A MULTIFACTORIAL STUDY ON MUSTARD MICROGREENS AND THE DEVELOPMENT OF SNACKS FROM MUSTARD MICROGREENS POWDER

DISSERTATION SUBMITTED

In Partial Fulfilment of the Requirement for

The Award of the Degree of

MASTER'S PROGRAMME IN

CLINICAL NUTRITION AND DIETETICS

BY

FATHIMA M.A

(Register No: SM19MCN006)

DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS

WOMEN'S STUDY CENTRE

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

APRIL 2021

CERTIFIED AS BONAFIDE RESEARCH WORK

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Signature of Internal **Signature of External**

DECLARATION

I hereby declare that the thesis entitled **"A multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder"** submitted in partial fulfilment of the requirement for the award of the Degree of **Master's Programme in Clinical Nutrition and Dietetics** is a record of original research work done by me under the supervision and guidance of **Ms. Swathilakshmi Venu**, Asst Professor, Department of Clinical Nutrition and Dietetics, Women's Study Centre, St. Teresa's College(Autonomous), Ernakulam. That the thesis has not previously formed on the basis for the award of any degree work has not been submitted in part or full or any other degree/diploma/ fellowship or the similar titles to any candidate of any other University.

Place: FATHIMA M.A

Date:

CERTIFICATE

I hereby certify that the dissertation entitled **"A multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder"** submitted in partial fulfilment of the requirement for the award of the Degree of **Master's Programme in Clinical Nutrition and Dietetics** is a record of original research work done by Ms. Fathima M.A during the period of her study under my guidance and supervision.

Signature of the HOD

Signature of the Guide

Ms. Swathilakshmi Venu

Assistant professor

Department of Clinical Nutrition and Dietetics

Women's Study Centre

St. Teresa's College, Ernakulam

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ABSTRACT

ABSTRACT

AIM: To do a multifactorial (multiple harvest,growth characteristics, nutrient analysis,physical properties of shelf life) study on mustard microgreens and the development of snacks from mustard microgreens powder.

INTRODUCTION: Microgreens are the immature greens developed from vegetables and herbs.

METHODOLOGY: Mustard microgreens are grown in soil mixed with cocopeat, eggshell powder. In the same soil double harvesting is done. At the regrowth phase, extra compost was added. Nutrient content, growth characteristcs of mustard microgreens was analysed. Microgreens powder was kept for 3 months for checking the physical properties of shelf life, there is no deterioration in the powder. Snacks were developed and kept for sensory evaluation.

RESULT: It was observed that regrowth was more wherein pinching off leaves and additional compost was added. Nutrient analysis was done mustard microgreens are rich in dietary fibre also the addition of compost enhances the nutrient content. Study finds that there is no physical deterioration of the powder. Snacks were also developed.

Key words: mustard microgreen, multiple harvests, growth characteristics, shelf life, nutrient analysis, dietary fibre, product development.

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INTRODUCTION

1. INTRODUCTION

Microgreens are the immature greens developed from vegetables and herbs. Microgreens are the state of greens with fully developed cotyledon leaves. Microgreens are harvested after a week or two. The study titled “A multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder” focuses on the study of multiple factors of mustard microgreens, in particular, which includes multiple harvesting of these microgreens and observation of their growth characteristics, its nutrient analysis and physical characteristics of the shelf life of mustard microgreens powder. This study also includes development of snacks from this powder, to commercialize the microgreen by including it in different forms.

MICROGREENS AS A SUPER FOOD

E. Sreenevasan, (2020) conducted a study “Preliminary Report on Multiple Harvests of Microgreens from Chickpea (Cicer arietinum) Seeds” he stated that “Microgreens are the immature edible vegetable that include seedlings of herbs, vegetables and other plant. microgreens are known as new super food. Microgreens are an exotic genre of edible greens, appearing in upscale markets and restaurants that have gained popularity as a new culinary trend over the past few years. Microgreens are tender immature greens produced from the seeds of vegetables and herbs, having two fully developed cotyledon leaves with or without the emergence of a rudimentary pair of first true leaves. Microgreens are usually 2.5−7.6 cm (1−3 in.) in height, harvested at

7−14 days after germination, depending on the species, and sold with the stem and attached cotyledons (seed leaves). Although small in size, microgreens can provide a large array of intense flavors, vivid colors and tender textures. Therefore, microgreens can be served as a new ingredient in salad, soups, and sandwiches, enhancing their colour, texture, and/or flavour, and also can be used as edible garnish to brighten up a wide variety of main dishes. Developed cotyledons and a pair of partially developed true stem base with simple harvesting tools".

NUTRITIONAL FACTS ON MICROGREENS

Paradiso et.al, (2018), conducted a study on nutritional characterization and shelf-life of packaged microgreens. The includes articulated nutritional profile of six genotypes of microgreens, belonging to three species and two families: chicory (Cichorium intybus L., Puglia's local variety 'Molfetta', CM, and cultivar 'Italico a costa rossa', CR) and lettuce (Lactuca sativa L. Group crispa, cultivar 'Bionda da taglio', LB, and 'Trocadero', LT), from Asteraceae; and broccoli (Brassica oleracea L. Group italica Plenk, Puglia's local variety 'Mugnuli', BM, and cultivar 'Natalino', BN) from Brassicaceae. The data obtained from this study point out the possibility to exploit genetic biodiversity in order to obtain tailored microgreens with the desired nutritional profiles, with particular regard to mineral nutrients and bioactive compounds.

Brazaityte et al, (2019) states that "Brassica vegetables are one of the most popular and widely grown vegetables in the world. These vegetables, including mustard it contains various health-promoting compounds such as carotenoids, chlorophylls,

phenolic compounds, glucosinolates, and minerals and are an excellent source of fiber".

Assessment of Vitamin and Carotenoid Concentrations of Emerging Food Products: Edible Microgreens was a study conducted by Xiao et.al (2013). In this study they stated that "microgreens have been claimed as nutritionally beneficial, to the best of our knowledge, no scientific data are available on the exact phytochemical content of microgreens. Limited studies have shown that some young seedlings may have much higher levels of vitamins, minerals, and other health giving phytonutrients than the mature leaves. In general, microgreens contain considerably higher concentrations of vitamins and carotenoids than their mature plant counterparts.

HARVESTING INTERVENTIONS

Turnek. R et,al, 2020 conducted a study on Microgreens nutrition, food safety, and shelf life: A review. The study found that preharvest and postharvest interventions, such as calcium treatments, modified atmosphere packaging, temperature control, and light, to maintain quality, augment nutritional value, and extend shelf life. The study also state that "One major limitation to the growth of the microgreens industry is rapid quality deterioration postharvest. Microgreens are difficult to store, due to their high surface area to volume ratio, high respiration rate, and delicate leaves that easily wilt, and rapid postharvest decay transpiration, leakage of nutrient rich exudates, tissue damage, and early senescence. Some growers sell microgreens as a "living product" so that the customer harvests and washes them as they are needed to serve the freshest quality. Hydroponic pads and soil-less substrates tend to be favoured for this practice for ease of trans-port and perception of cleanliness in a kitchen

environment. However, these microgreens still need to be used quickly to maintain peak quality".

Weber, (2017) conducted a study on Broccoli Microgreens: A Mineral-Rich Crop That Can Diversify Food Systems. He states that "Current malnourishment statistics are high and contemporary agricultural practices are a dominant force in damaging the very environments on which the production of nutritious food depends. In the U.S., food production utilizes 50% of land and is responsible for 80% of total freshwater consumption, which occurs at a rate that is faster than aquifer recharge in some regions. Food production also depends heavily of fertilizer and pesticide application, which is adversely impacting ecosystem biodiversity. Additionally, cultivation is increasingly focused on the mass production of fewer staple crops. This reduces the nutritional value of the average diet and makes food production less resilient to environmental change, should it be the demise of one or more of these relatively few crops. Therefore, simply upscaling current agricultural practices to increase crop yields is not a viable solution for feeding the World's population. It is a priority to establish dietary guidelines that satisfy human nutritional requirements with a diversity of foods that can be produced with minimized environmental impact, this is key to ensuring socioeconomic and sociocultural prosperity into the future".

The present study aims for the following objectives:

- To investigate multiple harvesting of mustard microgreens.
- To investigate growth characteristics of mustard microgreens.
- Analysis of nutrient composition of mustard microgreens in each harvesting.
- To evaluate the shelf life of dried mustard microgreens
- Development of product from mustard microgreens.

- Sensory evaluation of developed products.

RELEVANCE OF THE STUDY

Microgreens have gained a great deal of popularity in recent years. It is known for being nutrient-rich, especially vitamins, minerals, and fibre. But they are not quantified in particular hence nutrient analysis of dietary fibre, protein, calcium, iron were included in the study. Therefore, microgreens would be an excellent choice for enhancing one's well-being. The study entitled "A multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder" was inspired by one of the studies done by E. Sreenivasan, called "Preliminary Report on Multiple Harvests of Microgreens from Chickpea (Cicer arietinum) Seeds" which was done in the year 2020 in Kannur, Kerala. Mustards were chosen for the study as they are commonly available in our kitchen. Multiple harvesting provides a larger quantity of microgreens from one set of seed. Also, it reduces the quantity of seed for further growth. Multiple harvesting saves time, money and space and is affordable by the common people.

REVIEW OF

LITERATURE

2. REVIEW OF LITERATURE

The review of literature pertaining to the present study “A Multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder” is discussed under the following head:

2.1 SCIENCE BEHIND MICROGREENS

2.2 POSTHARVEST QUALITY AND SHELF LIFE OF MICROGREENS

2.3 NUTRITIONAL COMPOSITION OF MICROGREENS

2.4 HEALTH BENEFITS OF MICROGREENS

E. Sreenivasan (2020) conducted a study on multiple harvests of microgreens from chickpea seeds. He stated that “Microgreens are edible baby plants with huge potential for leafy vegetable production and many consider this plant-based functional food as the new “Super food”. Microgreens have many advantages over sprouts and they help to improve the nutritional value of our diet, with their high content of healthy compounds. During the microgreen cultivation, seeding is usually done as a broadcast or in rows and as the seeding density is difficult to recommend, most growers prefer to seed as thickly as possible to maximize production, but not too thickly because crowding encourages elongated stems which increases the risk of disease. Some growers have noticed the possibility of a “second harvest” of microgreens, just after the majority of tall-grown microgreens are carefully harvested from the substrate with very high density of seeds, leaving a certain percentage of

ungerminated seeds and sprouts to develop further". He investigated the possibility of harvesting more than one microgreen crop from a set of chickpea seeds through the regeneration of shoots after the first and the successive cuts above the lowest nodal portion of the shoots. He also included a study on the growth characteristics of the microgreens after the successive harvests.

2.1 SCIENCE BEHIND MICROGREENS

Di Gioia et.al (2017). Conducted a study on "Micro-scale vegetable production and the rise of microgreens" he says that "Microgreens is instead a marketing term used to describe a category of products that has no legal definition. They differ from sprouts because they require light and a growing medium and have a longer growth cycle (7–28 days); the edible portion is constituted by stem and cotyledons and often by the emerging first true leaves. By contrast, "baby leaf" vegetables are grown in the presence of light, either in soil or soilless systems, have a longer growth cycle (20–40), usually require the use of fertilizers and agrochemicals and are harvested after the development of the true leaves".

D. Frazie et.al (2017) conducted a study on "Health-Promoting Phytochemicals from 11 Mustard Cultivars at Baby Leaf and Mature Stages". They analysed the glucosinolates and their hydrolysis products, carotenoids, total anthocyanin and phenolic contents, and antioxidant capacity of the leaves of 11 mustard cultivars grown in a greenhouse at the baby leaf and mature stages. They found aliphatic glucosinolate sinigrin and its hydrolysis products allyl isothiocyanate and 1-cyano-2, 3-epithiopropane were the major phytonutrients in the mustard leaves. Carotenoids β-carotene, lutein, violaxanthin, and neoxanthin were also detected. The study found that phytonutrient concentration and their change with plant growth were cultivar-

dependent, Phenolic contents and antioxidant capacity also varied among cultivars and between physiological stages. The result of the study concludes that mustard leaves are rich in various phytochemicals and their composition depends on cultivar and the physiological stage.

Xiao et.al (2013) conducted a study on "Assessment of Vitamin and Carotenoid Concentrations of Emerging Food Products: Edible Microgreens". The study was conducted to determine the concentrations of ascorbic acid, carotenoids, phylloquinone, and tocopherols in 25 commercially available microgreens. The result of this study concludes that different microgreens provided extremely varying amounts of vitamins and carotenoids. Total ascorbic acid contents ranged from 20.4 to147.0 mg per 100 g fresh weight (FW), while β-carotene, lutein/zeaxanthin, and violaxanthin concentrations ranged from 0.6 to 12.1, 1.3 to 10.1, and 0.9 to 7.7 mg/100 g FW, respectively. Phylloquinone level varied from 0.6 to 4.1 μg/g FW; meanwhile, α-tocopherol and γtocopherol ranged from 4.9 to 87.4 and 3.0 to 39.4 mg/100 g FW, respectively. Among the 25 microgreens assayed, red cabbage, cilantro, garnet amaranth, and green daikon radish had the highest concentrations of ascorbic acids, carotenoids, phylloquinone, and tocopherols, respectively.

2.2 POSTHARVEST QUALITY AND SHELF LIFE OF MICROGREENS

Srividya et,al (2020) on Effect of Packaging and Coating Technique on Postharvest Quality and Shelf Life of Raphanus sativus L. and Hibiscus sabdariffa L. Microgreens. They assessed on the postharvest quality and shelf life of radish (RaS) and roselle (HbS) microgreens stored at 5 °C. Pre-harvest spray treatment (AGSC) was compared with postharvest dip coating (AGDC) using Aloe vera gel (AG) for the

first time in microgreens for postharvest quality improvement. The result found that PET–CS had a lower physiological loss in weight (PLW), respiration rate (RR), electrolyte leakage (EL), microbial counts (MCs), and higher overall acceptability (OA) than LDPE–SSB. AG-coated microgreens had significantly ($p \leq 0.05$) lesser deteriorative postharvest changes and higher ascorbic acid content than uncoated control. AGSC maintained better OA and postharvest quality than AGDC, especially at the end of the study period in terms of reducing EL, retaining greenness (−a*), and chroma value in HbS microgreens. In RaS microgreens, AGSC helped to maintain lower PLW, MC, and higher ascorbic acid levels.

2.3 NUTRITIONAL COMPOSITION OF MICROGREENS

"Assessment of Vitamin and Carotenoid Concentrations of Emerging Food Products: Edible Microgreens" is a study conducted by Xiao et.al (2013). There they stated "Microgreens are an exotic genre of edible greens, appearing in upscale markets and restaurants that have gained popularity as a new culinary trend over the past few years. Microgreens are tender immature greens produced from the seeds of vegetables and herbs, having two fully developed cotyledon leaves with or without the emergence of a rudimentary pair of first true leaves. Microgreens are usually 2.5−7.6 cm (1−3 in.) in height, harvested at 7−14 days after germination, depending on the species, and sold with the stem and attached cotyledons (seed leaves). Although small in size, microgreens can provide a large array of intense flavors, vivid colors and tender textures. Therefore, microgreens can be served as a new ingredient in salad, soups, and sandwiches, enhancing their color, texture, and/or flavor, and also can be used as edible garnish to brighten up a wide variety of main dishes".

Luo et.al, (2016) conducted a study on mineral content of Brassicaceae microgreens. This study was analyzed for 30 varieties of microgreens, representing 10 species within 6 genera of the Brassicaceae family. Brassicaceae microgreens were also assayed for concentrations of macroelements, including calcium (Ca), magnesium (Mg), phosphorous (P), sodium (Na), potassium (K), and of microelements, including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Determinations of mineral elements in microgreen samples were performed using an inductively coupled plasma optical emission spectrophotometer (ICP OES). Result obtained from this study conveys Potassium was the most abundant macroelement ranging from 176-387 mg/100 g fresh weight (FW), followed by P (52-86 mg/100g FW), Ca (28-66 mg/100g FW), Mg (28-66 mg/100g FW), and Na) 19-68 mg/100g FW. Among the microelements, Fe tended to be most abundant (0.47-0.84mg/100g FW), followed by Zn (0.22-0.51 mg/100g FW), Mn (0.17-0.48 mg/100g FW), and Cu (0.041-0.13 mg/100g FW). Based on this result it is clear that microgreens are good sources of both macroelements (K and Ca) and microelements (Fe and Zn.). Consumption of microgreens could be a health-promoting strategy to meet dietary reference intake requirements for essential elements beneficial to human health.

Lenzi et,al (2019) conducted a study on "Antioxidant and Mineral Composition of Three Wild Leafy Species: A Comparison Between Microgreens and Baby Greens" they compared three wild leafy species (Sanguisorba minor Scop., Sinapis arvensis L., and Taraxacum officinale Weber ex F. H. Wigg.) harvested at the microgreen and baby green stages. Seedlings were grown hydroponically in a half-strength Hoagland nutrient solution under controlled climatic conditions. While harvesting the yield was assessed, and chlorophylls, carotenoids, anthocyanins, phenolic index, nitrate, and mineral elements were measured in the two types of product. The potential

contribution to human mineral intake was calculated, and the possible risk due to the presence of metals potentially detrimental for health was estimated. Results found that micro/baby greens of these wild plants achieved competitive yields and could contribute to the dietary intake of macroelements, microelements, and non-nutrient bioactive compounds.

2.4 HEALTH BENEFITS OF MICROGREENS

McGrane (2020). Conducted a study on nutritional facts and health benefits of mustard microgreens. She concludes that free radicals are unstable molecules that can damage your cells. Research suggests that over time, this damage can lead to serious, chronic conditions, such as heart disease, cancer, and Alzheimer's disease. While levels of specific antioxidants vary between the different varieties of mustard greens, these leafy greens in general are a rich source of antioxidants like flavonoids, beta carotene, lutein, and vitamins C and E. Both raw and cooked mustard greens are a phenomenal source of vitamin K, providing 120% and 690% of the DV per one cup (56 grams and 140 grams), respectively . Vitamin K is best known for its vital role in helping with blood clotting. It's also been shown to be essential for heart and bone health. Mustard greens may also be good for your immune system. Just one cup (56 grams raw, 140 grams cooked) provides more than a third of your daily vitamin C needs. Vitamin C is a water-soluble vitamin that's essential for a strong immune system. Research shows that not getting enough vitamin C in your diet can weaken your immune system, making you more susceptible to getting sick. Additionally, vitamin A in mustard greens also supports your immune response. It does this by promoting the growth and distribution of T cells, which are a type of white blood cell

needed to help fight off potential infections. Mustard greens may also be good for your heart. They're loaded with antioxidants like flavonoids and beta carotene, which have been associated with a reduced risk of developing and dying from heart disease. It is good for eye and also it has anticancer effect.

Renna et.al (2018) conducted a study on production of microgreens with low potassium content for patients with impaired kidney function. In this study, for the first time, some chicory (local variety 'Molfetta' and cultivar 'Italico a costa rossa') and lettuce (cultivar 'Bionda da taglio') genotypes were grown using a hydroponic system with different potassium (K) levels (0, 29.1, 58.4, and 117 mg L−1) in order to produce microgreens with a low potassium content. The crop performances, cations content, proximate composition, and antioxidant activity was analysed. The results suggest that by using an nutrient solution NS without K or with low K concentrations, it is possible to obtain a useful reduction of K in microgreens, without negatively affecting the quality. Unlike conventional vegetables, the microgreens that were produced in this study could reduce the potassium intake in patients with impaired kidney function who were accustomed to eating vegetable-based dishes.

METHODOLOGY

3. METHODOLOGY

The methodology of the present study entitled “A multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder” is discussed under the following heads.

3.1 TO INVESTIGATE MULTIPLE HARVESTING OF MUSTARD MICROGREENS.

3.2 INVESTIGATE GROWTH CHARACTERISTICS OF MUSTARD MICROGREENS.

3.3 ANALYSIS OF NUTRIENT CONTENT OF MUSTARD MICROGREENS IN EACH HARVESTING.

3.4 EVALUATION OF THE SHELF LIFE OF DRIED MUSTARD MICROGREEN (PHYSICAL CHARACTERISTICS)

3.5 DEVELOPMENT OF PRODUCT FROM MUSTARD MICROGREENS.

3.5.1 GROWING, HARVESTING AND CLEANING OF MUSTARD MICROGREENS.

3.5.2 DRYING AND POWDERING OF MUSTARD MICROGREENS.

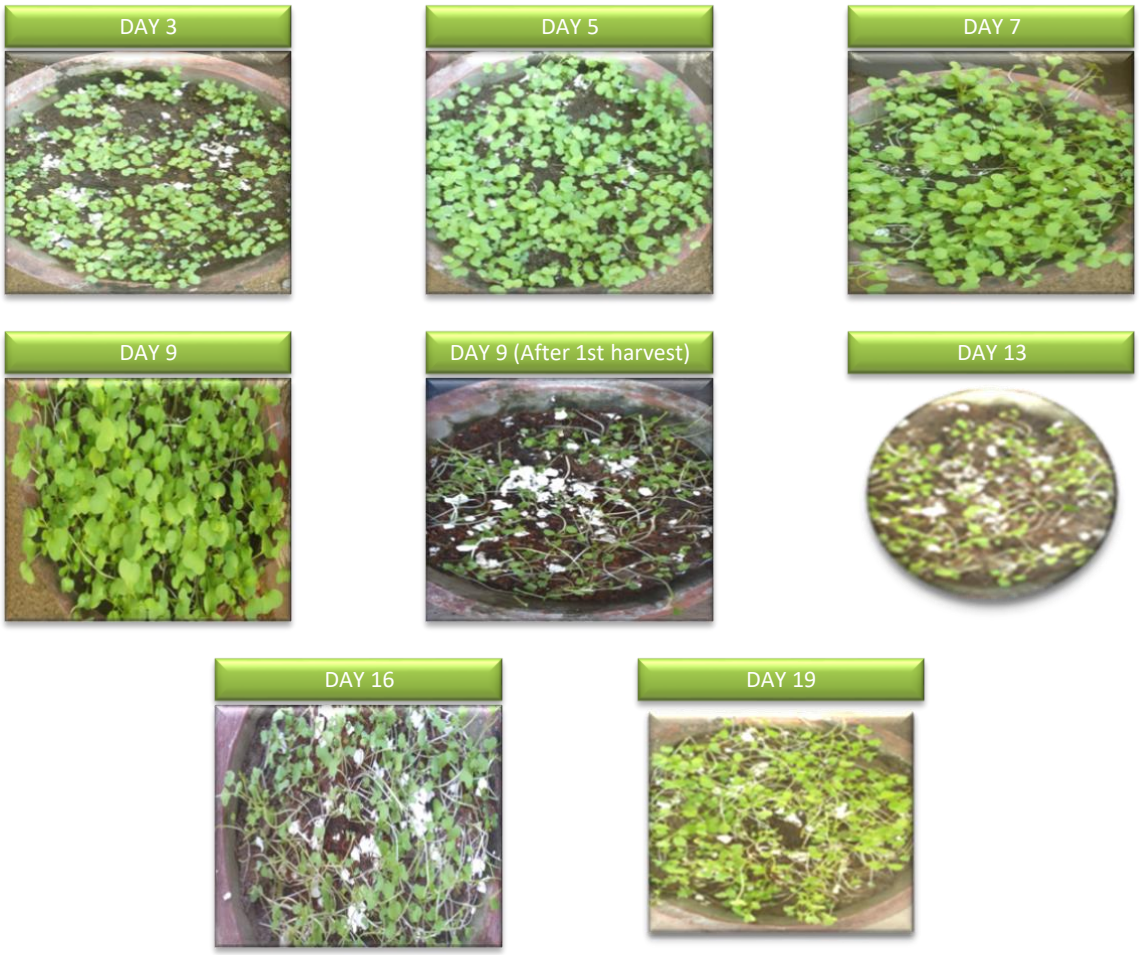
3.6 SENSORY EVALUATION OF DEVELOPED PRODUCT.

3.1 TO INVESTIGATE MULTIPLE HARVESTING OF MUSTARD MICROGREENS

Mustard microgreens were grown in soil that was mixed with coco peat and eggshell powder. After the first harvest, more compost was added to the soil to enhance its growth. Multiple harvesting of mustard microgreens was done by pinching the leaves above the lowest nodal portion of the shoots.

The germinated seeds were counted by the emergence of a 2 mm radicle at the time of observation. Germination/Regeneration percentage (GP/RP) was calculated at germination and the various stages of regrowth, using the formula: (GP or RP %) = g×100/50; Where 'g' is the number of germinated seeds/regenerated shoots. Where the number of seeds used for germination is 50. The formula for calculating (GP/RP) was referred from the study on Preliminary Report on Multiple Harvests of Microgreens from Chickpea (Cicer arietinum) Seeds done by E. Sreenivasan, 2020.

Figure 3.1: Multiple harvests in mustard microgreens



3.2 TO INVESTIGATE GROWTH CHARACTERISTICS OF MUSTARD MICROGREENS

Growth characteristics of mustard microgreens was analysed with regards to the following criteria's:

- First growth without compost
- Regrowth without compost
- First growth with addition of compost
- Regrowth with addition of compost after first harvest

The germinated seeds were counted by the emergence of a 2 mm radicle at the time of observation. Germination/Regeneration percentage (GP/RP) was calculated at germination and the various stages of regrowth, using the formula: (GP or RP %) = g×100/50; Where 'g' is the number of germinated seeds/regenerated shoots. Where the number of seeds used for germination is 50. The formula for calculating (GP/RP) was referred from the study on Preliminary Report on Multiple Harvests of Microgreens from Chickpea (Cicer arietinum) Seeds done by E. Sreenivasan, 2020.

3.3 ANALYSIS OF NUTRIENT CONTENT OF MUSTARD MICROGREENS IN EACH HARVESTING

The nutrient content analysis of mustard microgreens in multiple harvests was conducted at Neogen Laboratory located in Tripunithura of Ernakulam district. The test included Dietary fibre by IS 11062-1984 method, Protein by MOA/CH/N method, calcium by AOAC 21st Edn 927.02 method, Iron by AOAC 21st Edn 944.02 method.

3.4 EVALUATION OF THE SHELF LIFE OF DRIED MUSTARD MICROGREEN (PHYSICAL CHARACTERISTICS)

The shelf life of dried mustard microgreens was evaluated for physical characteristics such as colour change and odour. After drying the mustard microgreens, it was stored in an air-tight container in a cool dry place for evaluating physical characteristics.

3.5 DEVELOPMENT OF PRODUCT FROM MUSTARD MICROGREENS

3.5.1 GROWING, HARVESTING AND CLEANING OF MUSTARD MICROGREENS

Initially, grown mustard microgreens were harvested using scissors. Mustard microgreens were harvested on the 9th day. Then it was washed thoroughly with fresh potable water to remove dirt and other unwanted materials and then the excess water was removed.

Figure 3.2: Growing of mustard microgreens

3.5.2 DRYING AND POWDERING OF MUSTARD MICROGREENS

The washed mustard microgreens were dried using the sun drying method. The Sun drying method took 3 days, and then dried mustard microgreens were powdered using a blender.

Figure 3.3: Dried mustard microgreens

Figure 3.4: Powdered mustard microgreens

Present study includes the development of snacks using mustard microgreens powder such as diamond cuts, pappad, ladoo and halwa. All the ingredients for the preparation of snacks were measured using a weighing balance. Handling and preparation of snacks was done under hygienic conditions with minimum wastage of food materials.

3.5.3 DIAMOND CUTS

INGREDIENTS

- Refined wheat flour (Maida) -80g
- Mustard microgreens powder-10g
- Pepper- 2.5g
- Red chilli-5g
- Turmeric-2.5g
- Ginger, garlic paste- 2.5g
- Black seasam seed-2.5g
- Salt to taste
- Oil- 1tsp
- Oil for frying

METHOD OF PREPARATION

- Add all the ingredients except oil in a bowl mix well and knead into soft dough using enough water then add oil and keep it for 15 minutes.
- Divide the dough in to two equal parts.
- Sprinkle some refined wheat flour (maida) on rolling board and place the dough on it then spread it with the help of rolling pen in to very thin sheet. Finally cut it in to diamond shape.
- Fry till they turn golden brown and crispy from both sides.
- Drain them to the paper towel to remove excess oil.
- Store them in air tight container.

Figure 3.5: Developed diamond cuts 10% variant

3.5.4 PAPPAD

INGREDIENTS

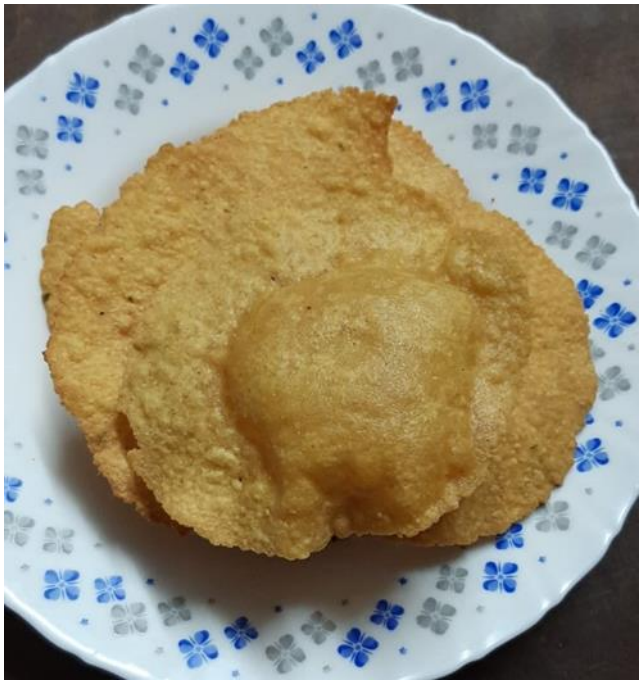
- Urad dal powder- 90g
- Mustard microgreens powder- 10g
- Baking soda-2.5g
- Salt to taste
- Seasam oil – 2tsp

METHOD OF PREPARATION

- Add all the ingredients except sesame oil in a bowl and knead using enough water. Then add 2 tsp. of sesame oil and knead well.

- Sprinkle some maida on the rolling board and place a small piece of dough on it then spread it with the help of a rolling pin into a very thin round shape.
- Fry till they turn golden brown and crispy from both sides.
- Drain them to the paper towel to remove excess oil.
- Store them in an airtight container.

Figure 3.6: Developed pappad 10% variant



3.5.5 LADOO

INGREDIENTS

- Rava-60g
- Coconut-10g
- Mustard microgreens powder -10g

- Sugar powder- 15g
- Ghee-5g
- Cashew- 2.5g
- Raisin-2.5g
- Salt a pinch
- Milk- half cup
- Turmeric powder -2.5g

METHOD OF PREPARATION

- Add 10g of coconut to a grinder and grind it well
- Then add Rava, ground coconut and mustard microgreens powder into a pan, roast in a medium flame by continuous stirring.
- When Rava turns crunchy add sugar powder, salt to balance sugar then immediately turn off the flame.
- Mix it well and keep it aside for cooling.
- Meanwhile boil the milk and add turmeric powder then stir well and keep aside for cooling.
- Roast cashew and raisin in ghee, keep aside.
- After cooling the milk, pour it into the roasted ingredients for having a yellow colour. Mix well and make into small balls.

Figure 3.7: Developed ladoo 10% variant

3.5.6 HALWA

INGREDIENTS

- Corn flour-60g
- Mustard microgreens powder- 10g
- Food colour-red
- Sugar-15g
- Lime juice- 1 tsp.
- Salt a pinch
- Ghee-10g

- Cashew-2.5g
- Black Sesame seed-2.5g

METHOD OF PREPARATION

- Firstly, add one cup of water into corn flour, mustard microgreens powder. Mix very well. There should be no lumps. Then add red food colour. Keep aside.
- For preparing sugar solution, add sugar to a Kadai. Add 3tbsp of water. Boil it in medium flame
- Once the sugar melt adds lime juice, mix well then reduce the flame.
- Now take the cornflour solution mix again as the corn starch settles down at the bottom.
- Then add the solution to sugar syrup
- As soon as adding mix very well. Continue to mix and stir nonstop on a low flame until the entire mixture thickens
- Once the entire mixture thickens add ghee in two to three parts. Again continue mixing.
- Then cook for 1 to 2 minutes till the mixture gathers in one mass. We can see the ghee releasing from the sides.
- Then pour it into a plate then add roasted cashew and black seas am seed to decorate halwa.

Figure 3.8: Developed halwa 10% variant

3.6 SENSORY EVALUATION OF DEVELOPED PRODUCT

The sensory evaluation was carried out by 10 untrained panel members located in vadanappally, Thrissur. The panel members were provided with samples of diamond cuts, pappad, ladoo and halwa in different variants along with sensory scorecards 9 points hedonic scale to rate the products according to their opinion. They were given instructions regarding how to carry out the assessment.

The 9 point Hedonic scale, also known as degree of liking scale, is the most common Hedonic scale for measuring product liking by customers based on numerical values to the response choices(from 1=dislike extremely to 9=like extremely) (Peryam and

Girardot,1952)Panellist were asked to evaluate the samples for each sensorial parameters which included appearance, colour, taste, texture and overall acceptability based on their degree of liking (1=dislike extremely,2=dislike very much,3=dislike moderately,4=dislike slightly,5=neither like nor dislike,6=like slightly,7=like moderately,8=like very much, and 9=like extremely)

Figure 3.9: Sample for organoleptic evaluation

Figure 3.10: Untrained panel members doing the sensory evaluation and filling the evaluation sheet

RESULT AND DISCUSSION

4. RESULT AND DISCUSSION

The results of the present study entitled “A multifactorial study on mustard microgreens and the development of snacks from mustard microgreens powder” has been presented and discussed under the following heads:

4.1 Multiple harvesting of mustard microgreens

4.2 Growth characteristics of mustard microgreens

4.3 Nutritional parameter of mustard microgreens in each harvest

4.4 Average mean sensory scores of the developed snack- diamond cuts

4.5 Average mean sensory scores of the developed snack- pappad

4.6 Average mean sensory scores of the developed snack- ladoo

4.7 Average mean sensory scores of the developed snack – halwa

4.8 Friedman’s test for overall acceptability of diamond cuts

4.9 Friedman’s test for overall acceptability of Ribbon pappad

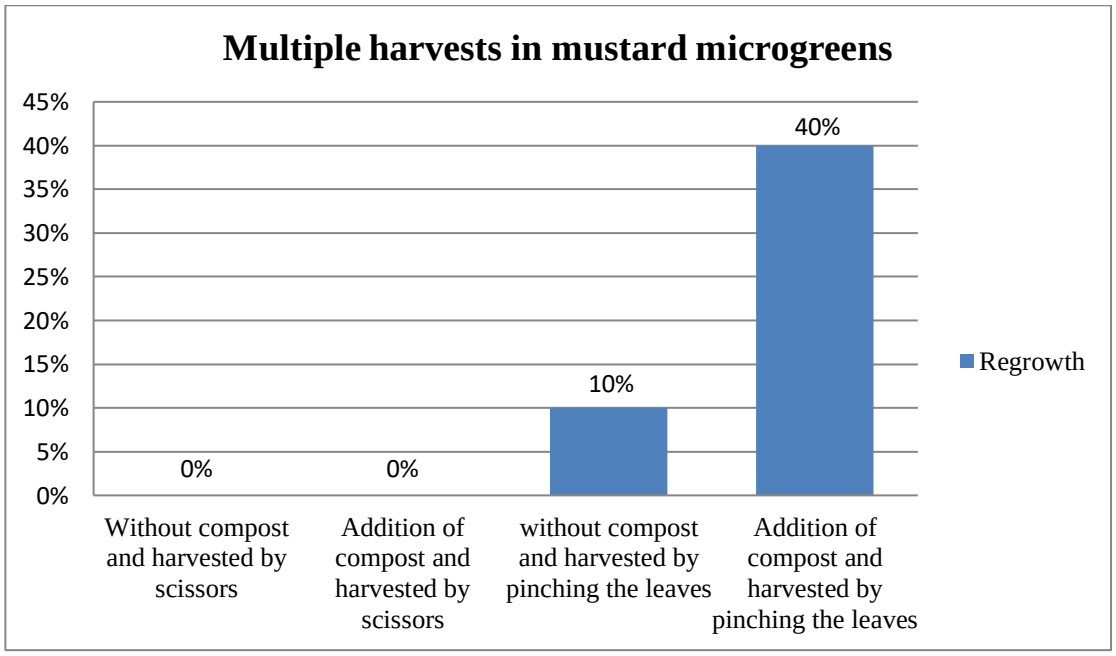
4.10 Friedman’s test for overall acceptability of ladoo

4.11 Friedman’s test for overall acceptability of halwa

Table 4.1: Multiple harvesting of mustard microgreens

S No.	Criteria	Growth Rate In %
1	Without compost and harvested by scissors	0
2	Addition of compost and harvested by scissors	0
3	without compost and harvested by pinching the leaves	10
4	Addition of compost and harvested by pinching the leaves	40

Figure 4.1: Multiple harvests in mustard microgreens



Multiple harvesting of mustard microgreens was analysed under two criteria's, 'without addition of compost' and 'with addition of compost'. This has been elicited in Table: 4.1. It was observed that with the addition of compost, regrowth of mustard microgreens was better as compared to without addition of any compost. It was also observed that the regrowth was better when they were picked by pinching when compared with cutting with the help of scissors.

Seed germination/ regeneration percentage was calculated using the formula GP/GR= g×100/50.

Where 'g' is the number of germinated seeds/regenerated shoots. Number of seeds used for germination is 50. The formula for calculating (GP/RP) was referred from the study on Preliminary Report on Multiple Harvests of Microgreens from Chickpea (Cicer arietinum) Seeds done by E. Sreenivasan, 2020.

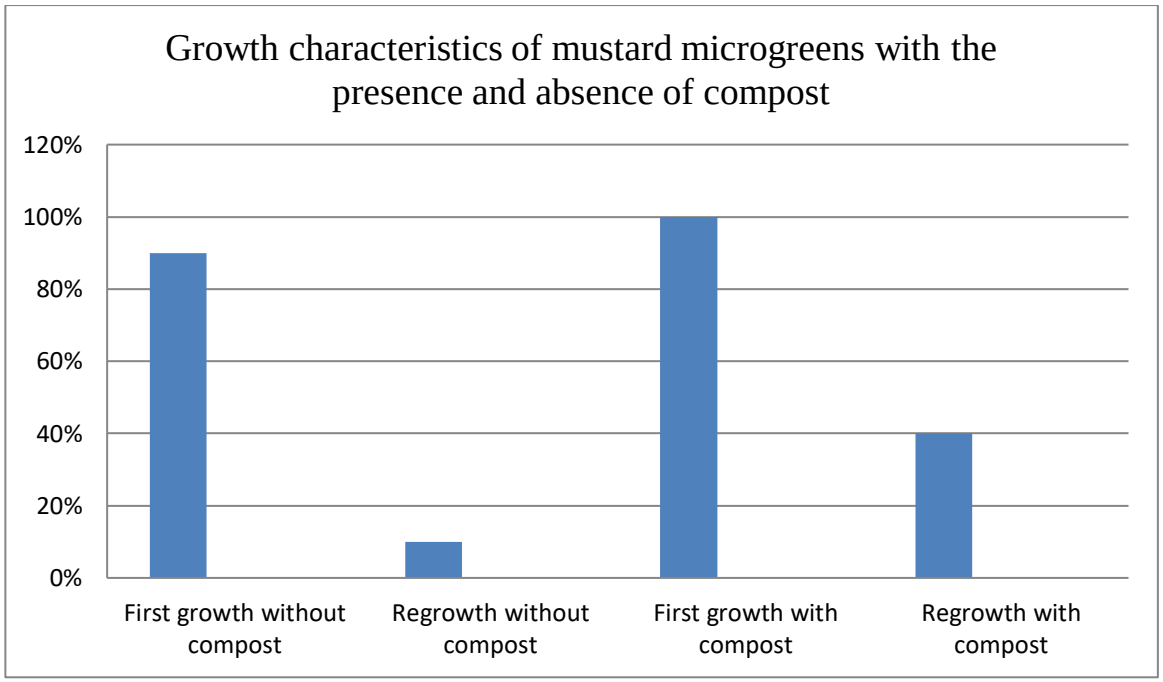
GROWTH CHARACTERISTICS OF MUSTARD MICROGREENS

Growth characteristics of mustard microgreens with the presence and absence of compost was calculated using the formula GP/GR= g×100/50, where 'g' is the number of germinated seeds/regenerated shoots. Number of seeds used for germination is 50. Formula for calculating (GP/RP) was referred from the study on Preliminary Report on Multiple Harvests of Microgreens from Chickpea (Cicer arietinum) Seeds done by E. Sreenivasan, 2020

Table 4.2: Growth characteristics of mustard microgreens with the presence and absence of compost

S No.	Growth Characteristics	Observation
1	First growth without compost	90% (takes 12 days to harvest)
2	Regrowth without compost	10% (takes 15 days to harvest)
3	First growth with compost	96% (takes 9 days to harvest)
4	Regrowth with compost	40% (takes 12 days to harvest)

Figure 4.2: Growth characteristics of mustard microgreens with the presence and absence of compost



The growth characteristics with the presence and absence of compost has been analysed and elicited in Table: 4.2. It was observed that addition of compost (coco peat, eggshell powder) enhances the growth rate, it takes 9 days to harvest, intensify the regrowth. Regrowth rate is higher when pinching the leaves.

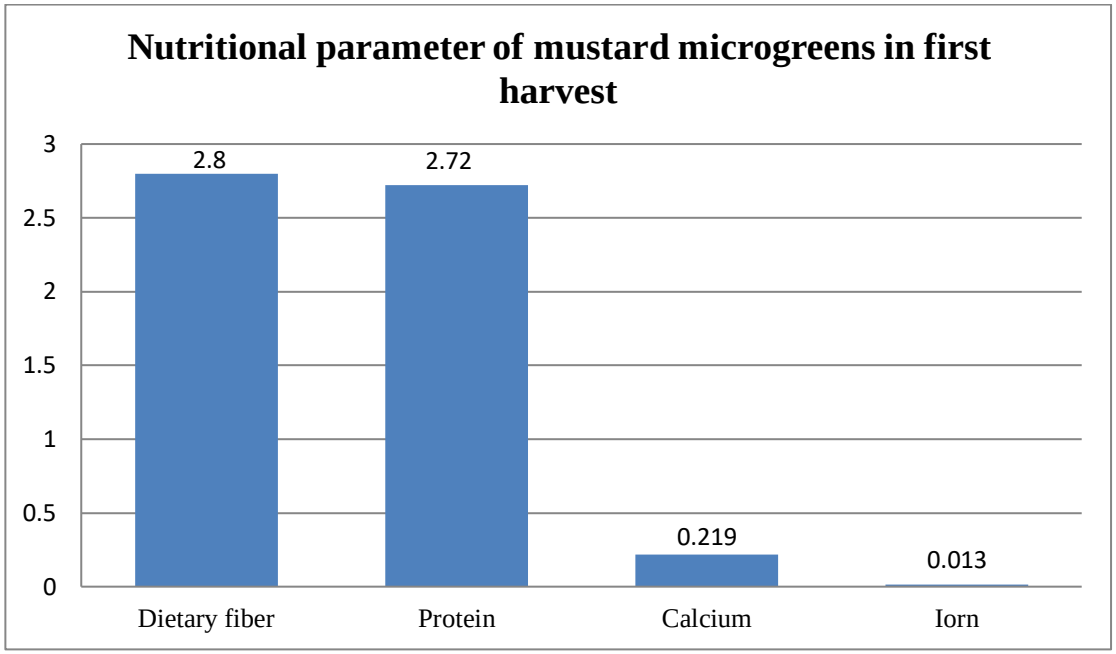
Growth characteristics in the absence of compost were analysed and it was observed that the growth rate was significantly lesser; it took 12 days to harvest. The regrowth was lesser, too as compared to the one with the presence of compost.

NUTRITIONAL PARAMETER OF MUSTARD MICROGREENS IN EACH HARVEST

Table 4.3: Nutrient content of mustard microgreens in first harvest (in 100 g)

S.No	Nutrients	Per 100g
1	Dietary fibre	2.80
2	Protein	2.72
3	Calcium	0.219
4	Iron	0.013

Figure 4.3: Nutritional parameter of mustard microgreens in first harvest

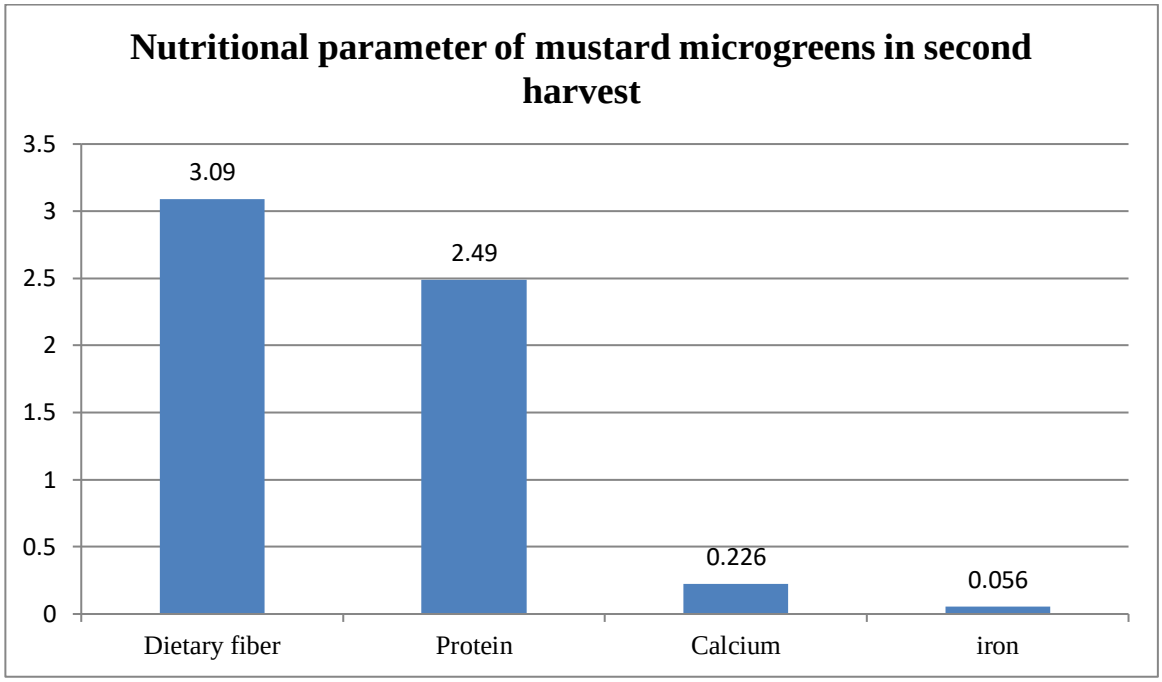


Nutritional parameter of mustard microgreens in first growth was analysed and bring about in the table: 4.3. The dietary fibre content of 100g mustard microgreens is 2.80g, protein 2.72g, calcium 0.219g, iron 0.013g. It is observed that dietary fibre content of mustard microgreens is high, whereas iron content is less.

Table 4.4: Nutrient content of mustard microgreens in second harvest (in 100g)

S.No	Nutrients	per 100g
1	Dietary fibre	3.09
2	Protein	2.49
3	Calcium	0.226
4	Iron	0.056

Figure 4.4: Nutritional parameter of mustard microgreens in second harvest



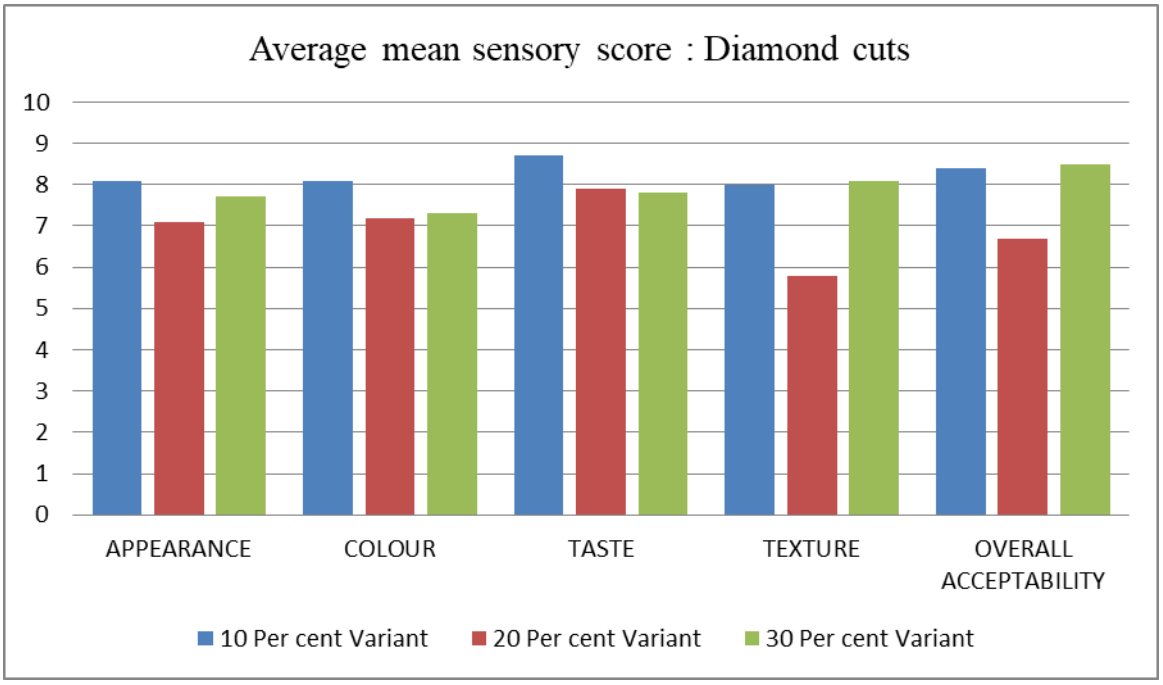
After addition of compost (coco peat and egg shell powder), the nutritional content of mustard microgreens in regrowth was analysed and elicited in the table: 4.4. The dietary fibre content of 100g mustard microgreens was found to be 3.09g, protein 2.49g, calcium 0.226g, iron 0.056g. It was observed that addition of compost enhanced the growth as well as the nutrient content of mustard microgreens. The dietary fibre, iron content is comparatively higher. While protein content is slightly lower, which could because of the lack of Nitrogen in the compost.

Table 4.5: Average mean sensory scores of the developed snack - diamond cuts

Percentage of mustard microgreens powder	Appearance	Colour	Taste	Texture	Overall acceptability
10 %	8.1	8.1	8.7	8	8.4

Variant					
20 % variant	7.1	7.2	7.9	5.8	6.7
30 % variant	7.7	7.3	7.8	8.1	8.5

Figure 4.5: Average mean sensory score - diamond cuts



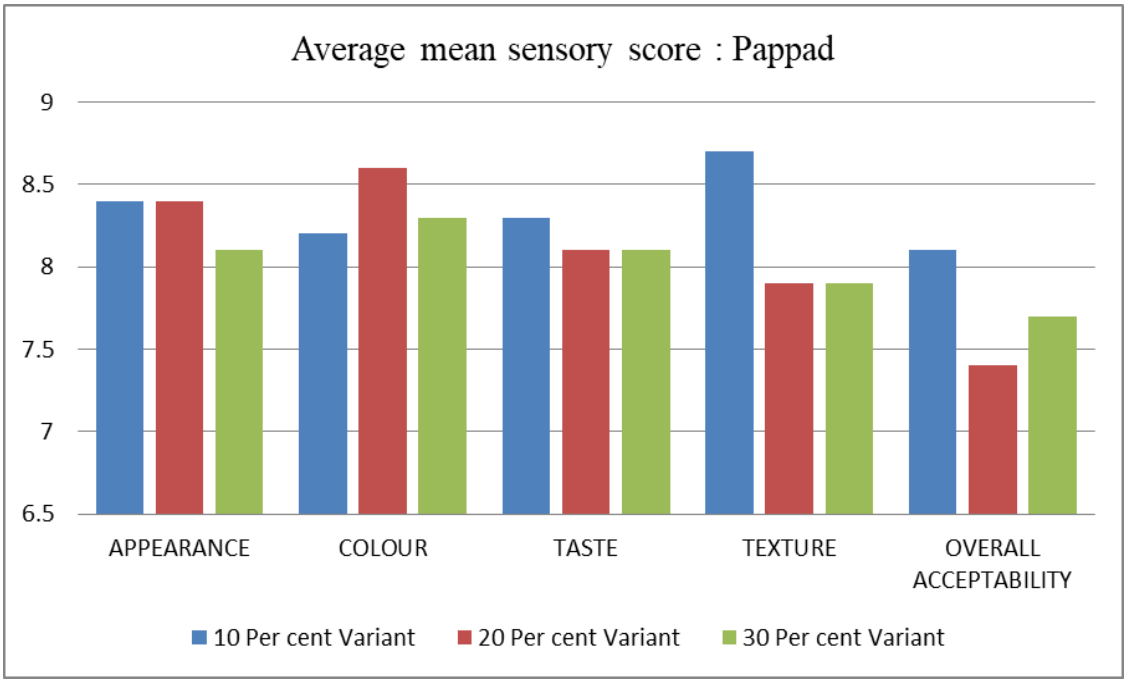
The organoleptic evaluation by 10 untrained panel members is depicted in Table: 4.5along with graphical representation.

Among the 3 samples kept for evaluation, 30% variant of diamond cuts was the most acceptable among the 3 different variations for all the 10 panel members. Therefore, it possessed highest score in organoleptic parameters.

Table 4,6: Average mean sensory scores of the developed snack - pappad

Percentage of mustard microgreens powder	Appearance	Colour	Taste	Texture	Overall acceptability
10 % variant	8.4	8.2	8.3	8.7	8.1
20 % variant	8.4	8.6	8.1	7.9	7.4
30 % variant	8.1	8.3	8.1	7.9	7.7

Figure 4.6: Average mean sensory score - pappad



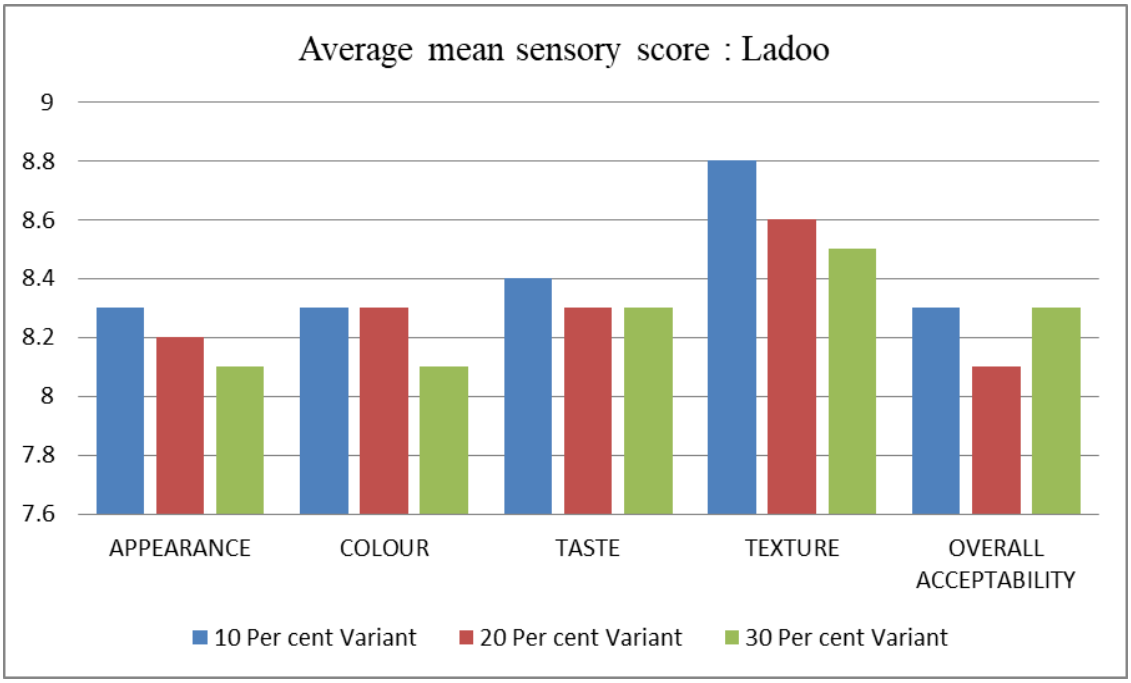
The organoleptic evaluation by 10 untrained panel members is depicted in Table: 4.6 along with graphical representation.

Among the 3 samples kept for evaluation, 10% variant of pappad was the most acceptable among the 3 different variations for all the 10 panel members. Therefore, it possessed highest score in organoleptic parameters.

Table 4.7: Average mean sensory scores of the developed snack - ladoo

Percentage of mustard microgreens powder	Appearance	Colour	Taste	Texture	Overall acceptability
10 % variant	8.3	8.3	8.4	8.3	8.3
20 % variant	8.2	8.3	8.3	8.6	8.1
30 % variant	8.1	8.1	8.3	8.5	8.3

Figure 4.7: Average mean sensory score - ladoo



The organoleptic evaluation by 10 untrained panel members is depicted in Table: 4.7 along with graphical representation.

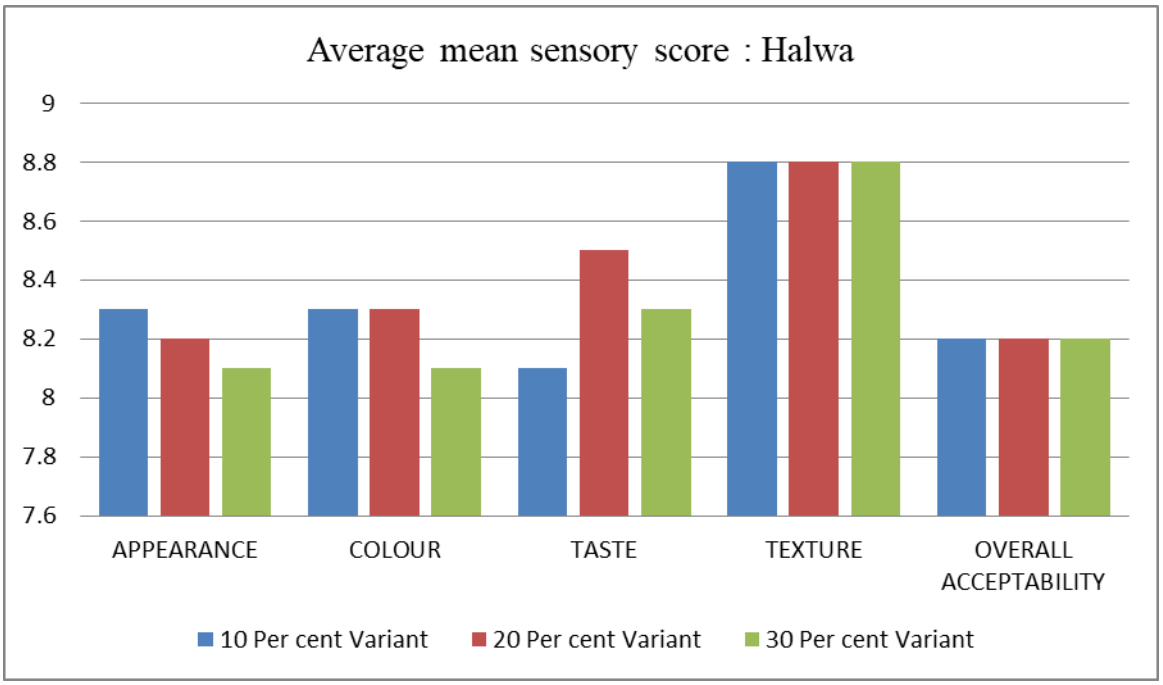
Among the 3 samples kept for evaluation, both 10% variant and 30% variant of ladoo was the most acceptable among the 3 different variations for all the 10 panel members. Therefore it possessed highest score in organoleptic parameters.

Table 4.8: Average mean sensory score of the developed snack - halwa

Percentage of mustard microgreens powder	Appearance	Colour	Taste	Texture	Overall acceptability
10 % variant	7.6	7.8	8.1	8.8	8.2
20 %	8.3	8.4	8.5	8.8	8.2

Variant					
30 % variant	8.1	8.1	8.3	8.8	8.2

Figure 4.8: Average mean sensory score - halwa



The organoleptic evaluation by 10 untrained panel members is depicted in Table: 4.8 along with graphical representation.

Among the 3 samples kept for evaluation, all the variant of halwa was equally acceptable for all the 10 panel members. Therefore it possessed highest score in organoleptic parameters.

Table 4.3: Friedman's test for overall acceptability of diamond cuts

No. of panel	Variation 1	Variation 2	Variation 3

members (N)			
1	9	7	9
2	9	7	9
3	9	7	9
4	9	6	9
5	8	7	9
6	8	7	8
7	8	7	8
8	8	7	8
9	8	6	8
10	8	6	8
Total	24.5	10	25.5

Calculated FM test statistics = 15.05

FM Critical value from table (Friedman's ANOVA by ranks critical value table) = 6.20

The null hypothesis for the test was that the samples differ from each other in their overall acceptability and alternate hypothesis was that the samples do not differ from each other in their overall acceptability. From the above result it was clear that the calculated FM statistics is larger than the FM critical value. So we reject the null hypothesis.

Table 4.10: Friedman's test for overall acceptability of pappad

No. of panel	Variation 1	Variation 2	Variation 3

members (N)			
1	9	9	9
2	8	9	9
3	8	7	7
4	8	7	7
5	8	7	7
6	8	8	8
7	8	7	7
8	8	7	8
9	8	7	7
10	8	6	8
Total	25	16	19

Calculated FM test statistics = 4.2

FM Critical value from table (Friedman's ANOVA by ranks critical value table) = 6.20

The null hypothesis for the test was that the samples differ from each other in their overall acceptability and alternate hypothesis was that the samples do not differ from each other in their overall acceptability. From the above result it was clear that the calculated FM statistics is smaller than the FM critical value. So we accept the null hypothesis.

Table 4.11: Friedman's test for overall acceptability of ladoo

No. of panel members (N)	Variation 1	Variation 2	Variation 3
1	9	9	9
2	9	9	9
3	9	9	9
4	8	8	7
5	8	8	9
6	8	8	9
7	8	8	8
8	8	7	8
9	8	7	7
10	8	8	8
Total	21	18	21

Calculated FM test statistics = 0.6

FM Critical value from table (Friedman's ANOVA by ranks critical value table) = 6.20

The null hypothesis for the test was that the samples differ from each other in their overall acceptability and alternate hypothesis was that the samples do not differ from each other in their overall acceptability. From the above result it was clear that the calculated FM statistics is smaller than the FM critical value. So we accept the null hypothesis.

Table 4.12: Friedman's test for overall acceptability of halwa

No. of panel members (N)	Variation 1	Variation 2	Variation 3
1	9	9	9
2	8	9	9
3	8	9	9
4	8	7	7
5	8	9	9
6	8	9	9
7	8	7	7
8	8	8	8
9	8	8	7
10	8	7	8
Total	20	20	20

Calculated FM test statistics = 0

FM Critical value from table (Friedman's ANOVA by ranks critical value table) = 6.20

The null hypothesis for the test was that the samples differ from each other in their overall acceptability and alternate hypothesis was that the samples do not differ from each other in their overall acceptability. From the above result it was clear that the calculated FM statistics is smaller than the FM critical value. So we accept the null hypothesis.

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

Microgreens are known as the new superfood. Microgreens are immature greens produced from vegetables and herbs, having two fully developed cotyledon leaves. Microgreens are usually harvested 7−14 days after germination.

Multiple harvesting of mustard microgreens was done by pinching the leaves above the nodal portion of the shoot. The study observed that Pinching of leaves and addition of the nutrients enhances multiple harvesting hence nutrient-rich soil is capable of regrowth.

The present study experimented with two kinds of soil; nutrient-rich and normal garden soil. After the first harvest additional compost was added to the soil. The study observed that the addition of compost in both phases enhanced the growth rate.

The study also evaluated the shelf life of dried mustard microgreens powder. The physical analysis of powder was done by sun drying the mustard microgeens and was kept for 3 month in an airtight container. It was placed in a cool dry place. The study observed that there is no characteristic change in colour, odour.

The nutrient content analysis of mustard microgreens in multiple harvests was conducted at Neogen Laboratory located in Tripunithura of Ernakulam district. The test included Dietary fibre by IS 11062-1984 method, Protein by MOA/CH/N method, calcium by AOAC 21st Edn 927.02 method, Iron by AOAC 21st Edn 944.02 method. The study observed that dietary fibre content in mustard microgreens was highest compared to others. From this we can conclude that the nutrient-rich soil also enhances the nutrient content in the mustard microgreens.

Since, the shelf life of microgreens as such is not long, dried and powdered form was used to make the snacks as that it can be stored for a longer period since mustard is commonly available in every kitchen these snacks can be incorporated by everyone regardless of their age. Snacks such as diamond cuts, pappad, ladoo and halwa were developed.

The sensory evaluation was carried out by 10 untrained panel members. Among the 3 samples kept for evaluation, 30% variant of diamond cuts was the most acceptable among the 3 different variations for all the 10 panel members. Therefore it possessed the highest score in organoleptic parameters. Among the 3 samples kept for evaluation, 10% variant of pappad was the most acceptable among the 3 different variations for all the 10 panel members. Therefore it possessed highest score in organoleptic parameters. Among the 3 samples kept for evaluation, both 10% variant and 30% variant of ladoo was equally acceptable among the 3 different variations for all the 10 panel members. Therefore it possessed highest score in organoleptic parameters. Among the 3 samples kept for evaluation, all the variant of halwa was equally acceptable for all the 10 panel members. Therefore the entire three variant possessed highest score in organoleptic parameters.

The reference study was carried out in a soil-less medium for growing microgreens while here we used a soil rich medium is used for growing the microgreens. Nutrient analysis of dietary fiber, protein, calcium and iron in multiple harvests was done in our study, which hadn’t been done in any studies so far for mustard microgreens.

The salient findings were,

Nutritional parameters of mustard microgreens in multiple harvesting are studied. Following was the findings:

- Nutritional parameters in first harvest: Dietary fibres 2.80g, protein 2.72g, calcium 0.219g, iron 0.13g.
- Nutritional parameters in regrowth: Dietary fibres 3.09g, protein 2.49g, calcium 0.226g, iron 0.056g.

Hence, from the study it can be concluded that only nutrient-rich soil is capable of regrowth which was not mentioned in the previous study. Nutrient-rich soil also enhances the nutrient content in the mustard microgreens. Physical analysis of shelflife of dried mustard microgreen powder was checked and there wasn't physical changes of deterioration seen to the powder. Snacks were developed from the mustard microgreens powder to popularise the mustard microgreens greens. These snacks can be incorporated from children to the elderly; it improves the nutritional quality of daily snack.

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LIMITATIONS

- As the quantity of dried mustard microgreens was less than 100g, microbial analysis of dried mustard microgreens could not be done.

RECOMMENDATIONS

- Microbial analysis of mustard microgreen could be studied in the future for a clear picture on the shelf life.
- Phytochemical analysis of mustard microgreens in multiple harvests could be studied.

- Micronutrient analysis of mustard microgreens in multiple harvest can be studied.

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5. BIBLIOGRAPHY

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APPENDIX

APPENDIX-1

ESTIMATION OF PROTEIN

Objective:

To determine the total protein present in mustard microgreens in multiple harvests

Application and limitation:

Applicable to food items. Total protein by Kjeldhal method is defined as the amount of nitrogen experimentally found and multiplied by an appropriate conversion factor

Principle:

The sample is oxidized by the presence of sulphuric acid and nitrogenous compounds are converted into Ammonium sulphate. Mercury is added to the digestion mixture as a catalyst and alkaline sulphate as a boiling elevator. Ammonia is liberated by adding an excess of alkali and is quantitatively distilled into a measured volume of standard alkali.

Apparatus and Reagents:

Kjeldhal Digestion and Distillation assembly ,Balance readable to 0.1 mg, Burette beakers, Sodium hydroxide solution=0.1N-Kjeldhasl nitrogen, Concentrated sulphuric acid 93-98% by mass -Nitrogen free, Mercuric oxide or metallic mercury-Nitrogen free, Sodium sulphate or anhydrous potassium sulphate , Zinc granules ,Sulphuric acid 0.1N standard solution (5gm H_2SO_4 make up to 12 of D/W) ,Methyl red indicator ,Dissolve 250 mg Methyl red in 100ml Ethanol ,Strong solution of Sodium hydroxide(50%),4% Potassium or sodium sulphide

Procedure:

- Weigh accurately 0.7 -2.2 gm of test sample into the Kjeldhal flask, taking precautions to see that particles of the material do not stick on to the neck of the flask.
- Add 0.7gm HgO or 0.65gm metallic Hg.
- Add 15g powdered K2SO4/anhydrous Na2SO4.
- Add 25 ml conc.H2SO4, If test portion >2.2gm is used .Increase H2SO4 by 10 ml for each gm test portions.
- Place flask in inclined position and heat gently until frothing ceases.
- Increase the temperature ,digest for more than 2 hours until the solution clears.
- Cool the contents of the flask –Transfer it quantitatively to the KB flask with Ca 200ml H2SO4, add 25ml of potassium or sodium sulphide solution and mix to precipitated Hg.
- Add few Zn granules to prevent bumping.
- Add layer of Na OH solution without agitation .Immediately connect this Kjeldhal flask with the protein distillation apparatus ,with tip of condenser immersed in standard H2SO4 and 5-7 drops indicator in receiver.
- Mix the contents of the flask by shaking and distill until all the ammonia has passed over in the standard H2SO4.
- Shut off the burner, remove receiver and rinse the condenser thoroughly with water into the receiver .Wash the tip of the condenser into the receiver.
- Titrate excess standard acid in distillate with standard NaOH solution.
- Correct for blank determination on reagents.

Calculations:

Nitrogen=(B-S)×N×0.014×100/W

Where,

B=ml of NaOH used for blank titration

S=ml of NaOH used for sample titration

N=Normality of NaOH

W=Weight of sample in gm

Protein =Nitrogen %× Protein facto

APPENDIX-2

ESTIMATION OF CALCIUM

Objective:

To determine the calcium present in mustad microgreens in multiple harvests

Applications and limitations:

This method is used to find the calcium content of milk, the hardness of water and the amount of calcium and calcium carbonate in various solid materials. It is a very established, reliable and accurate method but it take time to complete as done manually.

Reagents:

NaOH-1 N, Murexide solution (ammonium purpurdate) -0.100mg of murexide with 10g of solid sodium chloride and grinding the mixture to 300-425 microns, Standard EDTA solution =0.01N.

Procedure:

- Weigh accurately 5-10g of the material in previously weighed crucible.
- Dry for 2 hours in an air oven maintained at 105±2° C and ignite the divided material in the dish with the flame of a burner for about 1 hour.
- Complete the ignition by keeping in a muffle furnace at 600±20°C until grey ash results.
- Cool in a desiccator and weigh.

- Repeat the process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighing is less than 1mg.
- This ash is then dissolved in minimum amount of concentrated HCL and made up to 100ml using distilled water.
- Take 50 ml of this sample. Add 1N NaOH solution, a volume sufficient to produce a PH of 12-13.
- Add 2-3 drops of the murexide indicator. Titrate against EDTA with continues stirring.
- The end point is indicated by colour change from pink to purple.

Calculation

Calcium= (Titrate value*normality of EDTA*40.08*1000*dilution)/volume taken for test.

APPENDIX: 3

ESTIMATION OF IRON

Objective:

To determine the Iron present in the mustard microgreens in multiple harvests.

Applications and limitations:

This method is applicable to determine Iron by dry ashing and Flame Atomic Absorption Spectrometry.

Apparatus and reagents:

Air oven, Muffle furnace, Desiccator, Spectrophotometer, Concentrated HCL, Hydroxylamine hydrochloride, Ammonium acetate buffer, 1, 10 Phenanthrolin solutions.

Procedure:

- Weigh accurately 5-10g of the material in previously weighed crucible.
- Dry for 2 hours in an air oven maintained at 105±2° C and ignite the divided material in the dish with the flame of a burner for about 1 hour.
- Complete the ignition by keeping in a muffle furnace at 600±20°C until grey ash results.
- Cool in a desiccator and weigh.
- Repeat the process of heating for 30 minutes ,cooling and weighing till the difference in mass between two successive weighing is less than 1mg.

- This ash is then dissolved in minimum amount of concentrated HCL and make up to 100ml using distilled water.
- To 100ml of this sample and 50ml of blank, add 1ml hydroxylamine hydrochloride and add 0.5ml con .HCL.
- Then boil the contents to almost 20ml the volume for dissolution of all ions.
- Cool to room temperature. Add 10ml of ammonium acetate buffer and 10ml of 1,10 Phenanthrolin solution and mix well.
- Dilute to 100ml and place each standard solution and food solution into a separate cuvette.
- Measure and record the absorbance of each solution at 510nm using spectrophotometer.
- Prepare a standard curve (Beer's Law) of the standard concentrations vs. absorbance.

Calculation:

The amount of Iron present in the sample can be calculated from the standard graph in mg/dl

APPENDIX- 4

ESTIMATION OF DIETARY FIBER

OBJECTIVE:

To determine the Dietary fiber present in mustard microgreens.

APPLICATION:

This method determines soluble, insoluble and total dietary fiber content in processed foods and raw materials, such as cereal products, fruits and vegetables.

APPARATUS:

Dispenser, Fritted crucible, Gooch, fritted disk, Pyrex® 50 mL ,. Filtering flask, Rubber ring adaptors for use on filtering flasks,Vacuum source,Water bath,Balance, Ovens, Timer, Desiccator, pH meter,Dispensers, Cylinder, Magnetic stirrers and stirring bars,16. Rubber spatulas, Muffle furnace.

REAGENTS:

- Phosphate buffer, 0.08 M, pH 6.0. Dissolve 1.400 g disodium phosphate anhydrate (Na_2HPO_4) (or 1.753 g dihydrate) and 9.68 g disodium phosphate monobasic monohydrate (NaH_2PO_4) (or 10.94 g dihydrate) in approx. 700 mL distilled water. Dilute to 1 L with water. Check pH with pH meter.
- Sodium hydroxide solution, 0.275 N. Dissolve 11.00 g ACS grade NaOH in approx. 700 mL distilled water, using appropriate handling precautions, in 1 L volumetric flask. Cool and dilute to volume with water.

- Hydrochloric acid solution, 0.325 N. Dilute stock solution of known titer (i.e. 325 mL of 1.0 N HCl) to 1 L with water in volumetric flask.

PROCEDURE

- Weigh duplicate 1 g samples, accurate to 0.1 mg, into 400 mL tall-form beakers. Sample weights should differ by less than 20 mg from each other. Add 50 mL phosphate buffer (pH 6.0) to each beaker and check pH with pH meter. Adjust if pH does not equal 6.0±0.1.
- Add 50 µL heat-stable α-amylase solution.
- Cover beaker with aluminium foil and place in boiling water bath. Beaker must be incubated at 98-100°C for 15 min. Shake gently at 5 min intervals. Note: Increase incubation time when number of beakers in bath makes it difficult for beaker contents to reach internal temperature of 98-100°C. Use thermometer to indicate that 15 min at 98-100°C is attained. Total of 30 min in boiling water bath should be sufficient.
- Cool solutions to room temperature.
- Adjust to pH 7.5±0.1 by adding 10 mL 0.275 N NaOH solution. Check pH with pH meter.
- Add 100 µL of protease solution.
- Cover beaker with aluminium foil and incubate at 60°C with continuous agitation for 30 min.
- 8. Cool and add 10 mL 0.325 N HCl solution to adjust pH to 4.5±0.2. Check pH with pH meter.

- Add 200 µL amyloglucosidase, cover with aluminium foil, and incubate for 30 min at 60°C with continuous agitation.
- Add 280 mL 95% EtOH pre-heated to 60°C (measure volume before heating). Let precipitate form at room temperature for 60 min.
- Weigh crucible containing Celite to nearest 0.1 mg, then wet and distribute bed of Celite in crucible by using stream of 78% EtOH from wash bottle.
- Apply suction to draw Celite onto fritted glass as even mat. Maintain suction and quantitatively transfer precipitate from enzyme digest to crucible.
- Wash residue successively with three 20 mL portions of 78% EtOH, two 10 mL portions of 95% EtOH, and two 10 mL portions of acetone. In some cases, gums may form during filtration, trapping liquid in residue. If so, break surface film with spatula to improve filtration. Long filtration times can be avoided by careful intermittent suction throughout filtration.
- Dry crucible containing residue overnight in 70°C vacuum oven or 105°C air oven.
- Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weights to determine weight of residue.
- Analyse residue from one sample of set of duplicates for protein by using N x 6.25 as conversion factor.
- Incinerate second residue sample of duplicate for 5 h at 525°C. Cool in desiccator and weigh to 0.1 mg. Subtract crucible and Celite weights to determine ash.

$$\text{Dietary Fiber (\%)} = \frac{\frac{R_1 + R_2}{2} - p - A - B}{\frac{m_1 + m_2}{2}} \times 100$$

Where,

R1 = residue weight 1 from m1; R2 = residue weight 2 from m2 m1 = sample weight 1; m2 = sample weight 2 A = ash weight from R1; p = protein weight from R2 and

$$B = \text{blank} = \frac{BR_1 + BR_2}{2} - BP - BA$$

Where

BR = blank residue;

BP = blank protein from BR1

BA = blank ash from BR2.

APPENDIX-5

SENSORY EVALUATION FORM

Name: **Date:**

Product:

Please rate the product

9 point hedonic score rating card

Sample no.	Appearance	Colour	Taste	Texture	Overall acceptability
1					
2					
3					

Please evaluate the sample by ticking on any of the numbers according to your perception.

9 =Like extremely

8 =Like very much

7 =Like moderately

6 =Like slightly

5 =neither like nor dislike

4=Dislike slightly

3=Dislike moderately

2=Dislike very much

1=Dislike extremely

Suggestions: **Signature**

APPENDIX-6

NUTRITIVE VALUE OF MUSTARD MICROGREEN-CALCULATION METHOD

CALCULATION METHOD

1. Protein in 100gm = X

There total protein = X/100 x n

= n gm protein

2. Dietary fiber in 100g =X

Therefore total fiber =X/100×n

=n gm fiber

3. Calcium in 100g =X

Therefore total calcium =X/100×n81

=n gm of calcium

4. Iron in 100g =X

Therefore total iron =X/100×n

=n gm of iron

APPENDIX- 7

FRIEDMAN'S ANOVA BY RANK CRITICAL VALUE TABLE

k=3

N	α <.10	α ≤.05	α <.01
3	6.00	6.00	—
4	6.00	6.50	8.00
5	5.20	6.40	8.40
6	5.33	7.00	9.00
7	5.43	7.14	8.86
8	5.25	6.25	9.00
9	5.56	6.22	8.67
10	5.00	6.20	9.60
11	4.91	6.54	8.91
12	5.17	6.17	8.67

13	4.77	6.00	9.39
∞	4.61	5.99	9.21
k=4			
N	α <.10	α ≤.05	α <.01
2	6.00	6.00	—
3	6.60	7.40	8.60
4	6.30	7.80	9.60
5	6.36	7.80	9.96
6	6.40	7.60	10.00
7	6.26	7.80	10.37
8	6.30	7.50	10.35
∞	6.25	7.82	11.34

k=4			
N	α <.10	α ≤.05	α <.01
3	7.47	8.53	10.13
4	7.60	8.80	11.00
5	7.68	8.96	11.52
∞	7.78	9.49	13.28