proximate Analysis of Prepared Functional Smoothie

4.6.1 Ash

The ash value of functional smoothie for the samples C, S1, S2 and S3 were 0.71 ± 0.10, 0.74 ± 0.09, 0.79 ± 0.06, and 0.82 ± 0.06. The ash value were not affected significantly ($p > 0.05$) in smoothie. However the highest value was observed in S3 due to higher percentage of HGE and lowest value was observed in C in which the HGE was not present. The percentage of ash value was found in accordance with the composition of pineapple introduced by **Sew et al. (2012)** in development of multi-fruit smoothie. The ash value in Sorghum based smoothie, Finger millet based smoothie, and Chickpea based smoothie were 0.31±0.01, 0.26±0.018 and 0.395±0.03 as reported in **(Singh, 2014**).

4.6.2 Total Solids

The total solids value of functional smoothie for C, S1, S2 and S3 were 18.33 ± 3.15, 23.38 ± 0.20, 23.66 ± 1.83 and 23.69 ± 0.53 respectively. The smoothie shows highly significant ($p < 0.05$) difference because of control samples. The C contains lowest TS value in comparison to other sample due to absence of HGE and S3 contains highest TS value due to higher amount of HGE. This results of total solids were equivalent to **(Singh, 2014)**. The TS of sorghum based milk smoothie was 23.46 ± 0.07 and TS of finger millet based milk smoothie was 23.28 ± 0.10. And **Kumar, S. (2012)** the TS value was range from 23.12 to 23.51 in green gram milk based smoothie.

4.6.3 Antioxidant Activity

The antioxidant activity of functional smoothie sample for C, S1, S2 and S3 were detected as 24.59 ± 3.49, 28.18 ± 2.86, 31.11 ± 4.17, and 38.20 ± 5.04. There are highly significant variation ($p < 0.05$) between samples, the value of antioxidant in

Control was very low due to absence of HGE and S3 has high value of antioxidant activity due high percentage of HGE. The percentage of antioxidant activity (DPPH inhibition) is 52.56 ±0.75 in ungerminated flour and 60.76 ±0.64 in germinated horse gram flour. **(Moktan**, **2016)**

4.6.4 Crude Fibre

The crude fibre content in functional smoothie for C, S1, S2, and S3 were range from 0.76 ± 0.02 to 0.88 ± 0.01 significantly different ($p < 0.05$) to each other due to different concentration of HGE. The score of crude fibre in S3 was higher in comparison to others and C sample has lower value of crude fibre due to absence of HGE**. (S.K. et al. 2020)** studied that Fibre content was highest in horse gram malt (16.5%) and lowest in control sample (12.2%) was significantly increased in horse gram malt added weaning food in compared to control sample. Crude fibre content in Sorghum based smoothie, Chickpea based smoothie and Finger millet based smoothie were 0.60±0.01, 0.67±0.01 and 0.72±0.02 respectively **(Singh, 2014**).

4.6.5 pH

The pH of functional smoothie for sample C, S1, S2 and S3 were observed 5.86 ± 0.005, 5.87 ± 0.007, 5.93 ± 0.03 and 5.98 ± 0.006. The pH value was highly significant ($p< 0.05$) variation among each sample. The highest value of pH was observed in S3 due to high concentration of horse gram extract. **(Singh, 2014)** studied Chickpea based smoothie, Finger millet based smoothie and Sorghum based smoothie were 4.22±0.00, 4.21±0.003 and 4.22±0.00. And according to **(Mehta, 2013)** the pH content was 4.24±0.02 in soy protein isolate and chickpea flour milk based smoothie.

4.6.6 Titratable acidity

The titratable acidity range of functional smoothie sample for C, S1, S2 and S3 were range from 0.39 ± 0.06 to 0.41 ± 0.02. The pH value were observed non-significant ($p > 0.05$) differences between all the samples. The acidity value indicates proper handling condition and formation of lactic acid in milk products. It shows constant storage stability and handling treatment along with addition of other ingredients in to the smoothie. The titratable acidity value in milk smoothie was 0.23 ± 0.01 or ± 0.02 **(Kumar *et al*, 2020)** and 0.25±0.005 in dairy and plant based smoothie **Kumar, S. (2012)**

4.7 Proximate Analysis Of Control And Optimized Product

The functional smoothie of optimized and control sample were analyzed for proximate analysis as ash, total solids, fat, protein, and carbohydrate. These parameters are mentioned in the following table 4.7

Table 4.7 Proximate Analysis of Control and Optimized Product

Proximate Value	Control	Optimized
ASH	0.72 ± 0.1^{e}	0.81 ± 0.04^{e}
TS	18.38 ± 3.14^{a}	23.40 ± 0.44^{a}
FAT	2.66 ± 0.15^{d}	3.03 ± 0.15^{d}
PROTEIN	2.78 ± 0.10^{c}	3.38 ± 0.36^{c}
CARBOHYDRATE	12.20 ± 3.21^{b}	16.17 ± 0.10^{b}

Values are reported as **Mean ± SD (n=3) a,b,c,d different superscript differ significantly ($p < 0.05$)**

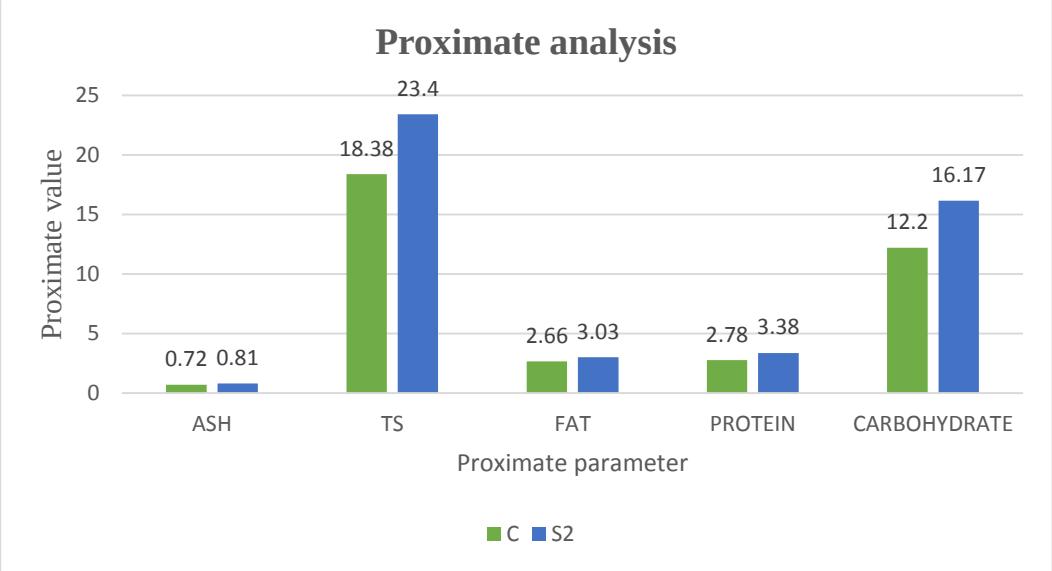

Fig 4.6 Proximate Analysis of Control and Optimized Product

4.7.1 Ash

Ash value of control (c) and optimized (S2) sample were 0.72 ± 0.1and 0.81 ± 0.04. There were non-significant variation ($p > 0.05$) between two samples. However higher percent of ash was observed in S2 than sample C, due to compatibility and composition of 10% of horse gram extract in the smoothie. The percentage of ash value (0.73 ± 0.04) was found in accordance with the composition of pineapple introduced by **Sew *et al*. (2012)** in development of multi-fruit smoothie. The ash value was studied 0.57 ± 0.02 in multi fruit smoothie **(Snigdha 2019)**

4.7.2 Total solids (TS)

The mean percentage of total solids for control (c) and optimized (S2) sample was noticed as 18.38 ± 3.14 and 23.40 ± 0.44. There were slightly non-significant variance ($p > 0.05$) observed between two samples. S2 shows higher value of total solids as it contains 10% of horse gram extract. The TS value was found similar to the

TS of sorghum based smoothie about 23.46 ± 0.07 and TS of finger millet based smoothie was about 23.28 ± 0.10 **(Singh, 2014**). And the TS value was range from 23.12 to 23.51 in green gram milk based smoothie **Kumar, S. (2012).**

4.7.3 Fat

The mean value of fat percentage in Control (c) and S2 sample were 2.66 ± 0.15 and 3.03 ± 0.15. Which are shows significant variation ($p < 0.05$). But maximum fat value was noticed in S2, due to the variation in milk and other ingredients like banana and horse gram extract. According to **(Kumar *et al.* 2020)** the fat percentage of banana based milk smoothie was range from 2.27 ± 0.04 to 2.30 ± 0.17. The fat percentage of dairy fruits pulse based smoothie contains 1.51% fat with 1.69% soy protein isolate **(Mehta, 2013).** In the dairy based plant food source of breakfast smoothie of 3% of green gram flour contains about 0.75% fat, studied in **Kumar, S. (2012)**

4.7.4 Protein

The mean percentage of protein value observed in Control (c) and S2 sample were 2.78 ± 0.10 and 3.38 ± 0.36. The protein value was slightly non-significant ($p > 0.05$) between the C and S2. S2 was noticed maximum protein value than C one. The variation could be due to the horse gram extract content. The percentage of protein content was equivalent to **(Kumar *et al.* 2020)** in banana based milk smoothie was range from 2.52 ± 0.01 to 2.83 ± 0.02. According to **Kumar, S. (2012)** the protein percent in green gram milk based smoothie was 1.94± 0.08. And the protein percent in Sorghum based smoothie, Finger millet based smoothie and Chickpea based smoothie were noticed about 0.87±0.05, 0.74±0.05 and 1.18±0.11 percent **(Singh, 2014**).

4.7.5 Carbohydrate

The mean value of carbohydrate percentage for Control (c) and S2 sample was 12.20 ± 3.21 and 16.17 ± 0.10. The carbohydrates percentage shows non-significant difference ($p > 0.05$) between control and optimized sample. The maximum percent was present in S2 due to 10% of horse gram extract. **Kumar, S. (2012)** was investigated the carbohydrates content in green gram smoothie was about 19.71±0.13, the Sorghum based smoothie, Finger millet based smoothie and Chickpea based smoothie the carbohydrates content were 13.93±0.34, 11.87±0.22 and 8.57±0.06 **(Singh, 2014)** and the carbohydrate percentage in canned mango pulp, pineapple and custard apple pulp were 17.64 ± 0.62, 13.27 ± 0.39 and 21.31 ± 0.48 according to **(Snigdha 2019).**

4.8 SHELF-LIFE ANALYSIS OF CONTROL AND OPTIMIZED PRODUCT

The shelf life study of optimized functional smoothie was analyzed for sensory characteristics by a panel of trained and semi trained judges, at minimum acceptability score of 2 into 9 point hedonic scale, The pasteurized product was stored in a polypropylene (PP) bottle throughout the experiment at temperature, under (7˚C). It was evaluated physicochemically (such as pH, acidity), sensory-wise, and microbiologically i.e. Yeast and Mould, TPC and Coliform test at 3 days interval for15 days.

In this shelf life study it was noticed that the stability in physicochemical properties of functional smoothie was degraded day by day up to 15days. The titratable acidity was increased whereas the pH value was decreased as days are increased and spores forming in TPC, yeast and mould, and coliform was increased after zero day. As well as overall acceptability was reduced upto 15^{th} days at a range from 5.58 to 2.08

Table 4.8.1 Microbial and Physicochemical Analysis of Control and Optimized Products

PARAMETERS	SAMPLES	0 DAY	3RD DAYS	6TH DAYS	9TH DAYS	12TH DAYS	15TH DAYS
pH	Control	5.63 ± 0.04^{a}	5.58 ± 0.03^{b}	5.53 ± 0.04^{c}	5.44 ± 0.01^{d}	5.38 ± 0.01^{e}	5.24 ± 0.02^{f}
	Optimized	5.97 ± 0.01^{a}	5.86 ± 0.02^{b}	5.66 ± 0.01^{c}	5.55 ± 0.03^{d}	5.43 ± 0.05^{d}	5.32 ± 0.2^{f}
Acidity	Control	0.39 ± 0.06^{a}	0.41 ± 0.04^{a}	0.43 ± 0.01^{a}	0.45 ± 0.02^{a}	0.48 ± 0.01^{b}	0.52 ± 0.01^{c}
	Optimized	0.50 ± 0.01^{a}	0.51 ± 0.01^{a}	0.54 ± 0.01^{b}	0.56 ± 0.02^{b}	0.61 ± 0.01^{d}	0.64 ± 0.01^{c}
YEAST & MOULD (log_{10}CFU/ml)	Control	Nil	Nil	Nil	1.63 ± 0.24^{c}	1.74 ± 0.63^{b}	1.89 ± 0.07^{a}
	Optimized	Nil	Nil	Nil	1.56 ± 0.01^{c}	1.66 ± 0.15^{b}	1.75 ± 0.49^{a}
TPC (log_{10}CFU/ml)	Control	0.10 ± 0.005^{f}	1.18 ± 0.47^{e}	1.23 ± 0.13^{d}	1.34 ± 0.32^{c}	1.67 ± 0.25^{b}	1.84 ± 0.05^{a}
	Optimized	0.02 ± 0.01^{f}	1.11 ± 0.01^{e}	1.19 ± 0.01^{d}	1.27 ± 0.01^{c}	1.45 ± 0.20^{b}	1.77 ± 0.41^{a}
COLIFORM (log_{10} CFU/ml)	Control	Nil	Nil	Nil	Nil	0.42 ± 0.01^{b}	0.57 ± 0.02^{a}
	Optimized	Nil	Nil	Nil	Nil	0.38 ± 0.06^{b}	0.45 ± 0.02^{a}

Values are reported as **Mean ± SD (n=3) a,b,c,d,e,f different superscript differ significantly ($p < 0.05$)**

4.8.1 Physicochemical and microbial Storage During Storage

4.8.1.1 Changes in pH

The changes in pH occurred during the storage of horse gram extract based functional smoothie have described in Table 4.8.1. It was observed that pH of both control and optimized samples kept decreasing gradually from 0^{th} to 15^{th} day. The score obtained for control and S2 from 0 to 15^{th} days was ranged from 5.63 ± 0.04 to 5.24 ± 0.02 and 5.97 ± 0.01 to 5.32 ± 0.2. There was significant ($p < 0.05$) difference between the samples throughout the storage period was observed between control and optimized sample. From the above table 4.8.1 it was shown the score of pH was decreased as the day was increased.

4.8.1.2 Changes in Acidity

The higher level of acidity indicates the more chances of microbial growth. It was observed that acidity of both control and optimized samples kept decreasing gradually from 0^{th} to 15^{th} day. It was observed that acidity increased non-significantly ($p>0.05$) from 0^{th} to 6^{th} day and then significantly ($p<0.05$) increased from 9^{th} to 15^{th} days between the samples throughout the storage period between control and optimized sample. During the storage period changes occurred in acidity found in optimized products for control and S2 was 0.39 ± 0.06 to 0.52 ± 0.01 and 0.50 ± 0.01 to $0.64 \pm 0.01$from 0 to 15^{th} days. From the above table 4.8.1 it was observed acidity increased day by day upto 15 days.

4.8.1.3 Yeast and Mould Count

The influence of yeast and mould found in storage of control and S2 is shown in table 4.8.1. the yeast and mould count for control and S2 were Nil from 0 days to 6^{th} days and for control 1.63 log 10 cfu/ml from 9^{th} days to 1.89 log 10 cfu/ml. at 15^{th} days. For S2 1.56 log 10 cfu/ml from 9^{th} days to 1.75 log 10 cfu/ml at 15^{th} days. From the above data it was found that no yeast and mould growth detected still 6^{th} day. However from 9^{th} day to 15^{th} days there was significant ($p < 0.05$) growth of Yeast and mould was detected. Similar result was observed by (**Snigdha, 2019).** The presence Yeast and mould are undesirable in dairy products because they cause colour defects and lipolytic alterations that result in the production of bad flavours in the final product. **(Kumar et al.,1975).**

4.8.1.4 Coliform Count

The coliform count found in optimized product is shown in table 4.8.1. In control and S2 the coliform were Nil upto 9th days. But in 12th and 15th days the coliform are studied for control and S2 were from 0.42 log 10 cfu/ml to 0.57 log 10 cfu/ml, and from 0.38 log 10 cfu/ml to 0.45 log 10 cfu/ml. From the above data it was found that no presence of coliform was detected still 9th day and then growth of Colliform was observed significantly ($p < 0.05$) from 12th days to 15th days. This investigation is supported by the results found by **Snigdha, 2019.**

4.8.1.5 Total Plate Count (TPC)

The Total plate count (TPC) was found in the storage of optimized product is described in Table 4.8.1 the TPC found in control from 0 to 15th days was 0.10 to 1.84 log 10 cfu/ml. and for S2 the TPC was 0.02 to 1.77 log 10 cfu/ml from 0-15th days. From the above data it was observed that total plate count increased significantly ($p<0.05$) from 0th day to 15th day. The values are in accordance with **(Singdha, 2019)** in multi fruit smoothie.

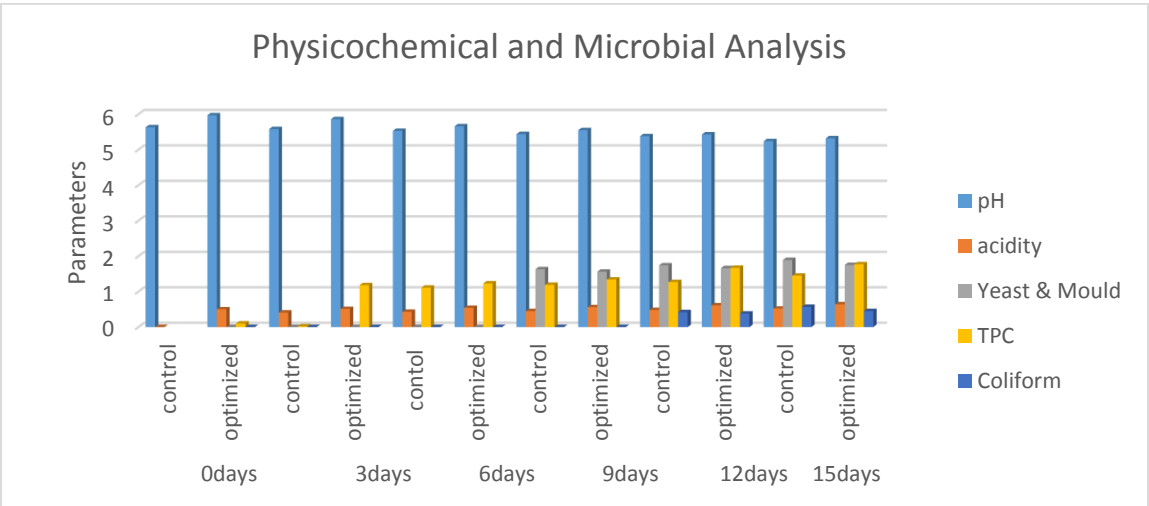

Fig 4.7 Physicochemical and microbial Storage during Storage

4.8.2 Sensory Evaluation of Control and Optimized Product

From the perspective of the consumer, it is one of the key characteristics that determines the product's quality. A product's shelf life is determined through sensory evaluation, which is important for both product development and/or enhancement. All deteriorative changes, including those that are sensory, physical, chemical, acidity development, and microbiological, are collectively represented in sensory quality and eventually cause the stored product to be unacceptable. The following table 4.8.2 represents the sensory scores of the storage functional smoothie.

4.8.2 Sensory Evaluation of Control and Optimized Product

Parameter	Control	S2
Flavour	2.08 ± 0.4^{d}	3.18 ± 0.05^{e}
Colour And Appearance	5.45 ± 0.54^{b}	5.14 ± 0.10^{a}
Body And Texture	4.61 ± 0.10^{c}	3.30 ± 0.14^{d}
Sweetness	5.42 ± 0.10^{b}	4.04 ± 0.49^{c}
Overall Acceptability	5.58 ± 0.04^{a}	4.51 ± 0.12^{b}

Values are reported as **Mean ± SD (n=3) a,b,c,d different superscript differ significantly (p < 0.05)**

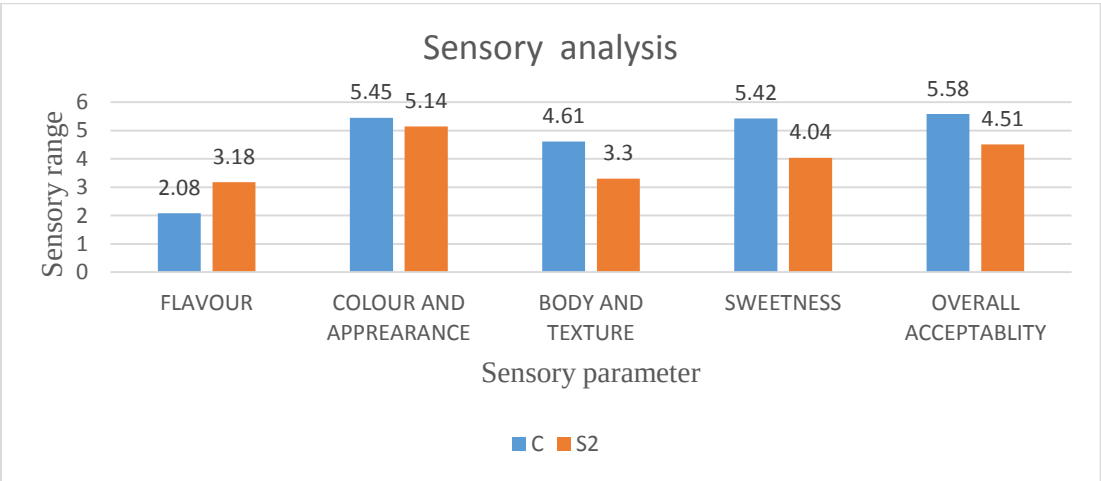

Fig 4.8. Sensory Evaluation of Control and Optimized Product

4.8.2.1 Changes in color and appearance

The score value in control and S2 were 5.45 ± 0.54 and 5.14 ± 0.10 out of 9 point hedonic scale. The score for control and S2 was decreased statistically non-significant ($p > 0.05$) difference. The changes in colour was due to development of Maillard reaction. Similar kind of changes was observed in the **(Mutoni, 2011)** in pearl millet milk based fermented product, **(Kumar, 2012)** in green gram cereal based breakfast smoothie and in curcumin based fortified functional lassi. **(Maurya 2012)**

4.8.2.2 Changes in sweetness

The sweetness score observed in control and S2 were 5.42 ± 0.10 and 4.04 ± 0.49. There was highly significant ($p < 0.05$) difference was noticed. The changes in sweetness occur due to long period of storage. As acidity was increased the sweetness was decreased.

Similar type of results for sweetness was observed in pearl-millet based fermented yoghurt during the time of storage by **Mutoni, 2011.**

4.8.2.3 Changes in flavour

The score value for flavour have been mentioned in Table 4.8.2. Control sample was 2.08 ± 0.4 and S2 was 3.18 ± 0.05. Changes in flavour is due to the storage of product. From the above table 4.8.2 it was noticed that values for control and S2 was significant variation ($p < 0.05$). The longer period of storage the lesser flavour will be found. Same result was observed by **Mehta 2012** in Dairy and pulse based breakfast smoothie, and in pearl milk based fermented product during storage of period by **Mutoni (2011).**

4.8.2.4 Changes in body and texture

The body and texture value observed in functional smoothie have mentioned in Table 4.8.2. Score for control and S2 was 4.61 ± 0.10 and 3.30 ± 0.14. Based on statistical analysis the values are significantly higher ($p< 0.05$) to each other. This could be caused by the product thinned by the protein sedimentation that occurred during storage.

4.8.2.5 Overall acceptability

The overall acceptability was decreases as time was passes. Results shows that Control was 5.58 ± 0.04 and S2 was 4.51 ± 0.12. The statistical analysis shows there was highly significant variation ($p < 0.05$) between the samples. The values are accordance with pearl milk based fermented product during the storage period by **Mutoni (2011)**

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CHAPTER-V

SUMMERY AND CONCLUSION

The current investigation entitled “Preparation of Horse gram (*Macrotyloma Uniflorum*) incorporated Functional Smoothie” was done at the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh.

Functional foods are foods that include essential nutrients that go beyond supporting an individual's normal growth and development. In view of the growing demand for food with functional properties, the current study emphasises the importance of applying new scientific knowledge to the exploration of underutilized crop; Horse gram as a source of functional and nutraceuticals compounds. Which is a great source of protein, carbohydrates, dietary fibre, micronutrients, and antioxidant properties. It minimizes the chances of intestinal diseases, diabetes, coronary heart disease, and dental caries, etc. Beside the Horse gram, banana and black carrot are used as a source of fruit and vegetable along with the dairy sources such as milk and dahi.

Preparation of Horse gram extract

- The horse gram extract was prepared by soaking around 12-18hrs followed by grinding and extraction of liquid. The extract was boiled for some time and analysis of parameters of HGE was done. The result obtained for fat, TS, carbohydrates, ash, antioxidant activity and crude fibre was 0.26 ± 0.14, 9.02 ± 0.91, 5.00 ± 0.95, 0.22 ± 0.03, 54.38 ± 1.59 and 0.72 ± 0.07.

Preparation of functional smoothie

- The functional smoothie was prepared by using milk, dahi (5%), Horse gram extract (5-15%), banana (15%), black carrot juice (3%), sugar (12%), tri-sodium citrate (0.04%), and pectin (0.2%).
- The sensory evaluation of functional smoothie was done by a group of trained and semi-trained judges of around 10 using '9-point hedonic scale'. The mean value of functional smoothie for treatment Control, S1, S2, and S3 were 8.01 ± 0.18, 6.9 ± 0.41, 8.35 ± 0.41, and 7.18 ± 0.96. There was significant variation ($p < 0.005$) were found in between treatments. S2 and control was obtained higher scored.
- The mean value for color and appearance of functional smoothie for treatment Control, S2, S2, and S3 were 7.64 ± 0.25, 6.98 ± 0.30, 7.77 ± 0.52 and 7.11 ± 0.30. The score were highest for C and S2. There was non-significant difference found in samples, due to equal level of black carrot juice.
- The mean score found in body and texture of functional smoothie for Control, S1, S2, and S3 were 7.46 ± 0.21, 8.37 ± 0.60, 6.67 ± 0.44, and 6.15 ± 1.4. S1 has highest and S3 has lowest in score due to high percentage of HGE in S3. There was slightly significant variation ($p < 0.05$) found in treatments.
- The mean value of sweetness found in Control, S1, S2, and S3 were 8.05 ± 0.32, 7.10 ± 0.74, 6.51 ± 0.29, and 5.92 ± 0.94 respectively. The score value were shows increase in significantly ($p < 0.05$).

- Overall acceptability was found for treatment Control, S1, S2, S3 and S4 were 7.19± 0.26, 6.68 ± 0.48, 8.09 ± 0.20 and 6.98 ± 0.13. The samples were varied significantly ($p < 0.05$) the control and S2 was chosen as product of acceptability.
- The physical analysis like viscosity, whey syneresis and sedimentation was investigated the results shows in viscosity for the treatments Control, S1, S2, and S3 were 718 ± 274.58, 740 ± 138.95, 843.33 ± 199.77 and 880 ± 24.97 cP. at 20 rpm. There was shows highly non-significant in variation ($p > 0.05$).
- The mean value of Whey syneresis for sample Control, S1, S2 and S3 were found 7.90 ± 3.12, 9.02 ± 1.77, 8.31 ± 0.85 and 6.19 ± 3.48.Which shows non-significant ($P > 0.05$) variation between the samples.
- Sedimentation result was found in Control, S1, S2 and S3 were 10.33 ± 3.73, 11.8 ± 5.99, 13.64 ± 1.94 and 14.14 ± 2.54. There were highly non-significant ($p > 0.05$) variation found in all sample.

Physicochemical analysis of functional smoothie

- The mean value for ash was absorbed in samples Control, S1, S2 and S3 were not affected significantly ($p > 0.05$) in smoothie. The ash content was 0.71 ± 0.10, 0.74 ± 0.09, 0.79 ± 0.06 and 0.82 ± 0.06. Highest value was observed in S3 due to higher percentage of HGE.
- The mean value of TS content was found 18.33 ± 3.15, 23.38 ± 0.20, 23.66 ± 1.83, and 23.69 ± 0.53.Which shows highly significant ($p < 0.05$) difference. Due to Control samples contains lowest TS value in comparison to other sample due to absence of HGE and S3 contains highest TS value due to higher amount of HGE.

- The mean value of antioxidant activity of functional smoothie for Control, S1, S2 and S3 were detected as 24.59 ± 3.49, 28.18 ± 2.86, 31.11 ± 4.17, and 38.20 ± 5.04 respectively. There are highly significant variation ($p < 0.05$) between samples, the value of antioxidant in Control was very low due to absence of HGE and S3 has high value of antioxidant activity due high percentage of HGE.
- The crude fibre content in functional smoothie for Control, S1, S2, and S3 were 0.76 ± 0.02, 0.82 ± 0.03, 0.86 ± 0.01 and 0.88 ± 0.01 significantly different ($p < 0.05$) to each other due to different concentration of HE
- Mean value in pH of functional smoothie for sample Control, S1, S2 and S3 were observed 5.86 ± 0.005, 5.87 ± 0.007, 5.93 ± 0.03 and 5.98 ± 0.006. The pH value was highly significant ($p < 0.05$) variation among each sample. The highest value of pH was observed in S3 due to high concentration of horse gram extract.
- The titratable acidity range of functional smoothie sample for Control, S1, S2 and S3 were range from 0.39 ± 0.06 to 0.41 ± 0.02. The pH value were observed non-significant ($p > 0.05$) differences between all the samples.

Proximate analysis of control and optimized sample

- Ash value of control (c) and optimized (S2) sample were 0.72 ± 0.1and 0.81 ± 0.04. There were non-significant variation ($p > 0.05$) between two samples.
- The mean percentage of total solids for control (c) and optimized (S2) sample was noticed as 18.38 ± 3.14 and 23.40 ± 0.44. There were slightly non-significant variance ($p > 0.05$) observed between two samples. S2 shows higher value of total solids as it contains 10% of horse gram extract.

- The mean value of fat percentage in Control (c) and S2 sample were 2.66 ± 0.15 and 3.03 ± 0.15. Which are shows significant variation ($p < 0.05$).
- The mean percentage of protein value observed in Control (c) and S2 sample were 2.78 ± 0.10 and 3.38 ± 0.36. The protein value was slightly non-significant ($p > 0.05$) between the two samples. S2 was noticed maximum protein value due to the horse gram extract content.
- The mean value of carbohydrate percentage for Control (c) and S2 sample was 12.20 ± 3.21 and 16.17 ± 0.10. The carbohydrates percentage shows non-significant difference ($p > 0.05$). The maximum percent was present in S2 due to 10% of horse gram extract.

Shelf-life study of control and optimized product

- In this shelf life study it was noticed that the titratable acidity was increased whereas the pH value was decreased as days are increased and spores forming in TPC, yeast and mould, and coliform was increased after zero day. As well as overall acceptability was reduced upto 15^{th} days at a range from 5.58 to 2.08.
- The microbial spores count for yeast and mold from 0-6^{th} days was Nil while from 9^{th} to 15^{th} days it was 1.63, 1.74, 1.89 log10cfu/ml for control and 1.56, 1.66, 1.75 log10cfu/ml for optimized product. However from 9^{th} day to 15^{th} days there was significant ($p < 0.05$) growth of Yeast and mould was detected.
- The microbial count for coliform from 0 to 9^{th} days was Nil. While from 12^{th} to 15^{th} days the spores count was 0.42, 0.57 log10cfu/ml for control and 0.38, 0.45 log10cfu/ml for optimized sample.

- TPC found in control from 0 to 15th days was 0.10 to 1.84 log10cfu/ml. and for S2 the TPC was 0.02 to 1.77 log10cfu/ml from 0-15th days. From the above data it was observed that total plate count increased significantly ($p<0.05$) from 0th day to 15th day.

Looking at the growing demand of functional food and dairy sector the current study on "Preparation of Horse gram (*Macrotyloma Uniflorum*) incorporated Functional Smoothie" was investigated at Department of Dairy Science and Food Technology, Banaras Hindu University, Uttar Pradesh. On the basis of organoleptic quality it was confirmed that S2 sample with 10% of HGE was found to be more acceptable by the judges. The under-utilized pulse Horse gram have a great source of nutritional as well as functional characteristics. In addition of HGE into the smoothie significantly increased the ash, carbohydrate, protein, crude fibre and antioxidant content.

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APPENDICES

APPENDIX-I

SENSORY SCORE CARD FOR

PREPARATION OF HORSE GRAM (MACROTYLOMA UNIFLORUM) INCORPORATED FUNCTIONAL SMOOTHIE

Date: ……..

PRODUCT:

……..

NAME OF THE PANELIST:

…….

DESIGNATION:

……..

Respected Sir/Madam

Kindly evaluate formulated samples of Functional Smoothie on 9-Point Hedonic Scale of following attributes.

Sample Code	Flavour	Colour & Appearance	Body & Texture	Sweetness	Overall Acceptability

1: Dislike extremely
2: Dislike very much
3: Dislike Moderately
4: Dislike slightly
5: Neither like not dislike
6: Like slightly
7: Like Moderately
8: Like very much
9: Like extremely

Remarks:……

……

(Signature of Judge)

APPENDIX-II

ANOVA OF SENEORY SCORWE CARD FOR FUNCTINAL LASSI

FLAVOUR

Source of Varia	SS	df	MS	F	P-value	F crit
Between G	4.189233	3	1.396411	4.31902	0.04351	4.066181
Within Gro	2.586533	8	0.323317			
Total	6.775767	11				

COLOR AND APPEARANCE

ce of Varia	SS	df	MS	F	P-value	F crit
Between G	1.345388	3	0.448463	3.446036	0.071814	4.066181
Within Gro	1.041109	8	0.130139			
Total	2.386497	11				

BODY AND TEXTURE

ce of Varia	SS	df	MS	F	P-value	F crit
Between G	8.503158	3	2.834386	4.069957	0.049895	4.066181
Within Gro	5.571333	8	0.696417			
Total	14.07449	11				

SWEETNESS

Source of Varia	SS	df	MS	F	P-value	F crit
Between G	7.432425	3	2.477475	6.078077	0.018494	4.066181
Within Gro	3.260867	8	0.407608			
Total	10.69329	11				

OVERALL ACCEPTABLE

Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	3.312216	3	1.104072	11.95526	0.002516	4.066181
Within Gro	0.738803	8	0.09235			
Total	4.051019	11				

ANOVA OF PHYSICOCHEMICAL EVALUATION OF FUNCTIONAL SMOOTHIE

VISCOSITY

Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	55544	3	18514.67	0.54762	0.663573	4.066181
Within Gro	270474.7	8	33809.33			
Total	326018.7	11				

WHEY SYNERESIS

ANOVA						
Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	12.94915	3	4.316385	0.669621	0.594123	4.066181
Within Gro	51.56813	8	6.446016			
Total	64.51729	11				

SEDEMENTATION

ANOVA						
ce of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	27.50952	3	9.169838	0.610295	0.626989	4.066181
Within Gro	120.202	8	15.02525			
Total	147.7115	11				

ANOVA OF PROXIMATE ANALYSIS OF SMOOTHIE.

ASH

	ANOVA						
	Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	Between G	0.020975	3	0.006992	0.965117	0.455022	4.066181
	Within Gro	0.057955	8	0.007244			
	Total	0.07893	11				

TS

	ANOVA						
	Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	Between G	62.05545	3	20.68515	6.055933	0.018675	4.066181
	Within Gro	27.32546	8	3.415683			
	Total	89.38091	11				

ANTIOXIDANT

	ANOVA						
	Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	Between G	299.8849	3	99.96164	6.314336	0.016694	4.066181
	Within Gro	126.6472	8	15.8309			
	Total	426.5322	11				

CRUDE FIBRE

	Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	Between G	0.024828	3	0.008276	12.26659	0.002316	4.066181
	Within Gro	0.005397	8	0.000675			
	Total	0.030225	11				

pH

Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	0.02403	3	0.00801	19.16241	0.000521	4.066181
Within Gro	0.003344	8	0.000418			
Total	0.027374	11				

ACIDITY

ANOVA						
Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	0.001177	3	0.000392	0.323685	0.808407	4.066181
Within Gro	0.009697	8	0.001212			
Total	0.010874	11				

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