

**DEVELOPMENT OF VALUE ADDED PRODUCTS
USING BRAHMI (*BACOPA MONNIERI*)**

By

**Santhiya priya C.B
(14PFD010)**

**Thesis Submitted to the
Avinashilingam Institute for Home Science and Higher Education for Women
Coimbatore – 641 043**

**In partial fulfillment of the requirement for the Degree of
Master of Science in Food Service Management and Dietetics**

April, 2016

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Certified as Bonafide Research Work

P. Arden Malani
Signature of the Guide
29/4/2016

P. Rasaramani
Signature of the
Head of the Department

Acknowledgement

ACKNOWLEDGEMENT

First and foremost the investigator expresses her deep sense of gratitude to **God almighty** for showering his blessings on her to carry out her work successfully.

The investigator expresses her heartfelt thanks and deep sense of gratitude to **Padmashri Dr.P.R. Krishna Kumar**, Chancellor, Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore, for providing the infrastructural facilities for the smooth conduct of the study.

The investigator expresses her reverential gratitude to **Dr. T.S.K. Meenakshi Sundaram, M.A.,M.Phil.,Ph.D.**, Former Chancellor, Avinashilingam Institute for Home science and Higher Education for Women, and Managing Trustee, Sri Avinashilingam Education Trust Institutions, Coimbatore, for providing the opportunity to conduct the research in this esteemed university.

The investigator owes her special thanks and gratitude to **Dr.(Tmt).Premavathy Vijayan, M.Sc., M.Ed., Dip.Spl.Edn., M.Phil., Ph.D, (Avinashilingam)**, Vice Chancellor (I/C), Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore, for the amenities provided for the successful completion of the study.

The investigator owes her special thanks and gratitude to **Dr. (Tmt) Sheela Ramachandran, M.Sc., P.G.Dip.Ed., Ph.D (Avinashilingam)**, Former Vice Chancellor, Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore, for all possible help for the smooth conduct of the study.

The investigator records her sincere gratitude to **Dr. (Tmt) Venmathi,M.Sc., Dip.,Ed, M.Phil., Ph.D.**, Registrar, Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore, for providing all the facilities to carry out the study.

The investigator owes her heartfelt thanks and deep dense of gratitude to **Dr.(Mrs.) Vasugi Raja M.Sc., M.Phil., Ph.D.**, Dean, Faculty of Home science, Avinashilingam

Institute for Home science and Higher Education for Women, Coimbatore, for her kind support and encouragement for the conduct of the study.

The investigator expresses her special thanks and sincere gratitude to **Dr.(Tmt) G.Vasanthamani M.Sc., M.Phil., Ph.D.**, Head, Department of Food service management and dietetics, Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore, for her keen interest, valuable help, concern and encouragement which has helped in the successful completion of the study.

The researcher is deeply debted in and it gives her an immense pleasure and proudness to offer profound gratitude to her guide **Dr. (Mrs) PL.Sridevi Sivakami M.Sc., M.Phil., Ph.D.**, Assistant Professor (SS), Department of Food service management and dietetics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, whose stimulating support, constructive suggestions, inspiring guidance and encouragement helped her in all the times of research and documenting the thesis.

The investigator owes her sincere thanks to all the **Staff Members** of the Department of Food service management and dietetics Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for being supportive and understanding in completion of the study.

The investigator is deeply thankful to the cooperation of the participants throughout the study.

No words are sufficient to express her deep sense of gratitude to her beloved and respected **Parents and Friends** for their affection, care, blessing and co-operation in all walks of her life.

I am grateful to each and every soul who had helped me in one or other way in making this study a great succes

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Introduction

I.INTRODUCTION

Health is a fundamental human right, recognized in the universal declaration of Human Rights. It is a universally accepted fact that good health plays an important role in human development. The constitution of WHO (1946) states that good health is a state of complete physical, social and mental well-being and not merely the absence of disease or infirmity.

Food is edible or potable substance consisting of nourishing and nutritive components such as carbohydrates, fats, proteins, essential mineral and vitamins which (when ingested and assimilated through digestion) sustains life, generates energy and provides growth, maintenance and health.

Food is the major source for serving the nutritional needs, but with growing modernization some traditional ways are being given up. Affluence of working population with changing lifestyles and reducing affordability of sick care, in terms of time and money involved, are some of the forces that are presently driving people towards thinking about their wellness. There has been increased global interest in traditional medicine. Efforts to monitor and regulate traditional herbal medicine are underway. The traditional systems of India, which are now called Indian System of Medicine (ISM), have a very strong conceptual base and have been practiced uninterruptedly for a very long time; hence they are considered as independent medical systems. Ayurveda, Siddha and Unani are the three important traditional systems practiced in India (Mishra, 2004)

Ayurveda the traditional Indian medicine, remains the most ancient yet living traditions. Derived from its ancient Sanskrit roots – “ayus” (life) and ved (knowledge), Ayurveda is the oldest surviving complete medical system in the world. The literal meaning of Ayurveda is “Science of Life” because it offers a complete system to live healthy life. The pharmacological treatment of disease began long ago with the use of herbs. According to WHO ayurvedic health is defined as “ Health is a state where in the Tridosha, digestive, fire, all the body tissues and components, all the physiological processes are in perfect unison and the soul, the sense organs and mind are in a state of total satisfaction (prasanna) & content”. Ayurveda is quite popular, being practiced throughout the country including tribal and remote areas where other modes of therapies are not readily available.

In India, about 70 percent of rural population depends on the traditional Ayurvedic system of medicine most healers/practitioners of the traditional system of medicine prepare formulation for their own recipes and dispense to the patients (Ballabh and Chaurasia, 2007).

Early humans recognized their dependence on nature for a healthy life and since that time humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of ailments. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or inactive, and also which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well defined herbal pharmacopeias (Solecki, 1975).

Indeed, well into the twentieth century, much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. The knowledge of plant-based drugs developed gradually and was passed on, thus, laying the foundation for many systems of traditional medicine all over the world. In some communities herbal medicine is still a central part of their medical system (Ahamad *et al.*, 2010)

Medicinal plants are widely distributed throughout the world but most abundantly in tropical countries. It was estimated that about 25 percent of all modern medicines are directly or indirectly derived from higher plants (WHO, 2005). Thus, herbal medicine has led to the discovery of a number of new drugs and non-drug substances (Pandey *et al.*, 2008).

Medicinal plants based traditional system of medicines are playing important role in providing health care to large section of population, especially in developing countries. India is the largest producer of medicinal plants. In India around 25,000 effective plant based formulations are used in traditional and folk medicine (Pandey *et al.*, 2008).

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Barnes *et al.*, 2007). Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value.

They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers (Sameet *et al.*, 2014)

Bacopa monnieri (Brahmi) is a nootropic medicinal plant, belonging to family Scrophulariaceae, is a creeping annual herb grows upto 1500m in altitude, perennial ground cover to 10cm height with sprawling stems that may root as they grow along the soil. Fleshly, oval shaped leaves 1-2cm long, form opposite along the stems. Small, tubular, white flowers with 5 petals develop in leaf terminals. This plant is found in wet, damp and marshy areas of tropical regions. *Bacopa monnieri* has been an important constituent of the “Ayurvedic Material Medica” which is the main revitalizing herb for the nerves and the brain cells. (Aguira and Borowski 2013)

Bacopa monnieri is a well known booster in a Indian herb commonly given to infants where it was observed to boost memory power intelligence and mental health. Studies revealed that bacosides which are the major phytoconstituents in *Bacopa monnieri*, help to repair damaged neurons by enhancing proteins involved in the regeneration of neural cell nerve impulses (Gohil and patel 2009)

The maximum bacoside yield was obtained during August to October (monsoon period) which also coincides with the favourable period for plant growth and would be suitable for plant harvest. Amongst the various individual plant parts, the highest content was recorded in the leaves followed by stem and least in the roots (Sharma *et al.*, 2013)

Bacopa monnieri can be viewed as a neural nourished, restoring depleted synaptic activity and leading to enhance memory functions (Jatwa *et al.*, 2014). *Bacopa monnieri* herb contains saponins as bacoside A and B; sapogenins- bacogenins A1 – A4, betulic acid and alkaloids as principal constituents. The alkaloid are brahmine, herpestine, hydrocotyline, dihydrocotyline and centoloides along with 3 bases like B-1-oxalate, B-2-acetate and B-3-chloroplantinate. The saponins are bacoside-A, bacoside – B, brahmoside, asiaticoside, monnierin, hersopin and made cacoside. Beside these α -amirin, three sugars, two glucose, rhyrnose, arabinose, arabinsol, bacogenins, δ -mannitol, β -sitosterol, stigmasterol, tannins along with acid as brahmic acid, isobrahmic acid, betulic acid, centoic acids. Two common flavonoids, luteolin and apigenin are present in *bacopa monnieri* (Avanigadda and Vangalapati 2011)

The herb is reported to have an extensive range of pharmacological properties Viz. Antioxidant, anti-inflammatory, cardiogenic and cognitive enhancing which are attributed due to the presence of triterpenoid, saponins and bacosides. The herb *Bacopa monnieri* has a specific pungent flavour and bitter taste (Al-snafi, 2013)

Bacopa monnieri in traditional medicine used as brain tonic to enhance memory development, learning, concentration and to provide relief patients with anxiety and also used to treat conditions such as fever, inflammation, pain, asthma, epilepsy and memory decline (Bammidi *et al.*, 2011)

Bacopa strengthens adrenal process that facilitate carbohydrate metabolism. It increases circulation in the brain and decreases senility and ageing. It fortifies the immune system both cleansing and feeding it. At the same time, it is a powerful blood purifier and is specified for chronic skin diseases, including leprosy and syphilis, as well as eczema and psoriasis and it also accelerates wound healing (Anju, 2011)

Bacopa monnieri extract may increase memory to formation by the enzyme Tryptophan Hydroxylase (TPH2) and increasing the expression of the serotonin transporter (SERT) (Charles *et al.*, 2011)

Brahmi (*Bacopa monnieri*) is used in several formulation and preparation of Ayurvedic medicine such as Brahmic rasayana, Brahmic prasha, Brahmi ghrita, Brahmi taila, Brahmi Ghana vati and many other proprietary preparations (Rameshwari *et al.*, 2013)

Bacopa monnieri is also known as neither lower nor epinephrine and increases the 5 – hydroxytryptamine level in hypothalamus and cerebral cortex. The higher doses of *Bacopa monnieri* extracts produced significantly greater anxiolytic effect compared to lorazepam a standard benzodiazepine (Shinomol *et al.*, 2011)

The role of diet rich in bioactive plant based phytochemicals as an adjunct therapy is gaining importance with the awareness on the role of such natural compounds. Many phytochemicals have been found to possess antioxidant property and additional functions such as preventing carcinogenesis (Lui, 2003)

Plant foods contain thousands of natural chemicals. They are called phytonutrients or phytochemicals. Phytonutrients can be defined as nutrients that have been scientifically

proven to provide health benefits. “Phyto” in greek means plants and phytonutrients fall into their own category because they are not related to fats, carbohydrates, proteins, vitamins or minerals (Tiwari *et al.*, 2013)

The regular consumption of polyphenols has been associated with a decreased risk of a number of degenerative diseases including cancer, cardiovascular diseases and neurodegenerative disorders. Rather than exerting direct antioxidants effects the mechanism by which polyphenols express these beneficial properties appear to involve their interaction with cellular signalling pathways and related machinery that mediate cell function under both normal and pathological conditions(Vanzour *et al.*, 2010)

Flavonoids are an important class of polyphenols which are widely distributed in plant foods as individual compounds or bound to saccharides as glycosides. In addition to their antioxidant properties, flavonoids have been reported to exhibit other multiple biological effects like antiviral, antibacterial, anti- inflammatory, vasodilatory, anti cancer and anti ischemic effects (Prochazkova *et al.*, 2011)

New evidence based science linking diet to disease and disease prevention has created an unprecedented opportunity to address public health issues through diet and lifestyle. Widespread interest in selected foods that might promote health resulted in the use of the term “functional foods”. But the term functional food conveys health benefits that extend far beyond mere survival. Food and nutrition science has moved from identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease (Fady Ntanios, 2001)

The institute of Medicine’s Food and Nutrition board (IOM/NAS, 1994) defined functional foods as “any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains”

All foods are functional to some extent because all foods provide taste, aroma and nutritive value. Functional foods are products that have been enriched with added nutrients or other substances that are considered to have positive influence on health. Functional food concept was first developed in Japan in the 1980s (Brown, 2014)

Functional foods can be considered to be those whole, fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients (eg. Vitamins and minerals). For normal maintenance, growth and development or provide

other biologically active components that impart health benefits or desirable physiological effects. Functional foods represent one of the most intensively investigated and promoted areas in the food and nutrition science today. It must be emphasized that these foods and ingredients are not magic bullets or panaceas for poor health habits. (Clare, 2002)

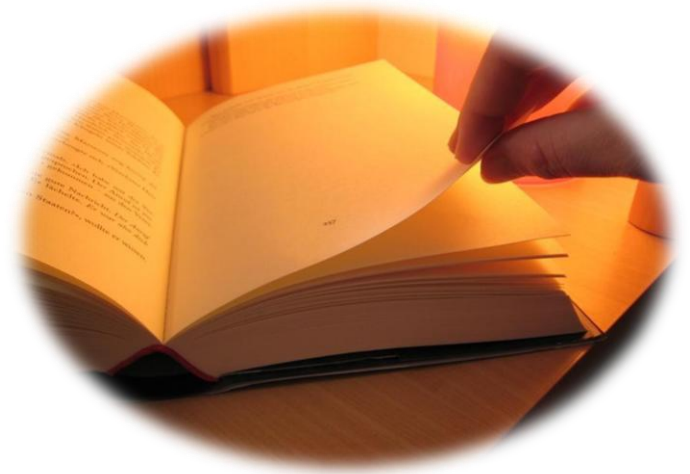
Functional foods includes a wide variety of foods and food components believed to improve overall health and well being, reduce the risk of specific diseases or to minimise the effects of other health concerns (cencic and chingwaru 2010)

Diet is only one aspect of a comprehensive lifestyle approach to good health, which could include regular exercise, maintenance of healthy body weight and other positive health practices. Only when all of these issues are addressed functional foods can become part of an effective strategy to maximize health and reduce disease risk. (Olga *et al.*,2009)

As a result of the change in physical state or the manner in which the agricultural commodity or product is produced and segregated, the customer base for the commodity or product is expanded and a greater portion of revenue derived from the marketing, processing or physical segregation is made available to the producer of the commodity or product (Huffman 2004)

Thus keeping all these perspectives the present study was conducted for development and organoleptic evaluation of value added products made by incorporating shade dried *Bacopa monnieri* powder in different proportions and shelf life of developed products was studied with the following objectives

1. To analyse nutrients and phytonutrients present *Bacopa monnieri* powder.
2. Formulation of value added products using *Bacopa monnieri* in different Indian recipes.
3. Standardization and acceptability of formulated products using organoleptic evaluation.
4. To analyse the nutrient content of the formulated value added product
5. To study the shelf life of developed value added products.



Review of Literature

II REVIEW OF LITERATURE

The literature pertaining to the study field “**Development of Value Added Products Using Brahmi (*Bacopa monnieri*)**” is reviewed under following headings:

- A. Medicinal properties of *bacopa monnieri*
- B. Chemical composition of *bacopa monnieri*
- C. Development of value added products
- D. Current trends in consumption of value added products

A. Medicinal properties of *bacopa monnieri*

Dhawan *et al.*, (1996) found that *Bacopa monnieri* rich in Pharmacological activities. An isolated plant extracts bacosides have been extensively investigated in several laboratories for their neuropharmacological effects, the active principles apart from the facilitating learning and memory in normal rats inhibited the amnesic effects of scopolamine, electroshock and immobilization stress. Plant also shown to enhance protein kinase activity in the hippocampus which could also contribute to its nootropic action.

Kishore *et al.*, (2005) found that bacosides facilitates anterograde memory and alternate anterograde experimental amnesia induced by scopolamine and sodium nitrite possibly by improving the acetylcholine level and hypoxic conditions, respectively. In addition bacosides also reversed BN52021 (a platelet – activating factor receptor antagonist) induced retrograde amnesia. Probably due to increase in platelet activating factor synthesis by enhancing cerebral glutamate level.

Ryan *et al.*, (2008) examined memory deficits following cholinergic blockage by scopolamine were reversed by Bacopa treatment. Bacopa improved memory functioning in cognitively intact cohorts, with improving working memory.

Limpeanchob *et al.*, (2008) demonstrated that Brahmi extracts protected neurons from the betaamyloid induced cell death, but not glutamate induced exotoxicity. This

neuro protection was possibly, due to its ability to suppress cellular acetyl – cholinesterase activity but not the inhibition of glutamate mediated toxicity

Morgan *et al.*, (2010) carried out a clinical trial to assess the effect of 12 – weeks administration of Bacopa monniera (300mg/day) on memory performance in people over the age of 55 years Bacopa significantly improved memory acquisition and retention in older persons.

Rao *et al.*, (2000) reported that fresh bacopa monnieri juice possess significance antiulcerogenic activity.

Elangovan *et al.*,(1995) concluded that plant extract of Bacopa monniera possess anti – cancer activity as it inhibited Sarcoma – 180 cell growth in vitro.

Goel *et al.*, (2003) reported that standardised Bacopa monnieri extracts in the dose of 1000mg/ml showed anti Helicobacter pylori activity in vitro.

Russo and Berrelli (2005) concluded that antioxidant properties of Bacopa monniera were widely recognised and have been discussed in various histological (in vitro) that the bacosides and extract improve system's defences against oxidation stress by decreasing the formation of free radical accumulation in the brain.

Cook and Samman (1996) study found that extract of Bacopa monniera was found to scavenge the free radicals such as peroxides, Superoxide's and hydroxyl radicals.

Deepak (2003) reported that antioxidant has the effect to prevent oxidative damage by free radicals.

Kikusaki and Nakataui (1993) studied free radicals contribute to more than one hundred disorders in humans including Atherosclerosis, Hypertension, Arthritis Ischemia, Gastritis, Cancer, Alzheimer's disease, Parkinsonism, Diabetics Mellitus and AIDS.

Monic *et al.*,(2012) reported extract of Bacosides have shown an antioxidant activity.

Sumathy *et al.*, (2002) found extract of Bacopa monnieri has shown to possess a significant protective effect against morphine induced liver and kidney functions in terms of serum, glutamate pyruvate transaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase activities and urea uric acid respectively

Tripathy *et al.*, (1996) research concluded that pre-treatment with bacoside A prevents the elevation of LPO (Lipid Peroxidase) and activity of serum marker enzymes and maintains the antioxidant system.

Kapoor *et al.*, (2008) research found that the *Bacopa monniera* extract exhibits interesting antioxidant properties, expressed by its capacity to scavenge superoxide anion and hydroxyl radical and to reduce H₂O₂ induced cytotoxicity and DNA damage in human fibroblast cells.

Dhanasekaran *et al.*, (2007) carried out a clinical study in patients with epilepsy, examined that bacopa was reported useful in improving the symptoms and occurrence of epileptic seizures.

B. Chemical Composition of *Bacopa monnieri*

Volluri *et al.*, (2011) concluded *Bacopa monniera* also referred to as *Bacopa monniera*, Herpestis, monniera water hyssop, and “Brahmi” has been used in the Ayurvedic system of medicine for centuries. The Whole plant methanolic extracts was investigated for any in vitro antioxidants properties and total phenolic content. Further, the antioxidant activity of the plant was studied using three in vitro models. The Nitric oxide scavenging activity, DPPH, free radical scavenging assay and reducing power activity was also concentration dependent with IC₅₀ value being $455.78 \pm 1.03 \mu\text{g/ml}$, $104.82 \pm 1.96 \mu\text{g/ml}$, and $96.13 \pm 0.86 \mu\text{g/ml}$, respectively. Total phenolic content was found out to be $27.76 \pm 1.87 \text{mg/gm GAE}$.

Prasad *et al.*, (2012) assessed the total phenolic content of aqueous extract of plant material of *Bacopa monnieri* belonging to family scropulariaceae. Folin ciocalteu reagent according to singleton and Rossi using gallic acid as a standard and vitro antioxidant activity determined according to the method of oyaizu, hydrogen peroxide scavenging method. Moreover *Bacopa monnieri* plant materials showed strong reducing powder and significant antioxidant activity. The total phenolic content measured by Folin ciocalteu was found to be 58mg GAE/g in hydrogen peroxide scavenging method the IC 50 value was found to be $254.70 \mu\text{g/ml}$.

Srinath *et al.*, (2014) concluded that the active constituents responsible for *Bacopa monniera*'s cognitive effects are bacosides A and B. The triterpenoid saponins and their bacosides are responsible for brahmi's ability to enhance nerve impulse transmission.

Sharma *et al.*, (2013) conducted a study to evaluate bacoside (expressed as sum of bacoside A3 and A2) production on monthly basis in net house grown plants of *Bacopa monnieri* (Accession BM001) collected from Jammu region. Bacoside content different during the course of the whole year. The whole herb is used commercially for extracting the memory enhancing molecule bacosides from the plant. The study significantly points out that the maximum bacosides yield was obtained during August to October (Monsoon period) which also coincides with the favourable period for plant growth and would be suitable for plant harvest. Among the various individual plant parts, the highest content was recorded in the leaves ($6.06\text{mg/g}^{-1}\text{DW}$) followed by stem ($5.13\text{mg/g}^{-1}\text{DW}$) and least in the roots ($3.19\text{mg g}^{-1}\text{DW}$)

Charoenphon *et al.*, (2015) concluded that the extract of *Bacopa Monnieri* revealed the presence of tannins, flavonoids glycosides, terpenoids saponins, bacosides, bacopasides bacopasaponins and steroids. The major chemical constituents isolated and characterized from *Bacopa Monnieri* are dammaranes of triterpenoid saponins with pseudojujubogenin glycosides or jujubogenin glycosides.

C.Development of value added products

Value – added products are defined by USDA as having: A change in the physical state or form of the product (such as milling wheat into flour or making strawberries into jam). The production of a product in a manner that enhances its value (Such as originally produced products).

Sharma and Joshi (2014) concluded that due to changing life style of the people and desire for more leisure time, there is considerable change in food habits with a strong demand for processed Ready – to Eat (RTE) and Ready – to – Cook (RTC) food products. This trend has resulted in huge demand in Indian Markets for use of convenience food.

Rao *et al.*, (2011) conducted a study on preparation of instant tomato pickle mix (ITPM) by blending optimized levels of tomato powder (TP) and other spice ingredients. The TP and ITPM were assessed for their storage stability. Total polyphenol content increased (1026 to 1608 mg/100mg) and lycopene content decreased (14.01 to 5.2

mg/100g) in samples packed in PE pouches during storage for six months. Sensory of reconstituted instant tomato pickle mix packed in MPE pouches with cooked rice scored very good (8.1) after six months of storage.

Sucharitha *et al.*, (2012) investigated on standardization of ber-pineapple jam. Three different proportions were carried out with different- ratios of ber to pineapple pulp. All the samples were subjected to sensory evaluation to determine their acceptability, using 5 point hedonic scale rating method. Sample III recorded highest score for overall acceptability. The data revealed that 370g of pulp was obtained from 510 of whole ber fruits.

Sindumathi and Amutha (2014) made an attempt to utilize the residual coconut pulp left in the tender coconuts after removed of coconut water. The coconut pulp was mixed with pineapple pulp and guava pulp in different preparations to increase the acceptability of the jam. The prepared coconut jam packed in plastic container and glass bottles and had the shelf life of 180 days at room and refrigerated temperatures. The results showed good sensory acceptability after 6 months of storage in glass bottles and plastic containers at room and refrigerated conditions on the basis of physicochemical and sensory attributes, coconut jam possess high nutritive value, safe and fit for consumption.

Mala *et al.*, (2014) conducted a study on novel pumpkin seed Chikki (PSC) and Pumpkin chocolate Chikki (PCC) with enhanced nutritional quality by incorporating flax seed, oats and peanuts was studied. The ingredients of the chikki were pumpkin seed (25%). The seed (8.0%) Oats (8.0%), Peanuts (9.0%) and jaggery (50%). The fatty acid composition of the total lipid should that chikki were rich in oleic and linoleic acids with unsaturated fatty acids constituting 65 and 63% of in PSC and PCC, respectively. The overall sensory quality determined on 9 point Hedonic scale indicated that PCC was the preferred one, with sensory score of 8.0 during three months storage at RT.

Gadhiya *et al.*, (2015) aimed at incorporating probiotics into chocolate and related products; the ways to enhance or sustain their viability in the presence of stressed surroundings throughout the manufacturing process, and their market potential with future prospects. A number of techniques have been developed to protect the probiotics from environmental stress in food matrices, processing and storage and GI tract passage. Among these, microencapsulation has been found to be, the most suitable and accessible technology to protect the tiny living organisms.

Jaiswal *et al.*, (2014) studied the physical properties of jamun fruits in order to facilitate its handling and processing. The work was intended to add value to fruits in jam production. The processed jam were compared with other commercial fruit jams in terms of proximate composition, total phenolic content, antioxidant activity and viscosity to test its overall quality parameters. According to the experimental findings, the proximate composition of jamun was found to be equivalent with other jams and was the richest source of total phenol with 5.58mg GAE/g. Antioxidant activity also highest. Viscosity of jamun jam was found to be acceptable with other commercial variety jams. Results revealed essential information that could promote the commercialization of jamun jam.

Khatoon *et al.*, (2011) carried out a study to analyse the chemical composition of dehydrated curry leaves and to prepare different products by the incorporation of these leaves. Organoleptic and nutritive values of the prepared products were analyzed. Nutritional composition was calculated using the food composition table. It was observed that calcium, iron, β -carotene content in prepared products increased significantly as the incorporation levels increased. Thus dehydrated curry leaves can be incorporated in the preparation of different food products to improve their nutrient contents.

Gupta *et al.*, (2015) studied the acceptability and analysed the micronutrient rich food products prepared from the micronutrient rich leaf mixture (*Spinacia Oleracea*), Fenugreek leaves (*Trigonella foenum graecum*), dill leaves (*Anethum Graveolens*) and coarse grains pearl millet, (*Pennisetum glaucum*) maize (*Zea mays*) Chickpea (*Cicer arietinum*). The products paratha and khichdi were prepared by incorporation of micronutrient rich indigenous foods and seemed as treatments T₁, T₂ and T₃ respectively. Organoleptic evaluation was carried out using the nine point hedonic scale, it was observed that T₁ was found to be best with regards to flavour and taste and overall acceptability, it is concluded that indigenous foods can be incorporated in the corporation of different food products to improve their sensory acceptability.

Sukhwal and Vyas (2015) conducted a study on cereal and pulse based mixes and were developed named as mix A,B,C the analysis of nutrients and antioxidants was done by using the standardised techniques (AOAC,2005). The total antioxidant activity was determined by using DPPH method. Result of the nutritional and antioxidant components highlighted that mix C therefore utilization of prepared mixes help in the overall well being of the society.

Kodandaram reddy and Waghay (2013) concluded that the papads are cereals or legume based preserved products, which are consumed after deep fat frying. Legume based papads are prepared using black gram, mint leaves at different levels (10%, 15%, 23%, 25%, 30%). All the chemical constituents like moisture content, ash content, PH, alkalinity, acid insoluble ash, fat, carotene content and crude fibre were evaluated and the values of all the levels of mint leaves incorporated papads were found to be reversible. The fried papads were subjected to sensory evaluation and all the samples subjected for sensory evaluation were accepted equally by the panellists flavour, minerals, vitamins and fibre contents of mint leaves increase.

Chauhan and Intelli (2014). attempted a study to utilize (Cauliflower greens) to develop low cost fibre rich products for people suffering from micronutrient deficiency and to assess the sensory quality of developed products. The fresh collected cauliflower green leaves were washed and sundried for 5-7 days to dry them. Three recipes (Pancake, Dhokla and Idli) were supplemented with 2g and 5g DCGLP per serving and sensory evaluation was done with the help of 9 point hedonic rating scale Biochemical analysis of DCGLP revealed moisture 3.4 percent, protein 21.6% crude fibre 10.23gm and iron 62gm / values as per 100gm). The prepared recipes were found to be acceptable at 2g incorporation of DCGLP. DCGLP, due to its high iron content can be used as supplement to make low cost iron rich recipes.

Gupta *et al.*, (2014) aimed to formulate and analyze the value added products prepared from the underutilized fresh Indian Sorrel leaves value added products were analysed for proximate constituents mineral and vitamin C. Results showed that the products were enriched with fresh Indian sorrel leaves are rich in protein, energy, carbohydrate, iron, calcium and vitamin C. Moisture content was higher in lemon drink, iron and calcium content was higher in peanut chutney, Fat, fibre, Protein, energy carbohydrate and vitamin C content was more in idli. Therefore it can be concluded that the incorporation of the leaves of Indian Sorrel in various food items can improve the nutritional qualities.

Vijayarani *et al.*, (2012) concluded the sensory analysis of the developed value added extruded product using spirulina showed that 5% spirulina incorporated product got the maximum mean score compared to 10% and 15% due intensification of colour and odour. Extruded products prepared by incorporating spirulina and whey water had

significant difference in its nutritional composition when compared with the control. The developed extruded products were subjected to microbial analysis for fungi and bacteria for one month and found to be safe.

Joshi and Mathur (2010) made an attempt to analyse the nutritional potential and acceptability of leaf mixtures (LM) prepared from the less utilized leaves of beat root (*Beta Vulgaris*), Carrot (*Daucus Carota*), Cauliflower (*Brassica oleracea*) and turnip (*Brassica rapa*). The leaf mixture was prepared by mixing the powders of above mentioned greens in a definite ratio (1:2:1:1). The LM was analysed for the proximate composition, mineral composition (Ca, P, Fe, Cu, Zn, Mn, and Mg) and antinutritional composition (oxalate and Phenols). Twenty different recipes with different levels (0, 5, 10, 15 and 20%) of LM incorporation were prepared and were assessed for quality on the basis of sensory attributes. The products were well accepted to the level of 10% protein, iron and calcium content were significantly ($P < 0.05$) higher in the LM incorporated recipes and the increase was directly proportional to the level of leaf mixture incorporated.

Mishra *et al.*, (2016) developed biscuits using creamery method and evaluated for physical properties, proximate composition, sensory characteristics and storability for 90 days at room temperature protein content in biscuits with makhana powder was higher than the biscuits prepared using potato powder in flour blends. Overall acceptability of biscuits made with makhans powder received the maximum scores.

Sunil *et al.*, (2016) assessed oil seed cakes being rich in proteins, antioxidants fibres, vitamins and minerals have been considered ideal for food supplementation. Rice bran pellets (RBP) stabilized rice bran (SRB). Coconut cake (CC) and sesame cake (SC) were taken up for the study. The sensory evaluation of cooked residue and extract showed overall higher acceptability by the panellists than the starting cakes and raw materials. The result can be concluded that these cooked residue and extract products are highly valuable for food supplementation than the raw ones.

Rawat and Darappa (2015) assessed the effect of substitution of brown flour (BF) with fibre rich ingredient mixture, FRIM (banana flour, Psyllium husk, Particularly defatted Coconut flour and oats) and proteins which ingredients mixture, PRIM (Chickpea flour, sesame, soya protein isolate and why protein concentrate) at the levels of 25, 50 and 75% on the rheological, nutritional and quality characteristics of baked energy bars (BEB) were studied. The overall quality score of BEB increased only up to the substitution of 50% of

BF with FRIM or PRIM. The BEB with 50% FRIM and PRIM remained chemically stable during storage up to 3 months and showed 9 times increase in dietary fibre content and about 2 times increase in protein content respectively.

Khan *et al.*, (2015) assessed the effect of spinach powder on the physico – chemical, rheological, nutritional and sensory characteristics on chapatti premixes was studied by incorporating spinach powder at different concentration form 1% to 10% based on wheat flour. Effect of incorporating of spinach powder on the alveo – consistographic, mixographic and pasting characteristics were studied. Addition of 5% of spinach powder to wheat flour was found to be optimum chapatti preparation.

Rajiv *et al.*, (2016) studied blackgram (*Phaseolus Mungo*) was roller milled into semolina (BGS) and was substituted at 25, 50 & 75% levels in vermicelli. There was an increase in ash and protein content as the inclusion of BGS in blends increased. The results concluded that 50% BGS was considered optimum in vermicelli.

Faion *et al.*, (2016) studied and evaluated the effect on the addition of dried extract from mate leaves and mesophilic culture on the chemical, microbiological and sensory characteristics of Prato cheese. The sensory evaluation showed that only the formulation added by adjunct culture and 0.2wt% of mate extract presented lower values for the attributed global acceptance, textures and flavour, compared to the control. The formulations added of mate leaves extract presented residual bitter flavour after 45 days of storage.

D.Usage of value added products in recent trends

Crassiva and Sudha (2015) carried out a study on wheat flour replacing it with mango ginger powder (MGP) at 0,5,10 & 15 % influence of MGP on rheological and baking characteristics was studied. Dietary fibre, minerals, polyphenols and antioxidants activity were determined using standard methods the results indicated that the soup stick showed further improvement in terms of texture and breaking strength with the addition of gluten powder, Potassium bromated and glycerol monostearate. The total dietary fibre and antioxidant activity of the soup sticks having 10% MGP increased from 3.31 to 8.64 and 26.83 to 48.06% respectively as compared to the control soup sticks MGP in soup sticks improved the nutritional profile.

Miglani *et al.*, (2016) conducted a study to develop baked and extracted functional foods for metabolic syndrome (MS) specific designed ingredient mixed with optimum amino acid make up using key foods ingredients with functional properties such as whole cereals, legumes, skimmed milk powder, along with flax seeds and fenugreek seeds. Three products namely baked, extruded snack and noodles were found to contain higher lysine, crude protein, ash and fibre and low carbohydrates when compare with control and whole wheat flour products.

Ahmed *et al.*, (2016) studied that the incorporation of green tea powder GTP in wheat flour at different level and its effect on quality parameters of flour and cookies. The addition of GTP to wheat flour increased the DPPH (2,2- diphenyl -1- picrylhydrazyl) percent inhibition and reducing power of cookies. Further, it also increases the acceptability for colour, aroma and taste.

Roy and Guha (2016) attempted to develop a novel cup cake by incorporating essential oil of betel leaf. All parameters of the developed cake were compared with different cup cakes available in the market for ascertaining consumer acceptability of newly developed product in terms of quality and market price. Results revealed that the novel cup cake developed with 0.005% (N/n) essential oil of betel leaf occupied the 1st place among the four developed novel cup cakes. Manufacturing of novel cup cake with essential oil of betel leaf would be a profitable and self sustaining entrepreneurship.

Fradinho *et al.*, (2016) carried out a experiential study to develop an enriched functional biscuit with Psyllium fibre and understand the impact of the new ingredient on physiochemical and sensorial properties of the dough and biscuits. The optimum biscuits formulation was determined using the response surface methodology (RSM). An optimum formulation was obtained by incorporating 6% psyllium and 50% flour. The biscuit has the enough amount of psyllium soluble fibre recommended by Food and Drug Administration (FDA) to prevent the risk of coronary heart disease.

Pasko *et al.*, (2016) suitability assessment of amaranth sprouts as a new functional food was carried out. The optimisation of sprouting process and the influence of selenium supplementation, in doses 10, 15, & 30 mg of selenium as sodium selenite, on amaranth growth and fatty acid profile were examined. The studies proved amaranth sprouts to be potentially attractive as functional food. The experiments revealed neither antibacterial or antifungal properties of sprouts.

Marques *et al.*, (2016) studies using acerola seed flours (ASF) and acerola base flours (ABF) to develop cereal bars (CB) in different combinations with brown oats. The sensory results concluded that CB4 and CB5 can be considered as product with enhanced nutritional value, containing iron with a low energetic value and high levels of dietary fibre besides being enriched with antioxidants.

Menon *et al.*, (2016) study elucidates the effect of utilizing Cereal- Pulse-Fruit seed composite flour in the development and quality analysis of learned bread. The composite flour was prepared using refined wheat flour (WF), high protein. Soy flour (SF), sprouted Mung bean flour (MF) and Mango Kernel flour (MKF). The study highlighted the nutrient enrichment of bread on incorporation of potential waste material mango kernel soy and sprouted legume.

Patel *et al.*, (2016) studied the effect of ragi (finger millet) to improve the nutritional value of ice cream by enhancing the iron and fibre content. Ragi, ice cream was prepared using 4 different flavours viz. Vanilla, Mango, chocolate and caramel. Chocolate flavoured ragi ice cream was suggested as best followed by mango, caramel and vanilla ice cream. In corporation of finger millet in ice cream resulted in reduction in the amount of stabilizer used and effectively functional as fat replacer in ice cream.

Bisharat *et al.*, (2015) studied the Phenolic content, antioxidant activity and sensory characteristics of vegetables – enriched extrudates. Phenolic content and antioxidant activity of broccoli enriched extrudates increased with extrusion temperature and broccoli addition and decreased with feed moisture content. The antioxidant activity of olive paste extrudates increased with natural ratio and decreased with feed moisture content and screw rotation.

Rasane *et al.*,(2015) carried out a study on shelf life model based on storage temperatures and developed for a nutriceal based fermented baby food formulation. The formulated baby food samples were packaged and stored at 10, 25, 37 and 45 °C for a test storage period of 180 days. A shelf life study was conducted using consumer and semi-trained panels, along with chemical analysis (moisture and acidity). Weibull hazard analysis was used to determine the shelf life of the product based on sensory evaluation. Considering 25 and 50 % rejection probability, the shelf life of the baby food formulation was predicted to be 98 and 322 days, 84 and 271 days, 71 and 221 days and 58 and 171 days for the samples stored at 10, 25, 37 and 45 °C, respectively.

Agrahar *et al.*, (2015) carried a study on composite flours (CF) using cereals, legumes, millets, soy-protein isolate, dairy ingredient and fruit without refined flour used for preparing multi-nutrient biscuits. Dough and biscuits were evaluated for physical, nutritional and textural properties, particle size, colour and sensory evaluation and compared against refined-flour biscuits (C). Effect of malting and sprouting on biscuit quality were also analyzed. The hardness, stickiness and cohesiveness values of CF doughs were significantly ($p \leq 0.05$) lower than C resulting in lower cutting strength and increased hardness of CF biscuits. Sprouting further decreased hardness of CF dough. Nutrient content of CF biscuits (sprouted and unsprouted) were significantly ($p \leq 0.05$) higher than C biscuits. Sensory evaluation showed CF biscuits especially with sprouted flour had higher acceptability and were superior to C.

Verma *et al.*, (2015) that millets have the potential to contribute to food security and nutrition, but still these are underutilized crops. The present study was undertaken with a view to analysed the physico-chemical, functional and nutritional composition of foxtail millet, barnyard millet and rice and to compare the sensory quality and nutritive value of food products from foxtail and barnyard millet with rice. The protein, fat and fibre content of the formulated products from foxtail and barnyard millet were higher than the rice products. Thus from all the parameters analysed in the present study concluded that the foxtail millet and barnyard millet are superior in nutritive value when compared rice and have potential for use in traditional food products

Kaur and das (2015), conducted a study on Barley flaxseed based functional dry soup mix (BFSM) developed from whole barely flour (46.296%), roasted flaxseed powder (23.148%) and the seasoning (30.555%) comprising several flavoring compounds and anticaking agent, using simple processing technique. Different extracts of BFSM revealed the presence of total phenols (0.57–1.86 mg gallic acid equivalent/g, sb) with antioxidants equivalence of DPPH (20.69–39.07%) and FRAP (120–331 $\mu\text{m Fe (II)}$ /g, sb).

Pilipezuk *et al.*, (2015), concluded that Coffee being the beverage consumed worldwide is also a very competitive commodity. The major bioactive components - chlorogenic acids (CAs) - detected by HPLC-DAD-MS included caffeoyl-, feruloyland dicaffeoylquinic acids. Roasting declined CAs content by 50 %, while caffeine content increased by about 30 % during this process. In roasted coffee brews studied, CAs content varied from 14.1 to 26.6 mg/g; smaller variations were seen in antioxidant potential (determined by spectrophotometric and cyclic voltammetry assays). Profiles generated by HPLC with post-column on-line antioxidant detection revealed that caffeoylquinic acids

were the most abundant antioxidants in samples studied. The highest antioxidative potential exhibited coffee enriched with green coffee extract confirming the soundness of such an approach to obtain healthier products.

Choudhury *et al.*, (2015) concluded that Bamboo shoot has attracted significant research and a commercial interest due to its many health promoting bioactive compounds as well as its effectiveness in decreasing blood pressure, cholesterol and increasing appetite. Bamboo shoot powder (BSP) was added in 0 % (control), 5, 10, and 15 % level in dry ingredients by replacing wheat flour and other ingredients were kept constant. Dough prepared are firstly analysed for basic characteristics. Then biscuits were prepared and analyzed for moisture, water activity, protein, fibre, fat, ash, phenolics, antioxidant activity, dimension, hardness, colour and sensory acceptability. Results showed that up to 10 % fortification level the biscuits were acceptable with improved functional and nutraceutical properties compared to the control.

Ortega and beltran (2015), suggested that the need to add value to cultivated and native plant products that have medicinal and nutritional properties is increasing due to the consumers demand. Dried Roselle calyces (*Hibiscus sabdariffa* L.) are commercially available and appreciated to obtain concentrated extracts which might be used in the food and pharmaceutical industries. It has been shown that ingestion of infusions of Roselle may help to reduce chronic diseases such as diabetes mellitus, dyslipidemia, and hypertension. This could be due to the activity of some compounds, mainly flavonoids and anthocyanins, found as natural antioxidants in Roselle extracts.

Devisetti *et al.*, (2016) studied the effect of alkali pre-treatment on the nutritional, anti-nutritional and functional properties of moringa (*Moringa oleifera*) leaf flour (MLF), and sensory assessment of MLF-based snack product . The HPLC analysis of phenolics revealed that chlorogenic and gallic acids were the predominant phenolic acids present in the raw leaf flour, whereas p-coumaric, caffeic and gallic acids were the major phenolic acids in the pre-treated leaf flour. Flavonoids such as catechin, kaempferol, rutin and luteolin were present in both MLFs and the prepared snack. Overall sensory quality indicated that the snacks had acceptable textural attributes and improved nutritional profile at the 20 % level of substitution. It is possible to develop a ready-to-eat convenience food product with good functional and nutritional properties using pre-treated moringa leaf.

Mridhula *et al.*, (2009) studied that the fortified bengal gram sattu was prepared following standardized sattu making procedure and fortified as per FDA using wheat flour with thiamin, riboflavin, niacin, Ca and Fe and stored at 25°C, 65% RH and 35°C, 65% RH and at ambient condition (16–39°C, 18–98% RH) in low density polyethylene and laminated aluminum foil pouches. Alcoholic acidity increased by 0.04% in different sattu samples during 180 days storage but was within the acceptable limit as per BIS standard. Storage temperatures and packaging materials did not affect the overall quality of fortified sattu except moisture content and total microbial load during 6 months storage. Fortified Bengal gram sattu, both fresh and stored, were within the acceptable sensory quality when used in the drink form.

Gupta and nagar (2009) conducted a study on the effect of cooking, fermentation, defueling and the use of utensils on flavonoids (quercitin and pelargonidin) – antioxidants present in pearl millet (*Pennisetum typhoideum*) rabadi, along with proximate composition and sensory acceptability of the product were studied. Study revealed an increased ash and flavonoids and reduced crude fibre after cooking in all samples, while fermentation enhanced the crude protein and reduced fat and crude fibre after 16 h in fermented-cooked-fermented rabadi prepared in steel and earthen pot and cooked-fermented rabadi in earthen pot. Sensory evaluation showed the acceptance of all samples in the range of liked extremely (fermented-cooked-fermented-steel pot) to liked slightly (fermented-cooked in earthen pot).

Kulkarni *et al.*, (2009) conducted a study that date palm (*Phoenix dactylifera*) were crushed and treated with 0.1% pectinase enzyme for 120 min to obtain maximum juice. Date juice was found to be rich in reducing sugars (16.1%) and total sugars (18.3%). Juice was pasteurized at 85°C to inactivate the enzyme, cooled and centrifuged at 3000 rpm to get clear juice. Date juice concentrate was stable during 6 months storage could be reconstituted for preparing ready-to-serve beverages with acceptable sensory quality.

Schumacher *et al.*, (2009) concluded that Quinoa (*Chenopodium quinoa* Willd) is a good source of vitamin E containing high quality protein. A dark chocolate with the addition of 12, 16 or 20% quinoa was developed. Cysteine, tyrosine and methionine increased by 104, 72, 70%, respectively in chocolate containing 20% quinoa. The amino acid pattern was as per WHO standards, which was adequate to human needs. The chocolate with quinoa was approved by 92% of the sensory panel. All the samples showed an index of

acceptance above 70%. Quinoa could be used at the levels evaluated in this study adding its potential health benefit to the dark chocolate.

Joshi and Sharma (2009) carried out a study on different combinations of fermented vegetables like carrot, radish and cucumber with pear and mango pulps were made separately and were processed. All the sauces prepared were having a constant TSS of 18°B using different combinations of fermented pulp viz., 25, 50, 75 and 100% with fruit pulps of mango and pear. Fermented carrot based sauce having blend of 75% fermented carrot + 25% pear was adjudged sensory as the best. The cost of production of fermented vegetable based sauces (200 ml bottle) ranged between Rs.15.54 and 24.14 and the lowest (Rs 15.54) cost was for radish based fermented sauce.

Mallik and Kulkarni (2009) studied that concentrated panner whey was utilized in place of water in the production of rusks. The studies indicated that panner whey concentrated to 30% total solids can be used without adversely affecting the sensory attributes.. The modified process required additional sugar incorporation to maintain the sweetness to that of market samples. The rusks prepared by the modified process had 2.5% moisture, 12.1% fat, 9.4% total protein, 1.9% ash and 74.1% total carbohydrate. Rusks with and without whey concentrate stored at 37°C for 9 days remained in good condition without significant variation in sensory attributes.

Dadasaheb *et al.*, (2010) suggested that appetiser in a need based product for defence forces deployed at high altitudes. Ready to eat appetisers in the form of munches i.e. pepper munch and lemon munch were developed by using response surface methodology and central composite rotatable design with active ingredients as variables and quality parameters such as acidity, sugars and sensory acceptability score as responses. The products had 8.7–9.8% fat, 6.5–7.9% protein and 72–73% carbohydrates supplying about 80 Kcals/20 g munch. The appetisers packed in metalized polyester pouches had a shelf-life of 9 months at room temperature (18–33 °C) as well as at 37 °C.

Ashish *et al.*, (2010) studied that a low fat mango ice cream (2.4% milk fat) was prepared in a mechanized 'ice and salt' type freezer using powdered sago at 2.5% as a natural bulking agent along with sodium alginate at 0.025% as adjunct. The low fat mango ice cream was compared with control mango ice cream having 10% milk fat and 0.15% sodium alginate as stabilizer. Both control as well as experimental ice creams contained 20% mango pulp solids. Incorporation of 2.5% powdered sago and 0.2% Cream Plus as

flavour adjunct is recommended in the manufacture of 'low-fat' mango ice cream. The energy values for control and dietetic mango ice cream was 202.8 and 142.9 kcal/100 g, respectively, which represents about 30% reduction in calorie.

Purohit and Rajyalakshmi (2010) concluded that defatted groundnut cake obtained from commercial oil processing units and prepared in laboratory oil expeller (LOE) were analyzed for quality parameters. Defatted groundnut cake flour (DGCF) was incorporated at 15–100% levels in laddoo, chutney powder, fryums (deep fried crisp and crunchy item), biscuits, noodles and extruded snacks. The products were studied for sensory, physico-chemical and shelf-life quality. Protein, fat and ash contents were 51.6 ± 0.06 , 4.5 ± 0.05 and $4.2\pm 0.11\%$, respectively. Shelf life studies indicated the maximum storability for 90 days for all the products except laddoo, which could be stored for 30 days at ambient temperature ($28\pm 2^\circ\text{C}$). All the products were acceptable and the order of preference for the products as indicated by adults and varied.

Pandey and Singh (2010) carried out a study on the possibility of using full fat soy flour (FFSF) for replacer for whole milk powder (WMP), stevia-mannitol blend as replacer for sugar and soybean oil (SBO) as replacer for cocoa butter in chocolate manufacture without impairing the sensory quality characteristics of chocolate was explored. Data on the sensory evaluation of WMP, sugar and cocoa butter substituted chocolates revealed that 40% (w/w) of WMP, sugar and cocoa butter can be successfully substituted by FFSF, stevia-mannitol blend and SBO, respectively in the preparation of high protein and low sugar chocolate without impairing the sensory attributes. The optimized chocolate was found acceptable (score ≥ 7.0) after 90 days of storage at $16\pm 1^\circ\text{C}$ and $\sim 65\%$ RH.

Koc *et al.*, (2010) studied that pasteurized liquid whole egg was subjected to spray drying to determine the effect of spray drying conditions on moisture content, water activity, peroxide value, emulsion stability, gel texture, foaming stability and colour change of the powder product. Drying process was carried out in a pilot scale spray dryer (Mobile Minor Niro-Atomizer, Denmark). Optimum spray drying conditions of whole egg powder were determined according to the specific end product requirements (bakery foods, omelette and mayonnaise and salad dressing) targeting to obtain the desired value of functional properties, i.e.; emulsion stability, gel texture, foaming stability and colour change.

Chakraborty *et al.*, (2010) conducted a study on five blends of refined wheat flour (RWF) (63.2 – 96.8, %RWF) and millet were used to manufacture biscuits baked for varying time (3.3–6.7 min) and temperature (166.6 – 183.4 °C). The manufactured biscuits were evaluated in terms of textural attributes (crispness, hardness and cutting strength) and overall acceptability (OAA). Results showed that increasing the amount of RWF in biscuits decreased ($p < 0.01$) hardness. The predicted responses in terms of crispness, hardness, cutting strength and OAA were 45, 0.3N, 27.2N and 8.9, respectively. The desirability of the optimum conditions was 0.98.

Chetana and sunkireddy (2010) made an attempt to achieve correct ratio of n-3 to n-6 fatty acids for health benefits, flaxseeds (*Linum usitatissimum*), a good source of n-3 fatty acids, were incorporated with optimized 20% replacement of peanuts in the formulation for the preparation of chikki. Addition of flaxseeds to chikki increased PUFA content, especially n-3 fatty acids, up to 9%, which were not present in chikki prepared only with peanuts. Thus the ratio of 18:2 to 18:3 increased with addition of flaxseed, which has significant health benefits.

Methodology

III. METHODOLOGY

The methodology pertaining to the study on the **Development of Value Added Products Using Brahmi (*Bacopa Monnieri*)** is presented under the following headings:

- A. Selection of sample
- B. Chemical analysis of *Bacopa monnieri*
- C. Development of value added products using *Bacopa monnieri*
- D. Organoleptic evaluation of the developed value added products
- E. Computation of nutrient content of developed value added products
- F. Study the shelf life of the developed value added products

A. Selection of sample

The fresh whole *Bacopa monnieri* plant was collected from the Malapuram district of Kerala, while selecting the leaves and stems from the plant various points were considered they are:

Fungi like cercosporaspp and septorialycopersic causing brown spot in the leaves and further turning the leaves yellow and kill them apart from fungi the most common pests on the leaves are grasshoppers, crickets and caterpillars. Therefore diseased, damaged, insects infected, bruised, discoloured, decayed and wilted leaves are discarded manually just after the collection of fresh green plant. As decayed and wilted leaves gives a bad flavour to the whole batch and can also leads to loss of nutrients too.



Different procedures adopted for preparation of the sample

(i) **Washing**

The fresh leaves and stems were separated thoroughly and washed in running tap water for 2 to 3 times to remove the adhering dirt particles and impurities. After decanting excess water completely the leaves were scattered in cotton cloth and kept in an airy space to drain away the moisture and to air dry the leaves and stem after decanting the residual moisture was evaporated at room temperature before the actual drying process on a clean paper with constant turning over to avert (stop) fungal growth. After air drying process excess water was removed and sample were equally divided for drying process.

(ii) Drying the sample

Sun drying

The air dried leaves were placed on a plate and was covered with a cotton cloth to keep off dust and insets. The plate covered with cotton cloth were placed in direct sunlight on a roof. The leaves were sundried for 5 to 7 days, after 7th day the sundried leaves and stem were collected in air tight container for pulverizing.

Shade drying

In shade drying process the air dried leaves and stem were spread on plate and kept inside the room, and the temperature was maintained through proper ventilation. The sample was shadow dried for 7 to 9 days , after 9th day ,shade dried leaves and stem were collected in air tight container for pulverizing.

Pulverizing

After complete drying the entire sample portion separately was reduced to fine powder with the help of mechanical mixer before grinding the mixer was cleaned to avoid contamination. The powder was stored in a suitable container to avoid contamination and for further process.

B. Chemical analysis of *Bacopa monnieri*

The shade dried *Bacopa monnieri* sample were analyzed for proximate composition of energy using bomb calorimeter. Estimation Protein, crude fiber, vitamin A, vitamin C, calcium using AOAC (Association of Official Agricultural Chemists) method, phenol content were determined using Folin - Ciocaltean reagent. Total antioxidant activity using FRAP (Ferric Reducing Antioxidant Power) method. Iron and zinc using Atomic Absorption Spectrophotometry (AAS), procedure adopted for the experiments was shown in Appendix – II

C. Development of value added product using *Bacopa monnieri*

Eleven recipes were selected for this study. The recipes selected for incorporation of *Bacopa monnieri* powder were instant peanut dalia dal dry chutney powder , instant curry leaf chutney powder, instant kootu mix, instant puliyogare mix, instant pan cake mix, instant adai mix, instant rasam powder, instant idli chutney powder which was prepared by complete removal of moisture which extends the shelf life of the product and to know the acceptability of bitter taste of *Bacopa monnieri* in sweet products, and was incorporated in chocolate and pineapple jam. To study the acceptance level *Bacopa monnieri* was incorporated in tomato pickle.

D. Organoleptic evaluation of the developed value added products

The sensory attributes such as appearance (visual appeal on sight), colour (adequacy of hue and uniformity), aroma (olfactory feeling on inhaling the head space volatiles), taste (response of taste bud on masticating), texture (force of chew and consistency on melting) and overall acceptability (likeness as compared to commercial products) was evaluated. The discipline of sensory examination requires the use of panel members, in which test outcome are recorded based on their responses to the product under test. Recipes were prepared and 5 point hedonic scale was used to evaluate the sensory characteristics of the developed value added products as shown in appendix. Ten panel members were selected for the sensory evaluation of developed value added products in the laboratory. Water was provided to panel members before and after tasting the product to rinse their mouth during evaluation otherwise the taste of the first sample will still linger in the mouth and affect the taste of the other sample. The mean score of the organoleptic evaluation were calculated on the basis of the total scores obtained.

Each product was developed with five variations namely A, B, C, D and E the shade dried *Bacopa monnieri* powder was incorporated in different variations like 1mg, 2mg, 3mg, 4mg, 5 mg respectively. Standardization of product was done using organoleptic evaluation by ten selected panels.

E. Computation of nutrient value of developed value added products

The developed value added products with each variations were calculated for its nutrient content like energy, proteins, fats, crude fiber, calcium, iron, zinc, vitamin A, and vitamin C based on nutritive value of Indian foods (Gopalan *et al.*, 2011).

F. Study the shelf life of the developed product using *Bacopa monnieri*

The developed products were analyzed for their shelf life stability for a period of sixty days, 25g of cooked variations of each developed products were stored in sterile pouches, the products like instant peanut dalia dal dry chutney powder, instant curry leaf chutney powder, instant idli chutney powder, instant rasam mix, instant pan cake mix, instant kootu mix, instant adai mix, instant puliyogare mix were kept in room temperature at 37°C and were analyzed for their shelf life. Pineapple jam, chocolate and tomato pickle were kept in refrigerator at 0 to 4°C and the product was also analyzed for their shelf life.

VALUE ADDED PRODUCTS USING BRAHMI (*bacopa monnieri*)





Results and Discussion

IV. RESULTS AND DISCUSSION

The result of the study entitled “**Development of value added products using Brahmi (*Bacopa monnieri*)**” is discussed under the following headings.

- F. Nutrient analysis of shade dried *Bacopa Monnieri* Powder
- G. Analysis of phytochemicals present in shade dried *Bacopa Monnieri* Powder
 - i.Total Antioxidant Activity
 - ii.Total Phenols
- H. Standardization and organopeptic evaluation of value added products
- I. Nutritive value obtained for developed value added products
- J. Shelf life of the developed value added products.

TABLE I

A .CHEMICAL ANALYSIS OF BACOPA MONNIERI

Nutrients	Brahmi Powder(100g)
Energy Kcal	290
Protein (g)	6
Iron (mg)	52
Crude Fiber (%)	14
Calcium (mg)	220
Vitamin A (µg)	164
Vitamin C(mg)	3
Zinc (mg)	13

Table I shows that the analysed nutrient composition of shade dried *bacopa monnieri* powder shows 220mg of calcium, 164µg of vitamin A, 52 mg of iron, 13mg of zinc, 6g of protein it also contribute 290kcal of energy.

B. Analysis of phytochemicals present in *Bacopa monnieri*

- i. Total antioxidant activity: The term antioxidant refers to free radical scavengers, inhibitors of lipid peroxidation and chelating agent. An adequate intake of natural antioxidant was found to protect macromolecule against oxidative damage in cells (Mittler, 2002).
- ii. Total Phenols: Phenols are considered to be the most abundant antioxidant in diet; some herbs are loaded with powerful antioxidant phenols. Phenolic compounds

passes a wide spectrum of biological effects including antioxidant and free radical scavenging activity (Kahkonen et al., 1999)

TABLE II
TOTAL ANTIOXIDANT ACTIVITY AND TOTAL PHENOLS CONTENT OF SHADE DRIED BACOPA MONNIERI POWDER

Phytochemical	Brahmi Powder
Total antioxidant activity (mg/g)	17
Total Phenols (mg/g)	1405

Table. II depicts that shade dried brahmi powder showed that total antioxidant activity was analysed by FRAP assay (Ferric Reducing Antioxidant Powder) and the phenolic content present in the sample was analysed using Folin – Ciocaltean reagent as per AOAC standards, both the phytochemical were calculated in milligrams in which total phenol content was found to be the highest value. Total antioxidant activity and total phenols present in *bacopa monnieri* (brahmi) was 17mg and 1405mg respectively.

C. STANDARDIZATION AND ORGANOLEPTIC EVALUATION OF VALUE ADDED PRODUCTS

Organoleptic evaluation was done using five point hedonic rating scale to find out the best variation of the developed value added products. The mean scores for the quality attributes like appearance, flavour, taste, colour, texture and overall acceptability of all the variations in developed products was done by trained panel members. The variation which scored highest score was considered as a standard product. The Mean sensory acceptability Score of Instant Peanut Dalia Dhal Dry Chutney Powder are given in Table III.

TABLE III
MEAN SENSORY ACCEPTABILITY SCORE OF INSTANT PEANUT DALIA DAL DRY
CHUTNEY POWDER

Variations	Appear Ance	Flavour	Taste	Colour	Texture	Overall Accept ability Score	Overall Accept ability Score %
V ₁	4.2 ± 0.79	3.2 ± 0.92	4 ± 0.94	4 ± 0.94	3.8 ± 0.79	4.3 ± 0.82	43
V ₂	3.5 ± 0.85	3.3 ± 0.48	3.5 ± 0.85	3.7 ± 0.82	3.5 ± 0.85	3.3 ± 0.48	33
V ₃	4 ± 0.47	3.8 ± 0.79	3.9 ± 0.32	3.9 ± 0.88	3.5 ± 0.53	3 ± 0.47	30
V ₄	3.3 ± 0.67	3 ± 0.82	3.1 ± 0.88	4.1 ± 0.74	3.1 ± 0.57	3 ± 0.82	30
V ₅	3.2 ± 0.92	3.1 ± 0.32	2.8 ± 1.23	3.1 ± 1.20	2.8 ± 0.63	3.1 ± 0.32	31

The highest score was recorded in variation 1 where *bacopa monnieri* powder was incorporated in 1mg mean scores obtained for appearance 4.2, flavour 3.7, taste 4, colour 4, texture 3.8 and the overall acceptability was 4.3 and thus this variation I was selected as standard. Variation 5 obtained least score for appearance, taste, colour, texture because 5mg of *bacopa monnieri* powder was incorporated in this variation.

Sensory evaluation Scores of Instant curry leaf chutney powder is presented in table IV

TABLE IV
SENSORY EVALUATION SCORES OF INSTANT CURRY LEAF CHUTNEY POWDER

Variations	Appear ance	Flavour	Taste	Colour	Texture	Overall Accept ability Score	Overall Accept ability Score%
V ₁	4.1 ± 0.74	3.7 ± 0.95	3.8 ± 1.03	3.4 ± 0.84	3.3 ± 1.16	4.1 ± 0.74	41
V ₂	3.8 ± 0.63	3.3 ± 0.48	3.5 ± 0.71	3.5 ± 0.97	3.3 ± 0.82	3.8 ± 0.63	38
V ₃	3.6 ± 0.70	3.4 ± 0.52	3.3 ± 0.48	3.6 ± 0.84	3.1 ± 0.74	3.6 ± 0.70	36
V ₄	3.4 ± 0.52	3.5 ± 0.71	2.7 ± 0.67	3.2 ± 0.79	3 ± 0.67	3.5 ± 0.71	35
V ₅	3.3 ± 0.48	3.7 ± 0.95	2.6 ± 0.97	3 ± .25	2.5 ± 0.85	3.2 ± 0.63	32

Table IV portrays that among the five variations in the formulation of instant curry leaf chutney powder incorporated variation 5 scored the least mean score for taste 2.6, texture 2.5, variation 1 was accepted by panel members with the overall acceptability of 41% thus variation 1 was selected as standard.

Table V gives the Effect of level of brahmi powder on sensory attributes of Instant Kootu mix

Table V

Effect of level of brahmi powder on sensory attributes of Instant Kootu mix

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	3.8± 0.79	3.5±0.53	3.7±0.57	3.9± 0.74	3.7± 0.82	3.7±0.48	37
V ₂	3.5± 0.71	3.6±0.70	3.2±0.82	3.3± 0.67	3.2±0.79	3.6±0.52	36
V ₃	3.6±0.70	3.3± 0.48	3.2±0.79	3.3± 0.95	3.3±1.06	3.3±0.48	33
V ₄	3.1±0.74	3.3±0.48	3±0.94	2.6± 0.52	2.5± 0.85	3.1±0.74	31
V ₅	3.3±1.66	2.9± 0.57	3±1.25	2.5±0.85	2.8± 0.92	2.9±0.00	29

The overall acceptability scores was high in variation 1 (3.7), appearance 3.8, taste 3.7, colour 3.9, texture 3.7. The taste of instant kootu mix was accepted in variation 1 with the overall acceptability score of 3.7% hence it was selected as standard. Variation 5 scored the least mean score for flavour 2.9 of colour 2.5 and least overall acceptability 29 per cent.

The overall acceptability scores of Instant Puliogare Mix is presented in table VI

Table VI

Overall acceptability Score of Instant Puliogare Mix

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	4.1±0.74	3.7± 0.82	3.5±0.85	3.3±1.16	2.9± 1.37	4.4± 0.70	44
V ₂	4.2±0.92	3.5± 0.71	2.9±1.37	2.8±1.32	3±0.82	3.9 ±0.74	39
V ₃	3.8±0.92	3.5± 0.71	3.3±1.42	2.8±1.32	3± 0.88	3.5±0.71	35
V ₄	3.4±0.82	3±1.05	2.9±0.74	2.9±1.29	2.9±1.10	3±1.05	30
V ₅	3.4±0.84	2.8± 0.92	1.9±1.20	2.6±0.84	2.2±0.79	2.8±0.92	28

Table VI shows that variation 1 scored the maximum for appearance 4.1, flavour 3.7, taste 3.5, colour 3.3. The amount of incorporation of *bacopa monnieri* powder was 1mg in variation 1 and thus scored highest for overall acceptability. In variation 5 bitter tastes was obtained due to the incorporation of *bacopa monnieri* powder at 5% level.

The Mean score obtained for Instant pan cake mix is presented in table VII

Table VII
Sensory acceptability scores of instant puliyogare mix

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	4.5±0.71	4.1± 0.88	4.5±0.85	3.8±1.23	2.5± 0.97	4.5 ±0.71	45
V ₂	4.1±0.57	3.6± 0.52	4±0.00	3.3±0.67	3.8±1.23	4.1±0.57	41
V ₃	3.5±0.71	3.5±0.71	4.2±0.63	3.3±0.82	3.5± 0.85	3.5±0.71	35
V ₄	3.5±0.97	3 ±0.94	3.3±0.82	3.4±1.07	3±.33	3±0.94	30
V ₅	3.3±1.16	2.8± 0.92	2.8± 0.6	2.8± 0.63	2.8±0.92	2.5±0.63	25

The mean score obtained for instant pan cake mix prepared from varying proportion of incorporation of shade dried *bacopa monnieri* powder was shown in table VII The overall acceptability score for variation 1 was 45% due to incorporation of 1mg of *bacopa monnieri* powder whereas 25% of scores was obtained for variation 5. Thus variation 1 was selected as standard.

Table VIII Gives the Acceptance of Instant Adai Mix by means of Mean Sensory Evaluation

Table VIII
Acceptance of Instant adai mix by means of mean sensory evaluation

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	4±0.82	3.7±0.67	4.1±0.74	3.8± 0.63	3.7±0.67	3.7±0.67	37
V ₂	3.8±0.63	3.6±0.70	3.6±0.70	3.8± 0.79	3.4±0.84	3.8±0.63	38
V ₃	3.7± 0.67	3.3±0.48	3.3±0.48	3.3± 0.67	3.4±0.84	3.3±0.48	33
V ₄	3.4±0.52	3.4±0.70	3.4±0.52	3.3± 0.67	3.2±0.63	3.4±0.52	34
V ₅	3.2±0.63	3.4±0.84	3.4±0.52	3±0.67	3.2±0.79	3.3±0.48	33

Table VIII portrays that among the five variations prepared for instant adai mix with incorporation of *bacopa monnieri* powder, variation 2 obtained mean scores 38% for overall acceptability score and also this table revealed that the combination of rice and pulse

dominates the pungent flavour and bitter taste of bacopa monnieri, incorporation of bacopa monnieri powder 2mg was accepted by panel members and it was selected as standard, variation 5 scored the least for all the quality attributed with 3.3% of overall acceptability.

The Sensory attributes of instant rasam mix is depicted in table IX

Table IX

Sensory attributes of instant rasam mix

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	4.1± 0.88	3.5±0.71	3.6±0.70	2.7± 0.82	3.1±1.10	4.1±0.88	41
V ₂	3.6±0.70	3.4±0.97	3±1.13	2.6± 0.84	2.4± 0.97	3.6±0.70	36
V ₃	3.2±0.63	2.6±0.84	3.9±0.88	2.9± 0.88	2.8± 0.63	3.5±0.71	35
V ₄	3.2± 0.63	3.4±.17	2.9±1.10	3.8± 1.32	2.7± 1.25	3.4±0.97	34
V ₅	3.6±1.17	2.5±1.18	2.7±0.67	2.9± 0.99	3.2± 1.23	3±1.33	30

The mean scores of instant Rasam mix prepared from varying proportion of incorporation of *bacopa monnieri* powder was depicted in the table IX Sensory attributes of Instant Rasam mix. The highest score was recorded in variation 1 (41%) for overall acceptability. Mean score obtained for various attributes for appearance 4.1, flavour 3.5, taste 3.6 and texture 3.1.

Table X depicts the organoleptic evaluation of instant idli chutney powder

**Table X
Organoleptic evaluation of instant idli chutney powder**

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	5±0.00	4.1± 0.67	3.3±0.52	5 ±0.00	5 ±0.00	4.9± 0.32	49
V ₂	4.9±0.32	3.7± 0.40	2.7±0.48	4.5±0.53	4.2±0.42	4.7± 0.67	47
V ₃	4±0.00	3 ±0.47	2.3±0.48	4±0.47	3.6±0.70	4.5± 0.53	45
V ₄	3.8±0.42	2.8± 0.42	1.7±0.48	3.8± 0.63	3±0.47	4± 0.00	40
V ₅	3.4±0.70	2.6±0.32	1± 0.00	3.4± 0.70	3.8±1.14	3.8±0.48	38

Sensory evaluation of the developed instant idli chutney powder revealed that the variation I(1mg) incorporated bacaopa monnieri gained the maximum scoring for appearance colour, and texture (5), flavour (4), taste, (3.3) and their overall acceptability scores was found to be 49 per cent when compared to the variation 5 which had least scoring for all the quality attributes and also obtained a scoring of 38 per cent for their overall acceptability.

Out of the two formulations, variation I scored the best mean score when compared to the variation 5 and hence based on the best quality attributes and their overall acceptability the variation 1 was selected as the standard.

Table XI Gives the Mean Score obtained for chocolate

**Table XI
Mean Score obtained for chocolate**

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	4.1±0.74	3.3± 0.82	3.7±1.16	2.9± 0.99	3.1± 1.10	4.1± 0.74	41
V ₂	3.6±0.70	3.4± 0.84	3.6±1.17	3.2± 0.92	3.1± 1.29	4±0.67	40
V ₃	3.4±0.52	3.3 ±0.82	4±0.67	3.7± 0.95	3.3± 0.82	3.6±1.17	36
V ₄	3.1±0.57	3.1± 0.74	3.4±0.70	3.5±1.27	2.8± 1.03	3.5±1.27	35
V ₅	3.3±1.16	3±0.94	2.9±0.74	3± 0.82	2.9±1.10	3.4± 0.52	34

The mean sensory scores of chocolate prepared with incorporation of *bacopa monnieri* with different variation from V1 to V5 were evaluated for different sensory characteristics. The variation 1 had secured a maximum over all acceptability score of (4.1) followed by maximum scoring for the quality attributes like appearance 4.1, flavour 3.3, and taste 3.7 were found to be recorded. Whereas the variation 2 had secured a maximum mean score for flavour 3.4.

While the variation 1 had secured the maximum scores for overall acceptability of 41 per cent. The chocolate taste hides the bitterness of *bacopa monnieri* in 1mg incorporation. Whereas in variation 5 the overall mean score acceptability percentage. 34 per cent was found to be noted. Based on the scoring of quality attributes Variation 1 was selected as standard due to its high overall acceptability

Mean Sensory attributes of pineapple jam is given in table XII

Table XII

Mean Sensory attributes of pineapple jam

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	3.9 ±1.10	4.1±0.74	3.9±0.99	4.1± 0.74	3.7± 0.95	4.6± 0.52	46
V ₂	4.1± 0.74	3.7±0.48	3.9±0.74	3.8± 0.63	3.9± 0.74	4.1± 0.74	41
V ₃	3.9±0.88	3.6±0.70	3.9±0.32	4±0.47	3.8± 0.42	4± 0.47	40
V ₄	3.7± 0.82	4.5±0.53	3.7±0.48	3.4± 0.52	3.4± 0.52	3.9± 0.99	39
V ₅	3.4±0.82	3.4±0.52	3.6±0.70	3.2±0.42	3.4± 0.52	3.7± 0.48	37

The mean scores obtained for pineapple jam prepared at varying proportion incorporated using shade dried *bacopa monnieri* powder was depicted in table XII The maximum mean scores obtained by variation 1 (46%) for overall acceptability percentage, whereas in variation 5 scored 37% for overall acceptability mean score percentage due to 5mg incorporation of *bacopa monnieri* , thus variation 1 was selected as standard.

Mean score for sensory acceptability of Tomato pickle is presented in table XIII

Table XIII

Mean score for sensory acceptability of Tomato pickle

Variations	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability Score	Overall Acceptability Score%
V ₁	4.2±0.79	3.8±0.63	4.3±0.82	4.4±0.70	3.9± 0.74	4.4±0.52	44
V ₂	3.9±0.74	3.4±0.97	4±0.47	3.9±0.88	4 ± 0.67	3.6±0.52	36
V ₃	3.9±0.57	3.9± 0.57	3.5±0.71	3.8±0.79	3.4±0.57	3.3±0.48	33
V ₄	4±0.67	3.5±0.53	3.6±0.70	3.6± 0.70	3.6±0.76	3.1± 0.74	31
V ₅	3.5±0.85	3.3±0.48	3.6±0.70	3.6±0.70	3.4±0.52	2.9± 0.00	29

The mean score obtained for tomato pickle prepared from varying proportion of incorporation of shade dried *bacopa monnieri* powder was shown in table XIII. The mean score obtained by variation 1 (1mg) incorporation of *bacopa monnieri* powder was 4.2, 3.8, 4.3, 4.4 for appearance, flavour, taste, colour respectively. Owing to 5mg incorporation of *bacopa monnieri* variation 5 scored 29% for overall mean sensory acceptability.

D. computation of nutritive value for developed value added products

a. Instant Peanut Dalia dal dry Chutney Powder

Peanut also known as ground nut, a crop of global importance. Peanut and roasted Bengal gram dhal are an excellent source of protein and B vitamins. Dry chutney powder was prepared using peanut and roasted bengal gram dhal, which facilitates protein rich breakfast accompaniment.

Nutrient Composition of Instant Peanut Dalia dhal dry Chutney Powder (per 100g) is given in table XI

TABLE XIV

Nutrient content of instant peanut dalia dal dry chutney powder (100g)

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	783	1073	1263	1653	1943
Protein (g)	25	25	25	25	25
Iron (mg)	9	9	9	9	9
Fibre (g)	5	5	5	5	5
Calcium (mg)	50	50	50	50	51
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	3	3	3	3	3

The proximate composition of standard instant peanut dalia dhal dry chutney powder has been shown in table XIV. The standard instant peanut dalia dhal dry chutney powder incorporated with shade dried brahmi powder in different percentage variations. was analysed for their nutrient contents. The Vitamin A content of variation1 was found to be 110 µg followed by calcium and protein with 50mg and 25g respectively. Whereas the protein, iron fibre, Calcium, Zinc content was found to be the same with the values of 25g, 9mg,5g,50g,3g respectively for all the other variations. Though the energy and phytochemical content was high in other variations, variation 1 was chosen as standard due to their best scoring of overall acceptability percentage.

b. Instant Curry leaf Chutney Powder

The major nutrients found in curry leaf was iron, calcium fibre, phosphorous magnesium, copper and minerals. It also contains vitamin A,B,C,E antioxidants, plants sterols and flavonoids.

Table XV gives the Nutrient Composition of Instant Curry leaf Chutney Powder

Table XV

Nutrient content of instant curry leaf chutney powder

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	551	841	1131	1421	1711
Protein (g)	74	74	74	74	74
Iron (mg)	5	5	5	5	6
Fibre (g)	8	8	8	8	8
Calcium (mg)	658	658	658	658	679
Vitamin A (µg)	3681	3791	3901	4011	4121
Vitamin C (mg)	135	135	135	135	135
Zinc(mg)	1	1	1	1	1

Table 15. Represents the computed nutritive value of instant curry leaf chutney powder using *bacopa monnieri* shade dried powder. The developed and analysed Instant curry leaf chutney powder was found to be rich in protein, Calcium, Vitamin A, Vitamin C, with 74g, 658mg, 3681µg, and 135mg respectively..

C. Nutrient Composition of Instant Kootu Mix

Kootu mix prepared with combination of pulses. Pulses typically contains about twice the amount of protein found in whole grain cereal which possess Key minerals like iron, Zinc, Potassium, Magnesium and pulses are particularly abundant in B Vitamins.

Nutrient Composition of Instant Kootu Mix is presented in table XVI

Table XVI

Nutrient Content of Instant Kootu Mix

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	626	916	1206	1496	1786
Protein (g)	20	20	20	20	20
Iron (mg)	9	9	10	10	10
Fibre (g)	8	8	8	8	8
Calcium (mg)	459	459	460	460	460
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	2	2	2	2	2

Table XVI Gives the changes in the Nutrient content of developed formulation Pulses typically contains about twice the amount of protein found in whole grain cereal like wheat, oats, rice etc.

The proximate analysis was carried out for all the five variation of the developed products and the results revealed that the Energy content of the variation V₁ was found to be 626 kcal, followed by V₂ (916 Kcal), V₃ (1206 kcal), V₄ (1496 kcal) and V₅ had 1786 kcal respectively. Whereas the protein content of all the five variation were found to be (20 g).

The vitamin A content of the five variations were found to be 110µg, 220 µg, 330 µg, 440µg, 550 µg for variations V1,V2, V3 V4 and V5 respectively. The calcium content were found to be 459 mg for variation VI, V2 followed by 460 mg were found to be noted for V3 , V4 and V5 respectively. Salient findings of nutrient analysis revealed that the variation V5 with incorporation of 5 per cent of bacaopa monnieri had maximum energy of 1786 kcal, vitamin A 550 µg, and calcium 460 mg when compared to the incorporation of one per cent of bacaopa monnieri in variation VI had obtained 626 Kcal followed by vitamin A 110µg and calcium 459 mg respectively thus enhancing the nutritional value by higher the percentage of bacaopa monnieri incorporation higher the nutrient content were found to be noted.

d. Instant Puliogare Mix

Tamarind was the major ingredient in puliyogara mix. As most ancient foots tamarind has a long history of medicinal uses. Many involve easing stomach discomfort, aiding digestion and used as a laxative. Tamarind preparations was used for fever, sore throat, rheumatism, inflammation and sunstroke.

Table XVII gives the Nutrient Composition of Instant Puliogare Mix

Table XVII

Nutrient content of Instant Puliogare Mix

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	967	1257	1547	1837	2127
Protein (g)	28	28	28	28	28
Iron (mg)	13	13	13	13	13
Fibre (g)	23	23	23	23	23
Calcium (mg)	490	490	490	490	491
Vitamin A (µg)	663	773	883	993	1100
Vitamin C (mg)	26	26	26	26	26
Zinc(mg)	5	5	5	5	5

Value addition was done by product preparation of standard instant Puliogara mix variations incorporated with shade dried bacopa monnieri powder is presented in Table. XVII. The Nutrients of standard instant Puliogara mix variations was analysed. The standard instant Puliogara mix prepared with incorporation of variations 1 had maximum energy and was found to be 967 kcal followed by vitamin A 663µg. Calcium 490mg, Protein 28g, vitamin C, 26g, Iron, Fibre and micro mineral Zinc was found to be 13mg, 23mg and 5mg in variations 1 respectively.

E. Nutrient Composition of Instant Pan Cake Mix

pancakes provides high calories from sugar and fat Pan cakes are also considered to be a good source of Vitamins A and minerals as they are prepared using milk and egg as an ingredient.

Table XVIII gives the Nutrient Composition of Instant Pan Cake Mix

Table XVIII

Nutrient Content of Instant Pan Cake Mix

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	595	885	1175	1365	1755
Protein (g)	8	8	8	8	8
Iron (mg)	1	2	2	2	2
Fibre (g)	2	2	2	2	2
Calcium (mg)	17	17	17	17	18
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	0.343	0.356	0.369	0.382	0.395

Table XVIII Represent the complete nutritive value of instant pan cake mix using shade dried *bacopa monnieri* powder.

The instant pan cake mix using shade dried *bacopa monnieri* powder was prepared and analysed for their proximate composition and found to be noted with Energy 595kcal. As pan cake was made out of milk egg which are considered to be the sources of vitamin A. 110µg of Vitamin A was found in variation 1. Zinc (0.343mg) was recorded as lowest nutrient content.

F. Nutrient Composition of Instant Adai Mix

Adai mix composed of pulse and rice combination which provides lysine and methionine (essential amino acids). They also posses B vitamins, minerals like calcium, iron, zinc etc.

Nutrient Composition of Instant Adai Mix is presented in table XIX

Table XIX

Nutrient Content of Instant Adai Mix

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	556	846	1136	1426	1716
Protein (g)	111	111	111	111	111
Iron (mg)	3	3	3	3	3
Fibre (g)	3	3	3	3	3
Calcium (mg)	156	156	156	156	157
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	1	1	1	1	1

Table XIX depicts the nutrient content of instant adai mix using shade dried *bacopa monnieri* powder. Protein, iron, fiber content was found to be sharing the same values of 111g, 3mg, 3g respectively. Calcium and vitamin A content was found to be high in variation 5 157mg and 550µg due to incorporation of 5mg of *bacopa monnieri* powder.

G. Nutrient Composition of Instant Rasam Powder

Rasam was prepared using tamarind, tomato and black pepper. Incorporating rasam in daily meal keeps the digestive system fit and healthy, rasam has the ability to prevent constipation, it is rich in vitamins and minerals which can be introduced to babies during weaning.

Nutrient Composition of Instant Rasam Powder is given in table XX

Table XX

Nutrient content of Instant Rasam Powder

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	590	880	1170	1460	1750
Protein (g)	14	14	14	14	14
Iron (mg)	10	10	10	10	11
Fibre (g)	23	23	23	23	23
Calcium (mg)	591	592	592	592	593
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	2	2	2	2	2

Instant Rasam Powder was prepared with using shade dried bacopa monnieri powder. Among the five variations the variation 1 was chosen as standard due to overall acceptability. Nutrient composition were analysed and the contribution of Energy 590 kcal was found in variation 1 followed by calcium 591 mg, vitamin A 110µg, fiber 23g. Iron and protein content of instant Rasam Powder was 10mg and 14g. Though the energy and Phytochemical content was high in other variations, variation 1 was chosen as standard based on their quality attributes and overall acceptability.

H. Nutrient Composition of Instant Idli Chutney Powder

Sesame seeds are an excellent source of copper, a very good source of manganese and a good source of calcium, phosphorous, iron, zinc, vitamin B1 and dietary fiber. Idli chutney powder was developed using sesame seed and pulses which provides good amount of protein, calcium in the diet.

Table XXI gives the Nutrient Composition of Instant Idli Chutney Powder

Table XXI

Nutrient Content of Instant Idli Chutney Powder

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	629	919	1209	1499	1789
Protein (g)	18	18	18	18	18
Iron (mg)	5	5	5	5	5
Fibre (g)	5	5	5	5	5
Calcium (mg)	504	504	504	504	505
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	3	3	3	3	3

The standard instant idli chutney powder variation was value added with shade dried *bacopa monnieri* powder with different percentage variation and analysed for the proximate composition. In variation 1 the energy contribution was 629kcal and calcium 504mg. Protein content was found to be 18g, iron and fiber had same values of 5mg and 5g respectively. Whereas the variation V5 Contribution of energy were found to be 1789kcal and calcium 505mg. The protein content was found to be 18g, vitamin A was 550 µg, followed by the iron and fiber had same values of 5mg and 5g respectively.

I.Nutrient Composition of Chocolate

Chocolate is made out of cocoa powder which is a source of flavonoids and alkaloids such as theobromine, phenethylamine and caffeine. Chocolate has become one of the most popular food in the world and thus was developed by incorporating *bacopa monnieri* a medicinal plant.

Nutrient Composition of Chocolate is given in table XXII

Table XXII

Nutrient Content of Chocolate

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	356	826	1116	1406	1696
Protein (g)	12	12	12	12	12
Iron (mg)	8	8	8	8	9
Fibre (g)	0.00014	0.00028	0.00042	0.00056	0.0007
Calcium (mg)	98	98	97	97	99
Vitamin A (µg)	110	220	330	440	550
Vitamin C (mg)	0.0028	0.0056	0.0084	0.011 2	0.014
Zinc(mg)	4	4	4	4	4

Table XXII reveals that nutrients presented in standard chocolate prepared with *bacopa monnieri*. The nutrients were analysed for all the variations and energy were found to be 826, 1116, 1406, 1696 Kcal for variation 2,3,4,5 whereas the calcium and vitamin wa found to be 98, 97, 99 mg and vitamin A content was found to be 220,330. 440 and 550µg in variation 2,3,4,5 respectively. The energy, protein, iron, calcium and vitamin A content was found to be 356 kcal, 12g, 8mg, 98mg and 110µg respectively in variation 1. Due to the highest overall acceptability score obtained during organoleptic evaluation, variation 1 was selected as standard.

J. Nutrient Composition of Pineapple Jam

Pineapple fruit contains a proteolytic enzyme bromelain that digests food by breaking down protein. Bromelain has anti inflammatory, anti clotting and anti cancer properties. Regular consumption of pineapple helps to fight against arthritis, indigestion and worm infestation. Pineapple is a good source of vitamin A and C

Table 23 gives the Nutrient Composition of Pineapple Jam

Table 23

Nutrient Composition of Pineapple Jam

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	1134	1424	1714	2004	2294
Protein (g)	0.6	0.0	0.6	0.6	0.6
Iron (mg)	798	798	798	798	798
Fibre (g)	0.56	0.56	0.56	0.56	0.56
Calcium (mg)	48	48	48	48	49
Vitamin A (µg)	128	238	348	458	568
Vitamin C (mg)	41	41	41	41	41
Zinc(mg)	0.12	0.12	0.12	0.12	0.12

The nutrient composition of standard pineapple jam variation was presented in table XXIII Variation 5 was found to have the highest energy of .2294kcal , followed by vitamin A 568µg Iron and vitamin C content was found to posses same values in all the five variations of 798mg and 41mg.

K. Nutrient Composition of Tomato Pickle

Tomatoes are an excellent source of vitamin C, biotin, molybdenum and vitamin K. They are also a very good source of copper, dietary fiber, vitamin A(in the form of beta-carotene), vitamin B6, folate. Tomato based foods has an incredibly beneficial effect on health. Thus tomato was made into pickle by incorporating *bacopa monnieri* powder.

Nutrient Composition of Tomato Pickle is presented in table XXIV

TABLE XXIV

Nutrient Content of Tomato Pickle

Nutrients	Variations				
	V ₁	V ₂	V ₃	V ₄	V ₅
Energy (Kcal)	373	663	953	1243	1533
Protein (g)	4	4	4	4	4
Iron (mg)	2	2	2	2	2
Fibre (g)	7	7	7	7	7
Calcium (mg)	102	102	102	102	103
Vitamin A (µg)	442	552	662	772	882
Vitamin C (mg)	30	30	30	30	30
Zinc(mg)	349	349	349	349	349

Tomato pickle prepared by shade dried *bacopa monnieri* powder with different variation and analysed for their nutrient composition of all the variations. Whereas the vitamin A 442µg content was high in variation 1 followed by energy 373kcal and micro mineral zinc 349mg, calcium obtained was 102mg. It was clearly shown that protein, iron and fiber content was less 4g, 2mg and 7g in variation 1 but due to maximum overall mean sensory scores obtained during organoleptic evaluation, variation 1 was considered as standard.

E. Observation of shelf life of the developed value added products

The shelf life qualities of developed products were assessed for a period of sixty days, the dry products like instant dalia dal dry chutney powder, instant curry leaf chutney powder., instant kootu mix, instant puliyogara mix, instant pan cake mix, instant adai mix, instant rasam powder, instant idli chutney powder was stored in room temperature 37°C. 25g of each variation was taken for shelf life study, where as formatted in Chocolate, tomato pickle and pineapple jam were kept in refrigerator at 2 to 4°C, 25g cooked portion of tomato pickle, pineapple jam and chocolate of each variation was taken for shelf life study and they were visually examined on daily basis for any microbial growth and the period at which the first microbial growth was observed, that period was taken as the shelf life of the product under normal room temperature (37°C) and in refrigerated temperature (2 to 4°C)

Major preservatives like Sugar, Salt, Oil, Vinegar and sodium benzoate was added during the preparation of the products which determined the shelf life of each product. Dry instant chutney powder were prepare after complete removes of moisture, which increase the shelf stability of the product jam, chocolate and pickle does not contained any artificial colours.

Shelf Life study on *Bacopa Monnieri* Incorporated value added products table

Dates of Observation for Microbial Growth	Peanut	Curry leaf Chutney	Kootu Mix	Puliyogara	Adai Mix	Pan Cake	Rasam Mix	Idli Chutney Powder	Chocola	Pineapple Jam	Tomato Pickle
1 to 6	X	X	X	X	X	X	X	X	X	X	X
8 to 14	X	X	X	X	X	X	X	X	X	X	X
15 to 21	X	X	X	X	X	X	X	X	X	X	X
22 to 28	X	X	X	X	X	X	X	X	X	X	X
29 to 35	X	X	X	X	X	X	X	X	X	X	X
36 to 42	X	X	X	X	X	X	X	X	X	X	X
43 to 49	X	X	X	X	X	X	X	X	X	X	X
50 to 60	X	X	X	X	X	X	X	X	X	Y	Y

X- Absence of Microbial growth

Y- presence of Microbial growth

Note: table XXV shows that the Microbial growth was observed on the 50th day of storage for tomato pickle and 52nd day in pineapple jam due to the Presence of moisture content.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

From dawn of history, man has relied so much on medicinal plants for health and food needs. The traditional use of medicinal plants for curing and preventing illnesses includes the promotion of both physical and spiritual well being of human beings.

Plant derivatives had been employed by population to prevent different kind of diseases from centuries. The knowledge of plant properties was acquired by ancient civilization that passed down from generation to generation until today.

Medicinal plants are those plants that was used in treating and preventing specific ailments and diseases that affects human beings. Most of the plant parts identified (bark, root, seed, fruit, leaf) serves as major source of active ingredients and products of secondary metabolites example. Alkaloids, terpenoids etc used in curing diseases.

The selected *bacopa monnieri* plant was used in Ayurveda, siddha and traditional medicine systems. They have various bioactive components to cure different types of diseases especially treating memory loss in patients with neurodegenerative disorders like alzheimer's disease and also used as anti inflammatory, analgesic antipyretic sedative and antiepileptic agent.

The study was taken up in order to incorporate medicinal plant into foods and to convert the valuable medicinal plants into functional foods.

For the development and standardisation of functional foods, the following methods were adopted. The medicinal plant was collected from the malapuram district of kerala.

In order to standardise the functional foods the nutrient analysis and medicinal properties of *bacopa monnieri* plant were identified using different types of methods. The nutrients such as protein, iron, calcium, vitamin A, vitamin C, crude fiber, zinc and medicinal properties such as total antioxidant activity, phenols were analysed.

The collected medicinal plant leaves separated from the stalks and washed under running water separately for 2-3 times and water was drained. The leaves were shade dried for seven to nine days for complete removal of moisture. Dried

leaves were ground into fine powder using mechanical mixer followed by sieving. The powder was stored in a suitable container to avoid contamination.

Value added products like instant peanut dalia dal dry chutney powder, instant curry leaf chutney powder, instant kootu mix, instant puliyogare mix, instant pan cake mix, instant adai mix, instant rasam powder, instant idli chutney powder, chocolate, pineapple jam and tomato pickle were prepared.

Organoleptic evaluation of the prepared value added products were done by participants who had knowledge on food science, using five point hedonic rating scale. A score card was developed to score the formulated functional foods.

The result obtained from the study was summarized as follows:

The nutrient and medicinal properties of shade dried *bacopa monnieri* plant was analysed nutrient composition of shade dried bacopa monnieri powder shows 220mg of calcium, 164µg of vitamin A, 52 mg of iron, 13mg of zinc, 6g of protein it also contribute 290kcal of energy.

Total antioxidant activity of the shade dried *bacopa monnieri* powder was analysed using FRAP assay (Ferric Reducing Antioxidant Power) and total phenols content was analysed using folin-ciocalteu reagent.

The developed value added products mean scores were also calculated. In instant peanut dalia dal dry chutney powder the highest score was recorded in variation 1 where *bacopa monnieri* powder was incorporated in 1mg mean scores obtained for appearance 4.2, flavour 3.7, taste 4, colour 4, texture 3.8 and the overall acceptability was 4.3 and the this variation was selected as standard. Variation 5 obtained least score for appearance, taste, colour, texture because 5mg of *bacopa monnieri* powder was incorporated in this variation.

For instant curry leaf chutney powder variation 5 scored the least mean score for taste 2.6, texture 2.5, variation 1 was accepted by panel members with the overall acceptability of 41% thus variation 1 was selected as standard.

In instant kootu mix the overall acceptability scores was high in variation 1 (3.7), appearance 3.8, taste 3.7, colour 3.9, texture 3.7. The taste of instant kootu mix was accepted in variation 1 with the overall acceptability score of 3.7% hence it

was selected as standard. Variation 5 scored the least mean score for flavour 2.9 of colour 2.5 and least overall acceptability 29%.

In instant puliyogare mix variation 1 scored the maximum for appearance 4.1, flavour 3.7, taste 3.5, colour 3.3. The amount of incorporation of *bacopa monnieri* powder was less 1mg in variation 1 and thus scored highest for overall acceptability 44% and was chosen as standard. In variation 5 bitter tastes was obtained due to the incorporation of *bacopa monnieri* powder at 5% level.

For instant pan cake mix variation 1 obtained 45% score for overall acceptability whereas 25% of scores was obtained for variation 5. Thus variation 1 was selected as standard.

Instant adai mix the mean score for variation 2 obtained the highest overall acceptability score 38% and also this table revealed that the combination of rice and pulse dominates the pungent flavour and bitter taste of *bacopa monnieri*, this 2mg incorporation of *bacopa monnieri* powder was accepted by panel members and it was selected as standard variation 5 scored the least for all the quality attributed with 3.3% of overall acceptability.

For instant rasam mix the highest score was recorded in variation 1 (41%) of overall acceptability score percentage and overall acceptability score with 4.1, appearance 4.1, flavour 3.5, taste 3.6 and texture 3.1.

For Instant idli chutney powder the maximum mean scores obtained by variation 1 (1mg) incorporated *bacopa monnieri* scores 5 for appearance, 4 for flavour, 3.3 for taste, 5 for colour, 5 for texture and overall acceptability of 49%. Variation 5 scored least for all quality attributes and 38% of scores was obtained for overall acceptability percentage. Variation 1 was found to have the maximum overall acceptability score when compared with other variations. Thus variation 1 was selected as standard

In chocolate the maximum mean score obtained by variation 1 (4.1) for overall acceptability, appearance 4.1, flavour 3.3, taste 3.7, variation 2 score the maximum mean score for flavour 3.4, variation 1 score the maximum scores for overall acceptability 41% the chocolaty taste hides the bitterness of *bacopa monnieri* in 1mg incorporation, whereas in variation 5 (34%) was obtained for overall mean score

acceptability percentage. Variation 1 was selected as standard due to its high overall acceptability %

For pineapple jam the maximum mean scores obtained by variation 1 (46%) for overall acceptability percentage, whereas variation 5 scored 37% for overall acceptability mean score percentage due to 5mg incorporation of *bacopa monnieri* , thus variation 1 was selected as standard.

For tomato pickle the mean score obtained by variation 1 (1mg) incorporation of *bacopa monnieri* powder was 4.2, 3.8, 4.3, 4.4 for appearance, flavour, taste, colour respectively. Owing to 5mg incorporation of *bacopa monnieri* variation 5 scored 29% for overall mean sensory acceptability.

In shelf life of the developed products microbial growth was observed in pineapple jam and tomato pickle on the 50th day of storage from the day of preparation. Other value added product does not had any microbial growth.

From the above findings *bacopa monnieri* powder incorporated at 1mg and 2mg level were found acceptable due to its mild flavour, colour and tasted good for all the incorporated value added foods. So it can be incorporated in daily menu pattern.

Conclusion

From the above study it was clear that *bacopa monnieri* plant posses a lot of memory boosting nutrients that can pose to increase the memory power of human beings. It is concluded that the incorporation of *bacopa monnieri* in the prepared value added products increases the nutrient density or nutritional qualities. Hence it can be incorporated in the daily diet.

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Appendices

APPENDIX I

INSTITUTIONAL HUMAN ETHICS COMMITTEE



Avinashilingam

Institute for Home Science and Higher Education for Women

University

(Estd. u/s 3 of UGC Act 1956)

Chairman

Dr. S. Ramalingam
Principal, PSG Institute
of Medical Sciences
& Research, Coimbatore

Member Secretary

Dr. P. R. Padma
Professor, Department of
Biochemistry, Biotechnology and
Bioinformatics

Members

Dr. S. Premakumari
Mr. K. Arulmoli (Legal Expert)
Dr. A. Saraswathy
Mrs. V. Mangayarkarasi
Dr. S. Kowsalya
Dr. N.S. Rohini
Dr. Subhashini K. Sripathi
Mrs. S. Radha Devi
Mrs. Judith Justin

11th March 2016

To
Ms. Santhiyapriya C.B
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Madam,

Ref : Your proposal No. IHEC/15-16/FSMD/04 entitled
“Development of value added product using brahmi
(*Bacopa monnieri*)” submitted for approval of the IHEC

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/15-16/FSMD/04 entitled “Development of value added product using brahmi (*Bacopa monnieri*)” submitted by you. The Approval number for the same is AUW/IHEC/FSMD-15-16/XMT-03.

We wish you all the best in your research endeavours.

Regards,



Dr.P.R.Padma
Member Secretary



APPENDIX II

PROCEDURE FOR NUTRIENT ANALYSIS

1. Determination of energy value of food

The amount of energy released from foods can be obtained by measurements of heat produced when food is burnt in air. Bomb calorimeter works on the principle of direct calorimetry.

Bomb calorimeter

It consists of a heavy steel bomb, with a platinum or gold – plated copper lining and cover held tightly in place by means of a strong screw collar. A weighed amount of sample is placed in a capsule, within the bomb, which is then closed except for the oxygen valve, charged with oxygen to a pressure of at least 20 atmosphere (300 pounds or more to the square inch), oxygen valve closed and the bomb is immersed in a weighed amount of water. The water is constantly stirred and its temperature taken at intervals of one minute by means of differential thermometer, capable of being read to one thousandth of a degree. After the rate at which the temperature of water rises or falls has been determined, the sample is ignited by means of an electric fuse and on account of the large amount of oxygen present, undergoes rapid and complete combustion. The heat liberated is communicated to the water in which the bomb is immersed and the resulting rise in the temperature is accurately determined. The thermometer reading is also continued through an "after period" in order that the "radiation correction" may be calculated and the observed rise of temperature corrected accordingly. This corrected rise multiplied by the total heat capacity of the apparatus and water in which it is immersed, shows the total heat liberated in the bomb. From this must be deducted the heat arising from accessory combustion to obtain the number of kilocalories arising from the combustion of the sample. and the observed rise of temperature corrected accordingly. This corrected rise multiplied by the total heat capacity of the apparatus and the water in which it is immersed shows the total heat liberated in the bomb. From this must be deducted the heat arising from accessory combustion to obtain the number of kilocalories arising from the combustion of the sample.

2. Determination of Total Carbohydrate by Anthrone method

Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into simpler sugars. The carbohydrates content can be measured by hydrolysing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides.

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

Materials:

- 25 N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 mL of ice –cold 95% H₂SO₄ . Prepare fresh before use.
- Standard glucose : Stock – Dissolve 100 mg in 100 mL water. Working standard – 10ml of stock diluted to 100 mL with distilled water. Store in refrigerator after adding a few drops of toluene

Procedure:

- ✓ Weigh 100 mg of the sample into a boiling water
- ✓ Hydroxyl by keeping it in a boiling water bath for three hours with 5mL of 2.5 N HCl and cool to room temperature.
- ✓ Neutralise it with solid sodium carbonate until the effervescence ceases.
- ✓ Make up the volume to 100 mL and centrifuge.
- ✓ Collect the supernatant and take 0.5 and 1 mL aliquots for analysis.
- ✓ Prepare the standards by taking 0.,0.2,0.4,0.6,0.8 and 1 mL of the working standard '0' serves as blank.
- ✓ Make up the volume to 1 mL in all the tubes including the sample tubes by adding distilled water.

- ✓ Then add 4 mL of anthrone reagent
- ✓ Heat for eight minutes in a boiling water bath.
- ✓ Cool rapidly and read the green to dark green colour at 630 nm.
- ✓ Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- ✓ From the graph calculate the amount of carbohydrate present in the sample tube.

Calculation:

Amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

3. Protein Estimation by Lowry's method

Protein can be estimated by the methods as given by Lowry and also by estimating the total nitrogen content. No method is 100% sensitive. Hydrolysing the protein and estimating the amino acids alone will give the exact quantification.. The method developed by Lowry *et al* (2010), is sensitive enough to give a moderately constant value and hence largely followed. Protein content of enzyme extracts is usually determined by this method.

Principle:

The blue colour developed by the reduction of the phosphomolybdic – phosphotungstic components in the **Folin-ciocalteu** reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry's method.

Materials:

- 2% sodium Carbonate in 0.1 N Sodium Hydroxide (Reagent)
- 0.5% Copper Sulphate, (CUSO₄ 5H₂O) in 1% potassium sodium tartrate (Reagent B)

- Alkaline copper solution: Mix 50 mL of A and 1 mL of B prior to use (Reagent C)
- Folin-ciocalteau reagent (reagent D); Reflux gently for 10 hours a mixture consisting of 100 g sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$), 25 g sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)
- 700 mL water, 50 mL of 85% phosphoric acid, and 100 mL of concentrated hydrochloric acid in a 1.5 L flask. Add 150 g lithium sulphate, 50 mL water and a few drops of bromine water. Boil the mixture for 15 min without condenser to remove excess bromine cool, dilute to 1 L and filter. The reagent should have no greenish tint. (determine the acid concentration of the reagent by titration with 1 N NaOH to a phenolphthalein end point)
- Protein solution (*Stock standard*)

Weigh accurately 50 mg of bovine serum albumin (Fraction V) and dissolve in distilled water and make up to 50 mL in a standard flask.
- *Working standard*

Dilute 10 mL of the stock solution to 50 mL with distilled water in a standard flask. One mL of this solution contains 200 μg protein.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500mg of the sample and grind well with a pestle and mortar in 5 – 10 ml of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of protein

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes.
2. Pipette out 0.1ml and 0.2 ml of the sample extract in two other test tubes.

3. Make up the volume to 1ml in all the test tubes. A tube with 1ml of water serves as the blank.
4. Add 5 ml of reagent C to each tube including the blank. Mix well and allow to stand for 10min.
5. Then add 0.5ml of reagent D, mix well and incubated at room temperature in the dark for 30 min. Blue colour is developed.
6. Take the reading at 660nm.
7. Draw a standard graph and calculate the amount of protein in the sample.

Calculation

Express the amount of protein mg/g or 100g sample.

4. Estimation of Crude Fibre

Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It presents only 60-80% of the cellulose and 406% of the lignin. The crude fibre content is commonly used as a measure of the nutritive value of poultry and live stock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

Principle:

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre control.

Materials

- Sulphuric acid solution (0.255± 0.005 N): 1.25g concentrated sulphuric acid diluted to 100mL (Concentration must be checked by titration)

- Sodium hydroxide solution (0.313± 0.005N): 1.25g sodium hydroxide in 100mL distilled water (Concentration must be checked by titration with standard acid)

Procedure

1. Extract 2g of ground material with ether or petroleum ether to remove fat (Initial boiling temperature 35-38 °C and final temperature 52°C) If fat content is below 1% extraction may be omitted.
2. After extraction with ether boil 2g of dried material with 200mL of sulphuric acid for 30min with bumping chips.
3. Filter through muslin and wash with boiling water until washings are no longer acidic
4. Boil with 200mL of sodium hydroxide solution for 30 min.
5. Filter through muslin cloth again and wash with 250 mL of boiling 1.25% H₂SO₄ there 50mL of portions of water and 25mL alcohol.
6. Remove the residue and transfer to ashing dish (Prewighed dish W₁)
7. Dry the residue for 2 h at 130± 2° C. Cool the dish in a desiccator and weigh (W₂).
8. Ignite for 30 min at 600± 15°C.
9. Cool in a desiccators and reweigh (W₃).

Calculation

$$\% \text{ crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample}} \times 100$$

5. Estimation of Vitamin A

Vitamin A (retinol or retinal) is a fat-soluble vitamin. It is important for proper vision. The vitamin A is supplied to the body in the form of its precursor, β -Carotene, which is present in fruits (mango, papaya), vegetables (carrot), green (moringa leaf) etc. Vitamin A is not stored in the body when consumed abundant. There are a few

methods available for estimating vitamin A: instrumental analysis using High Performance Liquid Chromatograph is fast and accurate but expensive. A rapid and colorimetric method to measure vitamin A is given below.

Principle

The blue colour produced by vitamin A and its palmitate with trichloroacetic acid (TCA) is proportional to its concentration, which is measured at 620 nm.

Materials

- ✓ Potassium hydroxide in 90% alcohol, 2N
- ✓ Petroleum ether
- ✓ Sodium sulphate
- ✓ Trichloroacetic acid – Dissolve 15 g trichloroacetic acid (clear) crystals in 25 mL of chloroform (alcohol-free - and store in dark.
- ✓ Standard vitamin A -1.5 mg vitamin A palmitate dissolved in 10mL chloroform

Procedure

Extraction of Vitamin A

Grind one to five g of the sample material to a fine paste and add 1.0 ml of saponification mixture (2N KOH in 90% alcohol) Reflux the tubes gently for 20 min at 60°C. Cool the tubes at room temperature; add 20 mL water and mix well. Extract vitamin A with 10 mL portions of petroleum ether in a separating funnel, twice. Pool the extract (organic layer) and add sodium sulphate (anhydrous) to remove the moisture for 30-60 min. Evaporate 5mL aliquot of the ether extract to dryness at 60°C: Dissolve the dried residue in 1.0 mL of chloroform.

Estimation

1. Pipette out aliquots of the standard (vitamin A acetate) to a series of clean, dry test tubes in the concentration range 1.5-7.5µg.
2. Make up the volume in each test tube to 1.0 mL with chloroform.

3. Add 2.0 ml of TCA solution from a fast delivery pipette, rapidly mixing the contents of the tube.
4. Record the absorbance, immediately, at 620 nm in a spectrophotometer.
5. Determine the absorbance of the sample tubes in similar manner.
6. Construct a standard graph plotting the A_{620} in the Y-axis and vitamin A concentration in the X-axis.
7. Calculate the amount of vitamin A/g tissue of the samples.

6. Ascorbic Acid (Vitamin C)

Principle

Ascorbic acid reduces the 2, 6-dichlorophenol indophenols dye to a colourless leuco-base. The ascorbic acid gets oxidised to dehydroascorbic acid.. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acid medium. Oxalic acid is used as the titrating medium.

Materials:

- Oxalic acid 4%
- Dye solution: weigh 42 mg sodium bicarbonate into a small volume of distilled water. Dissolve 52 mg 2, 6-dichloro indophenols in it and make up to 200 ml. With distilled water.
- Stock standard solution: Dissolve 100mg ascorbic acid in 100mL of 4% oxalic acid solution in a standard flask (1mg/mL)
- Working standard: Dilute 10 mL of the stock solution to 100 mL with 4% oxalic acid. The concentration of working standard is 100 μ g/mL.

Procedure:

Pipette out 5mL of the working standard solution into a 100mL conical flask.

Add 10mL of 4% oxalic acid and titrate against the dye (V_1 mL). End point is the Appearance of pink colour which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.

Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100mL) and centrifuge.

Pipette out 5mL of this supernatant and 10mL of 4% oxalic acid and titrate against the dye (V_2 mL).

Calculation

$$\text{Amount of ascorbic acid mg/100g sample} = \frac{0.5\text{mg} \times V_2 \times 100\text{mL} \times 100}{V_1 \text{ mL} \quad 5\text{mL} \quad \text{Wt of the sample}}$$

7. ESTIMATION OF CALCIUM

Titrimetric method

Principle

Calcium is precipitated as oxalate and is titrated with standard potassium permanganate.

Reagents

- 4% ammonium oxalate solution
- Dilute ammonia solution: 2 ml of liquor ammonia with 98 ml water.
- 1N H_2SO_4
- 0.01 N Potassium permanganate solution
- 0.01 N oxalic acid: Sodium oxalate is dried in an oven at 100-105°C for 12 h. exactly 0.67 g is dissolved in redistilled water. Five ml of concentrated H_2SO_4 is added and solution made up to 1 L after it has cooled down.

Standardisation of potassium permanganate solution: 25 ml of 0.01 N oxalic acid is transferred to an Erlenmeyer flask. One ml of concentrated H_2SO_4 is added, warmed to about 70°C and titrated against KMnO_4 solution, till the pale pink colour remains.

The normality of KMnO_4 solution = $(25 \times 0.01) / \text{Titre value (ml)}$

Procedure

Two ml of sample is taken into a 15 ml centrifuge tube. Add 2 ml of distilled water and 1 ml of ammonium oxalate solution and mix thoroughly and leave overnight. Again the contents are mixed and centrifuged for 5 min at 1500 rpm. The supernatant liquid is poured off and the centrifuge tube drained by inverting the tube for 5 min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube is wiped with a piece of filter paper. The precipitate is stirred and the sides of the tubes are washed with 3 ml of dilute ammonia. It is centrifuged again and drained as before. The precipitate is washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate is dissolved in 2 ml of 1N H₂SO₄. The tube is heated by placing it in a boiling water bath for 1 min and titrated against 0.01 N KMnO₄ solution to a definite pink colour persisting for at least 1 min.

Calculation

1 ml of 0.01 N KMnO₄ is equivalent to 0.2004 mg of calcium.

mg of calcium/ 100 ml serum = [(X-b) x 0.2004 x 100]/ 2

where, X = Volume in ml of 0.01 N KMnO₄ required to titrate the sample, b = Volume in ml of 0.01 N KMnO₄ required to titrate 2 ml of H₂SO₄ (blank). If the normality of KMnO₄ is 'a', the value obtained in the above formula should be multiplied by the factor, a/ 0.01.

8. Estimation of Phytochemical

Total Phenols

Phenols, the aromatic compounds with hydroxyl groups, are widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack. Phenols include an array of compounds like tannins, flavonoids etc. Total phenol estimation can be carried out with the Folin-Ciocalteu reagent.

Principle

Phenols react with phosphomolybdic acid in “**Folin-Ciocalteu**” reagent in alkaline medium and produce blue coloured complex (molybdenum blue)

Materials

- 80% Ethanol
- Folin – Ciocalteu Reagent
- Na_2CO_3 20%
- Standard (100 mg Catechol in 100mL Water)
- Dilute 10 times for a working standard

Procedure

1. Weigh exactly 0.5 – 1.0g of the sample and grind it with a pestle and mortar in 10-time volume of 80% ethanol.
2. Centrifuge the homogenate at 10,000rpm for 20 min. Save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants.
3. Evaporate the supernatant to dryness.
4. Dissolve the residue in a known volume of distilled water (5mL)
5. Pipette out different aliquots (0.2-2mL) into test tubes.
6. Make up the volume in each tube to 3 mL with water.
7. Add 0.5 min, add 2mL of 20% Na_2CO_3 solution to each tube.
8. After 3 min, add 2 mL of 20% Na_2CO_3 solution to each tube.
9. Mix thoroughly Place the tubes in a boiling water for exactly one min, cool and measure the absorbance at 650 nm against a reagent blank.
10. Prepare a standard curve using different concentrations of catechol

Calculation

From the standard curve find out the concentration of phenols in the test sample and express as mg phenols/100g material.

9. Antioxidant FRAP

Ferric reducing Antioxidant power (FRAP) of the sample extracts was estimated using the method by Benzie and stain (1996). FRAP assay uses antioxidants as reductants in a redox-linked calorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

Principle

At low PH, reduction of ferric tripyridyl teiazine (Fe111TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The reaction is no specific in that any half reaction that has lower redox potential, under reaction conditions, that of ferric ferrous half reaction, will drive the ferrous(Fe 111 to Fe 11) ion formation. The change in absorbance is therefore, directly related to the combined of “total” reducing power of the electron donating antioxidants presents in the reaction mixture.

Reagents

- a) A cetate buffer 300 mMpH 3.6: Dissolved 3.1g sodium acetate trihydrate in 16 ml of glacial acetic acid and made the volume to e litre with distilled water.
- b) TPTZ (2,4,6 – tripyridyl –s-triazime) (M.W)312.34) 10mm: Dissolved 0.31 g of TPTZ is 100ml of 40mM HCl.
- c) Fecl3 6H2O (m.W.270.30) 20mM: 0.54g of the salt was dissolved in 100ml of distilled water
- d) The working FRAP reagent was prepared by mixing a b&c in the ratio of 10:1:1 at the time of use.

Standard

Trolox was used as the standard and 200µm, 400µm, 600µm, 800µm, 1000µm and 1200µm of working standard solutions were prepared.

Procedure

Sample (100µm) (Plasma / food extract/standard) is mixed with 3ml of working FRAP reagent and absorbance (593) is measured at 0 minutes after vortexing. Thereafter, samples are placed at 37°C water bath and absorption is again measured.

10. Estimation of Iron using Atomic Absorption Spectrophotometry:

Principle:

Test portions are dried and then ashed at 450°C under a gradual increase ($\leq 50^\circ\text{C}/\text{h}$) in temperature 6m HCL (1+1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1m HNO₃ and the analytes are determined by flame and graphite procedures.

Apparatus

- a. Atomic absorption spectrophotometer – with an air – acetylene burner or nitrous oxide – acetylene burner for flame and a graphite furnace for electro thermal determinations, with appropriate back ground (non atomic) correction
- b. Hollow cathode or electrode less discharge lamps for all elements determined
- c. Furnace – programmable, or muffle furnace with thermostat maintaining $450 \pm 25^\circ\text{C}$. If muffle furnace is used, a separate pre-ashing device is required
- d. Hot plate – with heating control, to heat up to about 300°C
- e. Lamp – IR 250W, fixed to a retort stand in a way that allows adjustment of the distance to the plate
- f. Ceramic plate – eg. Desiccators plate on a low stand, with a diameter that suits the hot plate

- g. Glass cover – eg., crystallizing dish, 185mm diameter, 100mm height, to fit on (f) for equivalent
- h. Wash – bottle – “Scrubber”, containing H₂SO₄ for purification of air
- i. Quarts or platinum crucibles – 50- 75ml
- j. Polydiamine bottles – with leak-proof closures, 100ml, carefully clean and rinse all glassware and plastic ware with HNO₃ or HCL to avoid metal contamination. Cleaning procedure for glass and plastic ware – Acid solution: 500ml concentrated HNO₃ C (c) +4500ml deionized water c(a) wash first with water and detergent. Rinse with tap water, followed by deionized water, then with dilute acid. Finally rinse 4-5 times with deionised water.

C. Reagent

Reagents should be at least analytical reagent grade (p.a) preferably ultrapure (Suprapur) or equivalent.

- a. Water – Redistilled or deionized, resistivity ≥18M Ω.cm
- b. Nitric acid – 65% (w/w)
- c. Nitric acid – 0.1M dilute 7 ml HNO₃ (c) with water (a) to 1h.

D. Procedures:

a. Pre-treatment – Aomogenize if necessary using non containing equipment check for leaching metals if the apparatus consists of metal parts.

Drying – In a crucible, weight 10-20g test portion to nearest 0.01g. Dry in a drying oven, on a water – bath, or a hot plate at 100C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace

Ashing

1. Ashing in a programmable furnace – place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum tae of 50°C /h to 450°C. Let dish stand for at least 8h or overnight. Continue according to (c)

2. Ashing in a muffle furnace with thermostat following drying and pre-ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in β (d) – (h) place crucible with the test portion covered with the glass tube sweep over the product. Put IR lamp down at the cover. Pre-ash product sample by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should than be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200-250°C and slowly raise temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8h or overnight. Take crucible out of furnace and let cool. Wet ash with 3ml water and separate on water or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50 -100°C/h) to 450°C. Proceed with ashing 450°C for 1-2h or longer. Repeat procedure until product is completely ashed, ie.ash should be white /gray or slightly covered. Number of repetitions varies depending on type of product. Add 5ml 6M HCL, c(b), to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water – bath or hot plate. Dissolve residue in 10.0 – 30.0ml, to the nearest 0.1ml, of 0.1M HNO₃ (d) Swirl crucible with care so that all ash comes into contact with acid. Cover with watch glass and let stand for 1-2h then stirring solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blank with each analytical batch.

d. Atomic absorption spectrophotometry – Pb and Cd in foods generally require graphite furnace AAS for determination of iron

Wavelength, gas mixture/ temperature program and other instrumental parameters that are most appropriate for each metal are found in the manual provided with the instrument.

Background correction must always be used in flameless AAS and for flame application at low concentrations.

When results are outside for the linear range, the test solutions should be diluted with 0.1M HNO₃, c(d).

Flame technique – prepare calibration curves from a minimum of 3 standards.

Graphite furnace (flameless) technique – The method of addition should always be used. Measurements must be made in the linear range when method of addition is used. Measurements are preferably made with peak area rather than peak height.

11. Estimation of zinc using Atomic Absorption Spectrophotometry:

Principle:

Test portions are dried and then ashed at 450°C under a gradual increase ($\leq 50^\circ\text{C}/\text{h}$) in temperature. 6M HCL (1+1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1M HNO₃ and the analytes are determined by flame and graphite procedures.

Apparatus

- a. Atomic absorption spectrophotometer – with an air – acetylene burner or nitrous oxide – acetylene burner for flame and a graphite furnace for electro thermal determinations, with appropriate back ground (non atomic) correction
- b. Hollow cathode or electrode less discharge lamps for all elements determined
- c. Furnace – programmable, or muffle furnace with thermostat maintaining $450 \pm 25^\circ\text{C}$. If muffle furnace is used, a separate pre-ashing device is required
- d. Hot plate – with heating control, to heat up to about 300°C
- e. Lamp – IR 250W, fixed to a retort stand in a way that allows adjustment of the distance to the plate
- f. Ceramic plate – eg. Desiccators plate on a low stand, with a diameter that suits the hot plate
- g. Glass cover – eg., crystallizing dish, 185mm diameter, 100mm height, to fit on (f) for equivalent
- h. Wash – bottle – “Scrubber”, containing H₂SO₄ for purification of air
- i. Quarts or platinum crucibles – 50- 75ml

- j. Polydiamine bottles – with leak-proof closures, 100ml, carefully clean and rinse all glassware and plastic ware with HNO_3 or HCL to avoid metal contamination. Cleaning procedure for glass and plastic ware – Acid solution: 500ml concentrated HNO_3 C (c) +4500ml deionized water c(a) wash first with water and detergent. Rinse with tap water, followed by deionized water, then with dilute acid. Finally rinse 4-5 times with deionised water.

C. Reagent

zinc standard solution – 1mg/ml. Dissolve 1.000 g Zn in 14ml water + 7ml HNO_3 , (c), in 1 volumetric flask. Dilute to volume with water.

Working standard solutions – (1) For graphite furnace analysis, - Dilute standard solution, (e) – (i), with 0.1M HNO_3 , (d), to a range of standards that covers the linear range of the element to be determined. (2) For flame analysis – dilute standards, (e) – (i), with 0.1M HNO_3 , (d), to a range of standards that covers the concentration of the element to be determined.

D. Procedures:

a. Pretreatment – Aomogenize if necessary using non containing equipment check for leaching metals if the apparatus consists of metal parts.

Drying – In a crucible, weight 10-20g test portion to nearest 0.01g. Dry in a drying oven, on a water – bath, or a hot plate at 100C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace

Ashing

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product sample by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should than be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200-250°C and slowly raise temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8h or overnight. Take crucible out of furnace and let cool. Wet ash with 3ml water and separate on water or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50 -100°C/h) to 450°C. Proceed with ashing 450°C for 1-2h or longer. Repeat procedure until product is completely ashed, ie.ash should be white /gray or slightly covered. Number of repetitions varies depending on type of product. Add 5ml 6M HCL, c(b), to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water – bath or hot plate. Dissolve residue in 10.0 – 30.0ml, to the nearest 0.1ml, of 0.1M HNO₃ (d) Swirl crucible with care so that all ash comes into contact with acid. Cover with watch glass and let stand for 1-2h then stirring solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blank with each analytical batch.

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method of addition is used. Measurements are preferably made with peak area rather than peak height

APPENDIX III

Development of value added product using *bacopa monnieri*

Bacopa monnieri powder:- Fifteen recipes suitable for bacopa monnieri incorporation were collected. 11 ideal recipes were selected for the study. The recipes selected for incorporation were Instant Peanut Dalia Dal day chutney powder, Instant curry leaf chutney powder, Instant kootu mix, Instant puliyogara mix, Instant pan cake mix, Instant Adai mix, Instant Rasam powder, Instant Idli chutney powder, Chocolate, Pinapple Jam, Tomato pickle.

1. Peanut dalia dal dry chutney powder

Name of Recipes	Ingredients	A	B	C	D	E
Instant Peanut Dalia Dal Day Chutney Powder	Peanut	50g	50 g	50 g	50 g	50 g
	Dalia Dhal	50 g	50 g	50 g	50 g	50 g
	Garlic Pod	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
	Red chilli Powder	10 g	10 g	10 g	10 g	10 g
	Salt	As required	As required	As required	As required	As required
	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

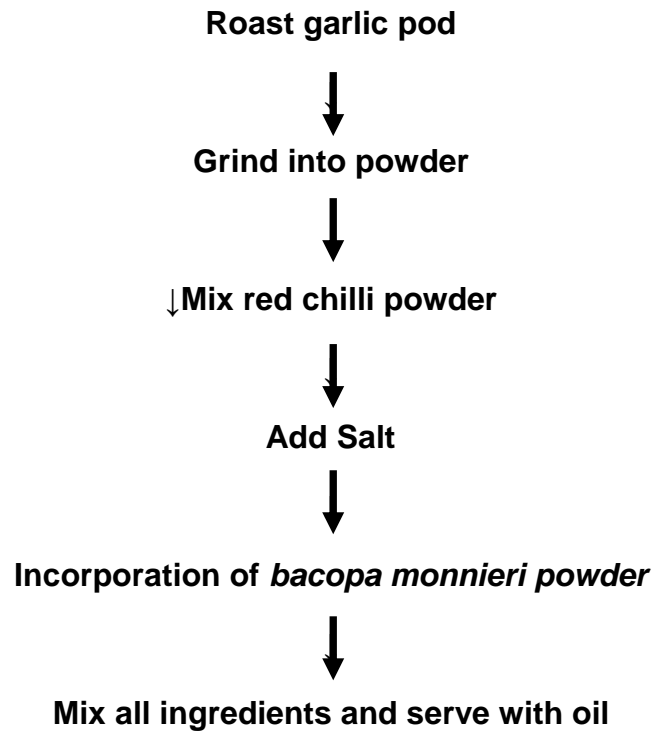
Preparation of peanut dalia dal dry chutney powder

Peanut dalia dal chutney powder



Dry roast peanut and roasted bengal gram dhal

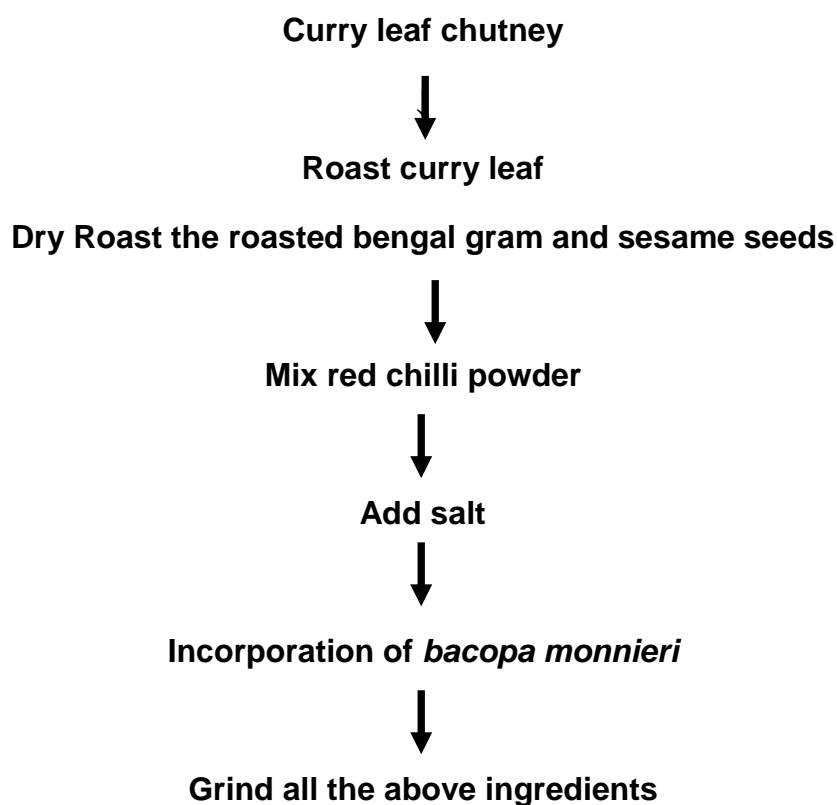




2. Instant curry leaf chutney powder

Name of Recipes	Ingredients	A	B	C	D	E
Instant curry Leaf Chutney Powder	Curry Leaf	50g	50g	50g	50g	50g
	Roasted Bengal gram	30g	30g	30g	30g	30g
	Red Chilli	5g	5g	5g	5g	5g
	Sesame seeds	20g	20g	20g	20g	20g
	Salt	As required	As required	As required	As required	As required
	Bacopa Monnieri Powder(mg)	1mg	2mg	3mg	4mg	5mg

Preparation of instant curry leaf chutney powder

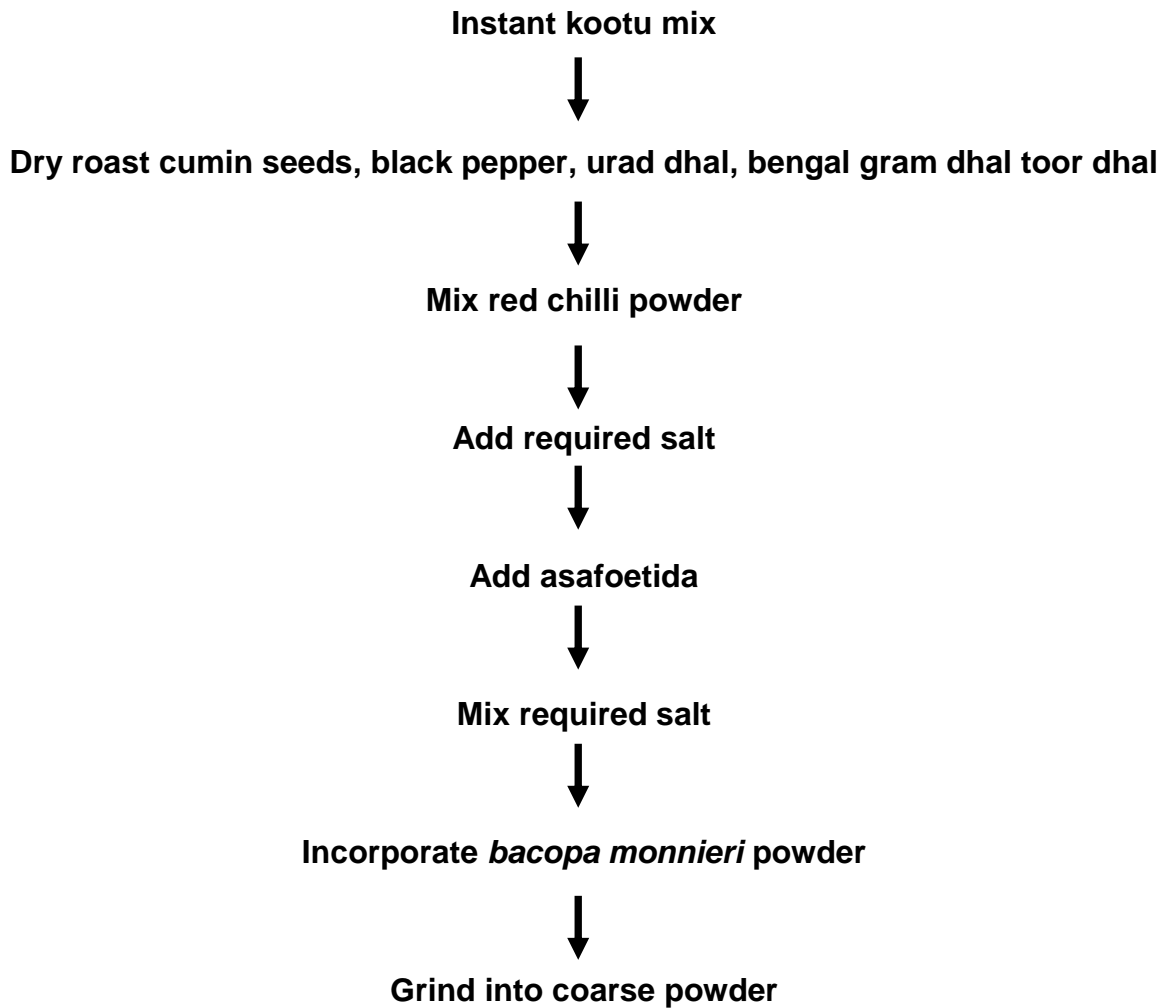


3. Instant kootu mix

Name of Recipes	Ingredients	A	B	C	D	E
Instant Kootu Mix	Cumin Seeds	25g	25g	25g	25g	25g
	Black Pepper	10g	10g	10g	10g	10g
	Urad dhal split	35g	35g	35g	35g	35g
	Bengal gram dhal	10g	10g	10g	10g	10g
	Toor dhal	10g	10g	10g	10g	10g
	Red Chillies	5g	5g	5g	5g	5g
	Asafoetida	A Pinch	A Pinch	A Pinch	A Pinch	A Pinch
	Salt	As required	As required	As required	As required	As required

	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg
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Preparation of instant kootu mix

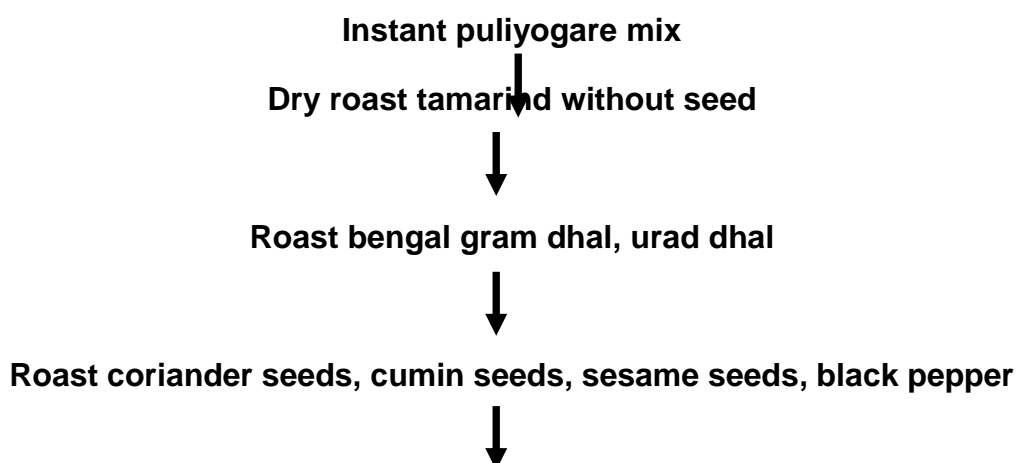


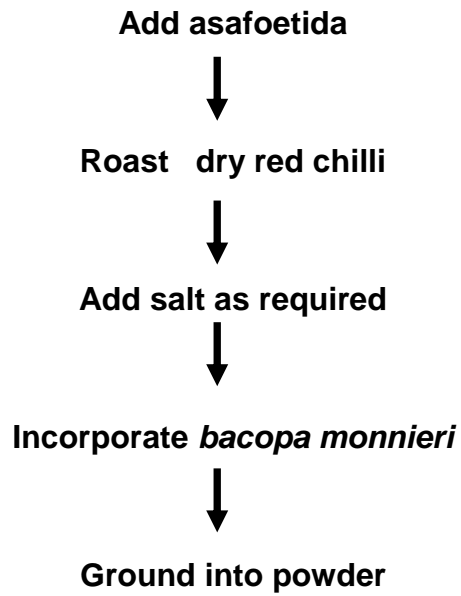
4. Instant puliyogare mix

Name of Recipes	Ingredients	A	B	C	D	E
Instant Puliyogara	Bengal gram dhal	20g	20g	20g	20g	20g
	Urad dhal	20g	20g	20g	20g	20g

Coriander Seeds	10g	10g	10g	10g	10g
Cumin Seeds	10g	10g	10g	10g	10g
Sesame Seeds	10g	10g	10g	10g	10g
Black Pepper	10g	10g	10g	10g	10g
Mustard Seeds	5g	5g	5g	5g	5g
Tamarind	10g	10g	10g	10g	10g
Turmeric Powder	A Pinch	A Pinch	A Pinch	A Pinch	A Pinch
Asafoetida	A Pinch	A Pinch	A Pinch	A Pinch	A Pinch
Dried red chilli	5g	5g	5g	5g	5g
Salt	As required	As required	As required	As required	As required
Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of instant puliyogare mix

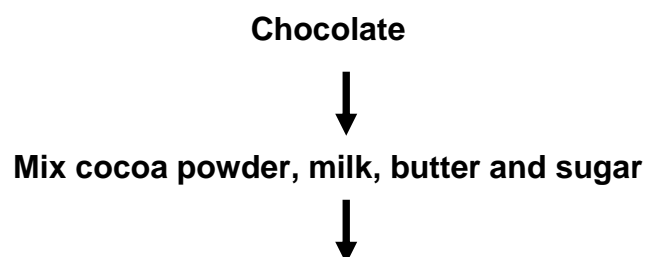




5.Chocolate

Name of Recipes	Ingredients	A	B	C	D	E
Chocolate	Coco Powder	60g	60g	60g	60g	60g
	Milk (ml)	15g	15g	15g	15g	15g
	Sugar	25g	25g	25g	25g	25g
	Butter	5g	5g	5g	5g	5g
	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of chocolate



Incorporate *bacopa monnieri* powder



mix well



Pour in mold and set into freezer

6.Pineapple jam

Name of Recipes	Ingredients	A	B	C	D	E
Pineapple Jam	Pineapple	100g	100g	100g	100g	100g
	Sugar	100g	100g	100g	100g	100g
	Lime Juice (mg)	5g	5g	5g	5g	5g
	Sodium Benzoate	A Pinch	A Pinch	A Pinch	A Pinch	A Pinch
	Bacopa Monnieri Powder (ml)	1mg	2mg	3mg	4mg	5mg

Preparation of pineapple jam

Pineapple jam



Grate the pineapple



In a bowl add the grated pineapple



Add sugar and cook well



Incorporate *bacopa monnieri* powder



Mix a teaspoon of lemon juice



Wait for the jam consistency and remove from fir

7. Tomato pickle

Name of Recipes	Ingredients	A	B	C	D	E
Tomato Pickle	Tomato	100g	100g	100g	100g	100g
	Gingerly Oil	50g	50g	50g	50g	50g
	Mustard seeds	5g	5g	5g	5g	5g
	Red Chilli Powder	15g	15g	15g	15g	15g
	Asafoetida	A Pinch	A Pinch	A Pinch	A Pinch	A Pinch
	Salt	As required	As required	As required	As required	As required
	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of tomato pickle

Tomato pickle



Blanch the tomatoes

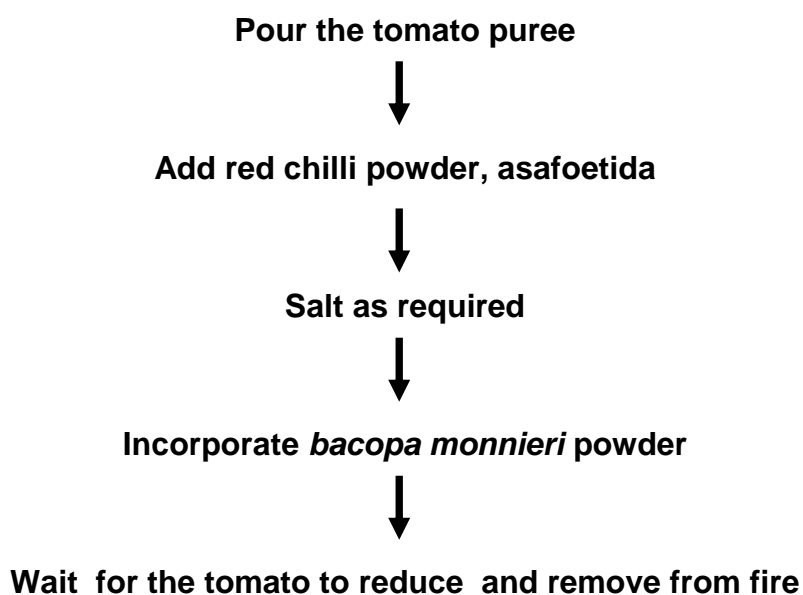


Make into puree



In a deep bottom pan add gingelly oil





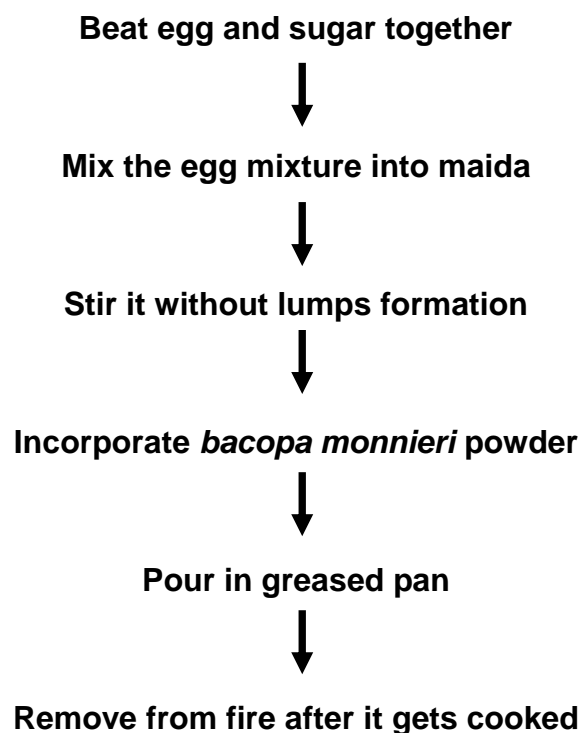
8. Instant pancake mix

Name of Recipes	Ingredients	A	B	C	D	E
Instant Pan Cake Mix	Maida	75g	75g	75g	75g	75g
	Baking Soda	5g	5g	5g	5g	5g
	Salt	As required	As required	As required	As required	As required
	Sugar	20g	20g	20g	20g	20g
	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of instant pancake mix

Instant pan cake





9. Instant adai mix

Name of Recipes	Ingredients	A	B	C	D	E
Instant Adai Mix	Rice	40g	40g	40g	40g	40g
	Urad dhal	15g	15g	15g	15g	15g
	Bengal gram Dhal	20g	20g	20g	20g	20g
	Curry leaf	20g	20g	20g	20g	20g
	Red Chilli	5g	5g	5g	5g	5g
	Salt	As required	As required	As required	As required	As required
	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of instant adai mix

Instant adai mix



Roast rice, urad dhal, bengal gram dhal,curry leaf



Roast dry red chilli



Add salt and incorporate *bacopa monnieri*



Ground the roasted mix into coarse powder



Add water and mix



Pour in pan and remove it after cooking

10.Instant Rasam powder

Name of Recipes	Ingredients	A	B	C	D	E
Instant Rasam Powder	Coriander Seed	40g	40g	40g	40g	40g
	Pepper	20g	20g	20g	20g	20g
	Cumin Seed	20g	20g	20g	20g	20g
	Red Chilli	20g	20g	20g	20g	20g
	Turmeric Powder	A Pinch	A Pinch	A Pinch	A Pinch	A Pinch
	Bacopa Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of instant rasam powder

Instant rasam mix



Dry roast coriander seed, pepper, cuminseed, redchilli powder



Add turmeric and *bacopa monnieri* powder



Grind well



Add in hot water with tomato and leave it for boil for preparing rasam

11. Instant idli chutney powder

Name of Recipes	Ingredients	A	B	C	D	E
Instant Idli Chutney Powder	Urad Dhal	50g	50g	50g	50g	50g
	Sesame Seed Black	20g	20g	20g	20g	20g
	Dried Red Chillies	5g	5g	5g	5g	5g
	Pepper	10g	10g	10g	10g	10g
	Curry leaf	10g	10g	10g	10g	10g
	Salt	5g	5g	5g	5g	5g
	Bacop Monnieri Powder (mg)	1mg	2mg	3mg	4mg	5mg

Preparation of Instant idli chutney powder

Instant idli chutney powder



Dry roast urad dhal, black sesame seed, dry red chillies, pepper and curry leaf one by one



Incorporate *bacopa monnieri* powder



Add salt and make into fine power



Add oil and serve

APPENDIX III
QUESTIONNAIRE
ON
SENSORY EVALUATION

NAME:

YEAR:

Name of the Product:-

Sample	Appearance	Flavour	Taste	Colour	Texture	Overall Acceptability
A						
B						
C						
D						
E						

Five Points hedonic scale:

5- EXCELLENT

4-VERY GOOD

3- GOOD

2- FAIR

1- POOR

DATE:

PARTICIPANT SIGNATURE