Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products

ASMA K

(20PFN003)

THESIS SUBMITTED TO

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE -641 043.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

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Certificate

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25/5/22

Signature of the Supervisor

25.5.22

Signature of the Head of the Department

Declaration

DECLARATION

I hereby declare that the dissertation entitled "**Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products**", submitted to the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, in partial fulfilment of the requirement for the award of the **Degree of Master of Science in Food Science and Nutrition** is a record of original research work done by me under the supervision and guidance of **Prof. (Mrs.). C. A. Kalpana**, Professor, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and that it has not formed the basis for the award of any Degree/ Diploma/ Associateship /Fellowship or similar title to any candidate of any other University and it represents entirely an independent work on the part of the Candidate.

Kalpana 25/5/22
Signature of the Supervisor

Asma. K 25.05.2022
Signature of the Candidate

Acknowledgement

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Introduction

I. INTRODUCTION

Vitamin D and Calcium play a vital role in bone mineralization. Vitamin D a fat soluble vitamin is also known as the sunshine vitamin, because the sterols present in the skin can be converted to vitamin D with the help of UV rays emitted from the sun. McCollum (1922) was first to coin the term vitamin D for its anti rachitic activity. Vitamin D is termed as calciferol by Augus (1931). Rickets is derived from an old English word wrickken meaning twist. Vitamin D is required to maintain normal blood levels of calcium and phosphate that are in turn need for the normal mineralisation of bone, muscle contraction, nerve conduction and general cellular function in all cells of the body (Chang *et al.,* 2019).

Vitamin D, either from diet or endogenously synthesized is transported bound to vitamin D binding protein to the liver, where it is hydroxylated into 25-hydroxyvitamin-D (25(OH) D or calcidiol), by 25-hydroxylase enzyme as the active form of Vitamin D (Cardoso *et al.*, 2018). It plays an important role in enhancing the absorption of calcium and phosphorus from the intestine and helps to maintain calcium homeostasis (Veldurthy *et al.*, 2016). Serum concentration of 25(OH) D is best indicator of vitamin D status which reflects vitamin D produced endogenously and obtained from diet and supplements .Recommended Dietary Allowance (RDA) of 600-800 IU is needed to maintain adequate levels of vitamin D (ICMR, 2020).

Vitamin D Deficiency has been a pandemic all over the world. Almost 50 percent of the world population has been affected by Vitamin D insufficiency (Holick *et al.*, 2017). Prevalence rates of severe vitamin D deficiency, defined as 25(OH)D <30 nmol/L (or 12 ng/ml), of 5.9 per cent (US), 7.4 per cent (Canada), and 13 per cent (Europe) have been reported. Estimates of the prevalence of 25(OH)D levels <50 nmol/L (or 20 ng/ml) have been reported as 24per cent (US), 37 per cent (Canada), and 40 per cent (Europe). Worldwide, many countries report very high prevalence of low vitamin D status. 25(OH) D levels <30 nmol/L (or 12 ng/ml) in >20per cent of the population are common in India, Tunisia, Pakistan, and Afghanistan. For example, it has been estimated that 490 million individuals are vitamin D deficient in India (Amrein *et al.*, 2020).

Such high prevalence is because of inadequate intake of vitamin D and calcium sources in the diet. Sunlight being the major source providing vitamin D is still not sufficient due to less exposure to our skin which results in less absorption of calcium in our body.

Vitamin D deficiency has been linked to several health outcomes, including musculoskeletal (rickets, bone fractures, osteomalacia, osteopenia, osteoporosis and muscle weakness) and non-skeletal complications (WHO, 2019).

Vitamin D deficiency is strongly associated with various cardiovascular and metabolic diseases such as hypertension, type 1 diabetes, myocardial infarction, and stroke. Moreover, vitamin D deficiency is related to several autoimmune diseases such as rheumatoid arthritis, systemic sclerosis, and systemic lupus erythematosus. Studies also have shown a negative correlation between serum vitamin D concentration and incidence of colorectal cancer and breast cancer (Kheiri *et al.*, 2018).

In foods, vitamin D has two main forms, D_2 (ergocalciferol) and D_3 (cholecalciferol). It differ chemically only in their side-chain structures. Ergocalciferol is of plant origin and cholecalciferol is of animal origin. The precursor for ergocalciferol is ergosterol and cholecalciferol is 7- dehydro cholesterol. Ergocalciferol is present in finger millet, mushroom, gardencress seeds,gingellyseeds, soya bean,rajmah , colocasia leaves and amaranthus seeds . Cholecalciferol is present in butter, milk, egg yolk, cod liver oil, cheese, liver (lamb) and shrimp.

Our Indian traditional diet lacks in fulfilling the daily Recommended dietary allowance (RDA) of Vitamin D and Calcium due to which their deficiencies prevails the most in our country. It is one of the most under treated and under diagnosed nutritional deficiencies which prevails about 70-100 per cent in the general population irrespective of age, gender, race, geography and socio religious and cultural practices (Ridhi and Ankita, 2018).

Considering that only few natural foods contain vitamin D, the best way to receive adequate daily amounts of vitamin D is obtaining it from supplements or fortified foods (Barnkob *et al.*, 2016). Despite the abundance and availability of vitamin D fortified products and supplements; the problem of vitamin D deficiency has not been solved yet. Some possible explanations could be an inadequate level of fortification or poor coverage of these foods by people due to food accessibility, allergic reactions to lanolin in supplements, or food avoidance due to veganism. Therefore, an alternative solution to meet the vitamin D requirement in the population is needed (Salemi *et al.*, 2020).

Dietary sources of Vitamin D are very less in vegetable diet and others which contain are from animal sources such as egg yolk, fish liver oil and fatty fish. Sunlight is the main source of Vitamin D (Roseland *et al.*, 2017). Button mushroom when exposed to UV light have the propensity to absorb ample amount of vitamin D (Loznjak and Jakobsen, 2018).

Agaricus is one of the largest genera of macrofungi, with several edible species that have medicinal and high nutritional values (Zhang *et al.*, 2017). Button mushroom is another name of *A. bisporus* and it is a valuable source of food and several important bioactive compounds.*A. bisporus* is rich in nutrients such as carbohydrates, amino acids, fats, and minerals and has potential anticancer, antioxidant, anti-obesity, and anti-inflammation properties The bioactive compounds extracted from this mushroom can be used for the treatment of several common human diseases including cancer, bacterial and fungal infections, diabetes, heart disorder, and skin problems (Usman *et al* ., 2021).

The white button mushroom (*Agaricus bisporus*) is very popular throughout the world and is the most important mushroom of commercial significance in India. The use of a UV-radiated mushroom food ingredient was effective in maintaining 25(OH) D in healthy, recreationally active volunteers. Mushroom-derived vitamin D_2 powder may offer an adjunct strategy as a more cost-effective and widely applicable food ingredient for populations, including vegans and vegetarians, with low vitamin D status in supporting their vitamin D intake (Pinto *et al.*,2020)

Vitamin D acquired from sun exposure, foods, and supplements is inert biologically. It has to undergo two steps of hydroxylation in the body for activation as calcidiol (in liver) and calcitriol (in kidney). It is essential for bone growth and bone remodelling (osteoblasts and osteoclasts). Inadequate vitamin D may prone bones to become thin or brittle.

Along with calcium, vitamin D aids to protect older adults by preventing osteoporosis. Vitamin D is critical for maintaining calcium homeostasis and assists in the active transport of calcium across the intestinal lumen (Hess *et al.*, 2016). Phosphorus, magnesium, and zinc are all critical to maintaining a healthy skeleton (Thorning *et al.*, 2016). Vitamin D has also other roles such as reduction of inflammation, modulation of cell growth, neuromuscular and immune function, and glucose metabolism. It also modulates many genes encoding proteins which regulate cell proliferation, differentiation and apoptosis. Calcium is an essential element that serves an important role in skeletal mineralization (Elaine Yu *et al.*, 2021).

Calcium is the most abundant cation found in the human body and plays an integral role in neural transmission, enzyme activity, myocardial function, coagulation and other cellular functions. Most of the calcium is found in the bones as calcium phosphate while a small percentage is found in the cells and extracellular fluids. It is a vital component of bone architecture and is required for deposition of bone mineral throughout life (Sadiq *et al.*, 2022).

Calcium requirements are high during all stages of life. Dietary reference values for individuals over 19 years of age vary from 1000 mg to 1300 mg, depending on the reference guidelines. High intakes of calcium and vitamin D potentiate the bone loss prevention effects of hormone replacement therapy in postmenopausal women. Pregnancy and lactation are not risk factors for skeletal fragility, although lactation is associated with a transient loss of bone that cannot be prevented by calcium supplementation. Low calcium intake has been implicated in the development of hypertension, colon cancer, and premenstrual syndrome, and it is associated with low intakes of many other nutrients (Cormick *et al.*, 2019).

Dietary calcium deficiency is considered to be widespread globally, with published es timates suggesting that approximately half of the world's population has inadequate access to dietary calcium. Evidence from HICs suggests that intakes below 800 mg/day in adults is suboptimal, although most populations in LMICs have intakes closer to 400–500 mg/day without strong evidence that these levels cause adverse bone outcomes. Extended breast-feeding without sufficient vitamin D and complementary food sources of calcium, as well as extremely restrictive diets, including vegan diets, also increase the risk of nutritional rickets (Shlisky *et al.*, 2022).

Calcium rich foods are dairy products, especially hard cheese that can provide 1 g of calcium per 100 g, whereas milk and yogurt can provide between 100 mg to 180 mg per 100 g. Cereals usually have around 30 mg per 100 g, however if they are fortified, the amount can reach 180 mg per 100 g. Nuts and seeds are also rich in calcium, especially almonds, sesame and chia that can provide between 250 to 600 mg per 100 g. Vegetables rich in calcium are kale, broccoli and watercress, which provide between 100 and 150 mg per 100 g (USDA ,2016).

Globally approximately (70%) of adults are deficient in intestinal lactase, the enzyme required for the digestion of lactose, therefore they cannot rely on dairy products for their Ca requirement. Incidentally, most of these lactose-intolerant people live in Asian and African regions which are predominantly dependent on agriculture-based economies (Forsgard and Richard, 2019). Finger millet (*Eleusine coracana*) is an important millet grown extensively

in various regions of India and Africa, constitutes as a staple food for a large segment of the population in these countries. It ranks sixth in production after wheat, rice, maize, sorghum and bajra in India. It has the highest calcium content of 344 mg /100 g.

Finger millet possesses all the quantitative and qualitative traits to serve as a model for Ca bio fortification. Anti-nutritional factors found in finger millet include tannins, non-starch polysaccharide (glucans), protease inhibitors, oxalates, and phytates, each of which can directly or indirectly affect nutrient digestibility. Soaking and sprouting can greatly reduce the anti-nutrient factor and improve protein digestibility (Kumar *et al.*, 2016).

Depending on the type of processing, calcium bioavailability either increased or decreased. One in vitro study showed that calcium bioavailability from finger millet was (28.6%) boiled, whereas three studies on processing show that certain processing can double the calcium bioavailability to (61.4%). Irrespective of the type of processing, finger millets contribute to high calcium retention and extremely high bio available Calcium and could be useful for healthy growth and in dealing with complications related to calcium deficiency (Anitha *et al* ., 2021).

An observational study revealed that food sources have immense benefit when compared with supplement (Muscogiuri and Barrea *et al.*, 2019). New technologies and ingredients are being introduced worldwide to fulfil nutritional needs. The changing demography, increasing purchasing power, increasing women work force and staying away from home have lead to increased demand for more convenient Ready To Eat (RTE) and Ready To Cook (RTC) products. Convenient foods are commercially prepared processed foods, which are designed for the ease of preparation and consumption.

Utilisation of millets is restricted due to non-availability of processed foods in ready to eat form. Millets can be effectively utilised for developing value added products which can improve the overall diet quality. The changes in life style and dietary pattern, increasing awareness about healthy food habits has made healthy convenient foods a perfect choice. As a major source of energy, cereals have important role in our daily diets. Millets are small seeded cereals known as nutri cereals which represent rich sources of photochemical and micronutrients (Zacharia *et al.,* 2020)

Due to so many advantages, the addition of vitamin D and calcium ingredient in food matrices such as cookies, flakes and soup mix is pertinent. Baked products are popular because of their availability, ready to eat convenience and having good shelf life. Therefore large scale production and distribution is possible. Common bakery products include breads, cookies, pastries, muffins, cake, bread etc.

Cookies are widely consumed and generally, they are rich in carbohydrates, fats and calories, but low in fibre, vitamins and minerals. Currently, fortification of cookies has evolved to improve its nutritional and functional quality (Awolu *et al.*, 2016). Cookies occupy an important position in the snack food industry due to their variety in taste, crispiness and digestibility, consequently, they represent a valuable vehicle of supplementation with nutrients because of their popularity, relatively low cost, varied taste, ease of availability, high nutrient density and long shelf life (Dourado Gomes Machado *et al.*, 2021).

Soup is one of the traditional foods which can be classified as an appetizer, warm food during cold and sick. In the modern world commercially prepared instant soup has replaced homemade soup as preparing a soup is a time consuming process (Ansari et *al.*, 2021).

Flakes is a leading source of nutrients in people's diets, making it one of the most nutrient dense choices for the number of calories it provides. If the millet is made available to the community in convenient forms, the nutritional and nutraceutical benefits of the millet will reach the community. Flakes consumed with milk also enhances the nutrient absorption .Millet based soup is extremely beneficial for health and a better alternative for other conventional corn starch and all-purpose flour soups.

With this view the present study entitled "**Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products"** was conducted with the following objectives:

To

- Formulate food products from vitamin D_2 and calcium rich sources.
- Evaluate the Organoleptic properties of the developed food products
- Analyse their physicochemical properties
- Assess the shelf life of the developed food products

Review of literature

II. REVIEW OF LITERATURE

The review of literature pertaining to the study entitled "Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products" is presented under the following headings.

A. Prevalence of Vitamin D and Calcium

B. Sources of Vitamin D and Calcium

C. Nutritive value of finger millet and button mushroom

D. Products and supplementation available for Vitamin D and Calcium

A. Prevalence of Vitamin D and Calcium

Calcium and vitamin D are inseparable nutrients required for bone health. In the past half a century, the dietary calcium intake of rural, tribal, and urban India has declined. A study revealed that 85 per cent of the Indian population are vitamin D deficient despite abundant sunlight. Dietary calcium deficiency can cause secondary vitamin D deficiency. Though India as a nation is the largest producer of milk, there is profound shortage of calcium intake in the diet with all negative consequences on bone health. There is a decline in dietary calcium in the background of upward revision of RDI/RDA. There is a gap in the production-consumption-supply chain with respect to dietary calcium (Harinarayanan *et al.*, 2021).

The prevalence of Vitamin D deficiency is reported worldwide, both in sunshine deficient and sunshine sufficient countries. Still, it is the most under diagnosed and undertreated nutritional deficiency in the world. However, various studies showed poor Vitamin D status irrespective of age, sex, and geography. As there is no standard guideline which is followed all over the world for classifying the Vitamin D status, these studies had different cut off values for the deficiency. The vast majority of these studies used serum 25(OH) D level of <20 ng/ml as Vitamin D deficiency (Aparna *et al.*, 2018).

India is coming to grips with a stage of nutrition transition. Preventable micronutrient deficiency is arising public health precedence in India. However, the foremost public health

concern is the lack of national prevalence data (*fssai*, 2021). A study carried out to estimate the pooled age-wise prevalence of six preventable micronutrient deficiencies (vitamin A, vitamin B_{12}, vitamin D, iron, iodine and folic acid) in India. The results revealed to reinforce the data on micronutrient deficiency in India and warrant the immediate need for further active public health interventions to address these deficiencies (Venkatesh *et al.*,2021)

Deficiency of vitamin D or hypovitaminosis D is widespread irrespective of age, gender, race and geography and has emerged as an important area of research. Vitamin D deficiency may lead to osteoporosis (osteomalacia in adults and rickets in children) along with calcium deficiency. Its deficiency is linked with low bone mass, weakness of muscles and increased risk of fracture. A high prevalence of hypovitaminosis D was observed among different age groups. Hypovitaminosis D ranges from 84.9 to 100 per cent among school-going children, 42 to 74 per cent among pregnant women, 44.3 to 66.7 per cent among infants, 70 to 81.1 per cent among lactating mothers and 30 to 91.2 per cent among adults was there (Kamboj *et al.*, 2018).

India is a diverse country with diverse cultural and dietary habits. The dressing and dietary habits vary between different regions and different socioeconomic classes. Indians come under the skin class category V (dark skin). With modernization and mechanization of lifestyle, the indoor working hours has increased, limiting sun exposure, and different dress codes in different regions of the country and population sects, increasing pollution, and increased usage of skin creams with SPF (Skin protection factor) >15 could account for the vitamin D deficiency (Harinarayanan and Akhila , 2019).

A study conducted by (Srimani *et al.*, 2017) revealed that high prevalence of metabolic syndrome as well as vitamin D insufficiency and deficiency existed among Post menopausal women of Singur block, West Bengal, India. 25(OH) D had significant inverse and direct relationship with fasting blood glucose and waist circumference. Low 25(OH) D may be one of the potential risk factors for developing metabolic syndrome in premenopausal women .A school based on 1222 school children aged 6–18 years, showed the prevalence of 81 per cent and 80 per cent in Kangra and Kullu districts of Himachal Pradesh respectively (Kapil *et al.*, 2017).

A study carried out by (Gaddas *et al.,* 2022) revealed that vitamin D and calcium supplementation to obese and prepubescent children, led to the reversibility of the pancreatic β-cells dysfunction. A study revealed that vitamin D deficiency is highly prevalent in this urban south Indian population and was higher among individuals with T2DM and prediabetes compared with those with NGT (Jayashri *et al.*, 2021). One of the most commonly observed

conditions that hamper immunity is Vitamin D. It has been linked to the onset and the alteration of course of the disease in COVID – 19 patients and is also being explored as a potential drug supplement (Saxena *et al.*, 2022).

The analysis by (Murdaca *et al.*, 2019) demonstrated an inverse association between vitamin D and the development of several autoimmune diseases, such as SLE, thyrotoxicosis, type 1 DM, MS, iridocyclitis, Crohn's disease, ulcerative colitis, psoriasis vulgaris, seropositive RA and polymyalgia rheumatica. A study by Sharma *et al.,* 2019 revealed that there was very high prevalence of vitamin D deficiency in SUI patients with (75%) patients showing deficient levels and (17.5%) showing insufficient levels in SUI patients. Vitamin D is essential for gastrointestinal calcium absorption, mineralization of osteoid tissue and maintenance of serum ionized calcium level. It is also important for other physiological functions, such as muscle strength, neuromuscular coordination, hormone release, subduing autoimmunity, and curtailing the development of certain cancers (Wimalawansa *et al.*, 2017).

Vitamin D plays a major role in suppressing Rheumatic Heart Disease (RHD), the major cause of cardiac related deaths (Sarkar *et al.*, 2016). The role of vitamin D in preventing liver diseases is associated with its potential to down regulate several signal transduction pathways, which allows for expression of interferons, chemokines and pro-inflammatory genes such as TNF-α, IL-4, IL-6 and the toll-like receptors, reducing the risk and providing protection against hepatitis B, liver inflammation, liver cirrhosis and hepatocellular carcinoma (HCC) (Elangovan *et al.*, 2017).

Globally, 800 million people are undernourished. More than 3.5 billion people are at risk of calcium deficiency and 90 per cent of those at risk, are in Africa and Asia. Review of the global calcium map reveals that there are many countries in which calcium intake is very low, under 400 mg/day are clustered in the Asia-Pacific region and include countries with large populations such as China, India, Indonesia, and Vietnam, among others. Countries in the next lowest intake categories, 400 to 500 and 500 to 600 mg/day, are clustered in South America (Argentina, Bolivia, Brazil) and scattered throughout the far East, North Africa, and elsewhere (Balk *et al* ., 2017).A study by Meenakshi and Hajistha (2019) revealed the high Prevalence of calcium deficiency among adolescent girls in Tamil Nadu.

There is a widespread dietary calcium and vitamin D deficiency in India. For over half a century, the intake of dietary calcium, milk, milk products, and cereals has declined drastically. There is a clear rural–urban divide in consumption of milk, milk products and cereals. The deficiency of these nutrients manifests as rickets/osteomalacia and/or osteopenia /osteoporosis, which is realized at much later age. Stunting and some of the calcium paradox

diseases could be attributed to the deficiency of these nutrients. Fortification of food with only vitamin D in India in the background of dietary calcium deficiency is doing a disservice to the population/society (Harinarayanan and Akhila, 2019)

B. Dietary source of vitamin D and Calcium

Vitamin D present in animal foods as cholecalciferol (Vitamin D_3) and in plant foods as ergocalciferol (Vitamin D_2) found in mushroom. Vitamin D_3 is present in fatty fish, egg, liver etc., and Vitamin D_2 is present in finger millet, mushroom, soya bean , rajma etc., (IFCT,2017). Mushroom is an excellent vegan source when compared with supplement as it has no significant difference in increasing the serum vitamin D level (Neufinger and Eliander, 2021).

As dietary staples, relatively small improvements in grain composition (especially in starch and fibre) have the potential to translate into significant health gains at the population level when they are incorporated into food Millets are one of such kind of the cereal. Millet is a cereal crop plant belonging to the grass family, *Graminae*. Millets are highly nutritious, non-glutinous, non-acid forming, least allergenic and most digestible available grains. Hence, it is also called as “nutri-cereals”. Various types of millets are cultivated in the world. Among these, finger millet (*Eleusine coracana*) is the most widely grown millet in India. Finger millet is originally native to Ethiopian highlands and was introduced in India 4000 years ago. Its grain is rich in methionine, tryptophan, cysteine, tyrosine, calcium, phosphorous, and iron, making the crop an excellent nutritional source compared to other major cereals (Gupta *et al.*, 2017).

Finger millet has the potential to grow in marginal agro ecological zones where other crops may not, and the grains have a long shelf life (Onyango, 2016). These attributes make it a valuable food crop and genetic resource that is critical for global food security. Its production is 4.5 million tons per annum with 2.0 million tons being produced in Africa (Sakamma *et al.*, 2018). Given that new finger millet products such as bakery products, snacks, pasta, and sweet products are becoming increasingly popular, the demand for this crop is steadily increasing (Onyango, 2016).

Due to rich in dietary nutritional profile it has numerous therapeutic effects. Finger millet seed coat portion hinder the intestinal pancreatic amylase and α-glucosidase which leads to control postprandial hyperglycemia. Therefore, regular intake of finger millet as a staple food and whole meal based product will help in administrating unusual disorders of our

body by regulating the proper blood glucose level. Wound healing is impaired in diabetic patients due to damage in nerve growth factor and various research work have shown that finger millet extracts outcomes in ameliorating this impairment via improving the nerve growth factor production and antioxidant level (Chandra *et al.*, 2016)

Finger millet has high calcium content (350 mg/ 100 g). Milled finger millet is rich in dietary fibre and micronutrients. Finger millet's fat consist oleic acid, linoleic acid, palmitic acid and traces of linolenic acid. Layers of Seed coat of the finger millets contain tannins and polyphenols. Phenolic compounds (both free and bound forms) are present in very good amount in finger millet. Several flavonoids such as orientin, isoorientin, vitexin, isovitexin, saponarin, violanthin, lucenin-1 and tricin (antitumour and anti-leukemic properties) founded in finger millet's leaves. Finger Millet has antioxidant, antimicrobial and antihypocholestrolemic, antifungal and antibacterial, antidiabetic (type 2 diabetes), nephroprotective, wound healing and anticataractogenesis properties (Ektasingh and sarita, 2016). Even though finger millet is nutritionally superior to other staple crops; presence of anti nutrients makes the micronutrients to become less bioavailable to the body (Netravati *et al.,* 2018)

The amount of vitamin D_2 generated depends on the time of day, season, latitude, weather conditions, and exposure time. Since these mushrooms have a higher surface area to volume (hence, more ergosterol is exposed), sun-exposed sliced mushrooms produce more vitamin D_2 than whole mushrooms from the same amount of UV radiation exposure (Urbain *et al.*, 2016)

Mushrooms are valuable nutritional foods with recognized bioactive properties, leading the application of UV irradiation to the production of significant amounts of vitamin D_2. UV-irradiated mushrooms present a high rate of conversion from ergosterol to vitamin D_2 at short treatment time and have the potential to increase serum 25-hydroxyvitamin D levels (Taofiq, 2017). Commercial dried mushrooms have a much longer shelf life than fresh mushrooms, often with a 'best-before' date of 2–3 years after packaging. They have about 15% of the original weight of fresh mushrooms, making them cheaper to transport and, potentially, a cheaper source of vitamin D_2 (Cardwell *et al.*, 2018).

Sun-drying is one method used for drying mushrooms in Asian countries. Analysis of vitamin D_2 and ergosterol content of 35 species of dried mushrooms sold in China revealed they contained significant amounts of vitamin D_2, with an average of 16.9 µg /g DM (range of 7–25 µg/g DM (Huang *et al., 2016*). A randomized controlled trial (RCT) data on the

label of serum 25-hydroxyvitamin D in healthy individuals that consumed UV light treated edible mushrooms was reported by Cashman *et al.*, (2016).

Agaricus bisporus is considered an important factor for biosynthesis of silver nanoparticles (AgNPs) which possess anti-microbial factor (Owaid *et al.*, 2017 and Owaid and Ibraheem, 2017). Yang *et al.*, (2016) demonstrated that lovastatin exerts anti-cancer effects in the triple-negative breast cancer cell line MDA-MB-231.A. bisporus contains high levels of dietary fibres and antioxidants including vitamin C, D, and B12; folates and polyphenols that may provide beneficial effects on cardiovascular and diabetic diseases (Calvo *et al.*, (2016).

A.bisporus may provide significant support against malnutrition due to high nutritional values especially in developing and undeveloped countries. Consumption of A. bisporus is not only useful in case of nourishment, but also existing the medicinal benefit of mushroom, especially as anticancer, anticardiovascular disease, anti diabetes, antioxidant, and antimicrobial. In the last decades, edible mushroom has been used as a source of treatment or health food supplements increasingly (Atila *et al.*, 2017).

To maximize absorption, frequent consumption of calcium sources spread throughout meals is recommended; prioritize low oxalate leafy greens such as bok choy, broccoli, napa cabbage, collard greens, dandelion greens, kale, turnip greens, and watercress; be wary of excessive intakes of sodium, protein, caffeine, and phosphorus (as from sodas); and ensure normal serum vitamin D levels (Hever and Crosine ,2017).

C. Nutritive value of Finger millet and Button mushroom

Finger millet grain has a carbohydrate content of (81.5%), protein (9.8%), crude fiber (4.3%), and mineral (2.7%) which is comparable to other cereals like rice, wheat, maize and millets. Its crude fibre and mineral content is remarkably higher than those of wheat (1.2% fibre, 1.5% minerals) and rice (0.2%fiber, 0.6% minerals); its protein profile is relatively well balanced; as it contains more lysine, threonine, and valine than other millets. In addition to this black finger millet contains 8.71 mg/g dry weight fatty acid and 8.47 g/g dry weight protein. Finger millet varieties contain calcium (220–450) and iron (3–20%) respectively. The finger millet contains important amino acids viz., isoleucine (4.4 g), leucine (9.5 g), methionine (3.1 g) and phenyl alanine (5.2 g) which are deficient in other starchy meals. Millets also contains B vitamins, especially niacin, B6 and folic acid calcium, iron, potassium, magnesium and zinc (Rathore *et al.,* 2019)

Germinated and raw finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) were investigated for their physicochemical (pH, total titratable acidity (TTA), proximate, mineral analysis), phytochemical, and antioxidant properties. The results showed that there were decreases in pH (8.50–7.60) with a corresponding increase in TTA (0.0038–0.18 g/L) during germination of the millets. Proximate composition of the millets revealed slight increases in protein (7.61%–7.81%; 10.57%–11.87%) and crude fibre (5.54%–8.81%; 1.07%–2.55%) with reductions in fat (3.84%–2.73%; 7.69%–2.30%) after germination for finger and pearl millet, respectively (Owheruo *et al.,* 2018).

The millets were found to be rich sources of minerals, which include magnesium (1,028.42–1,763.50 ppm), calcium (36.42–4,158.40 ppm), sodium (150.00–510.00 ppm), potassium (470.00–4,500 ppm), zinc (20.00–40.00 ppm), and iron (66.00–121.00 ppm) which either decreased or increased with germination. The results of the phytochemical composition revealed that during germination, alkaloids (36.03–74.53 mg/g) and saponins (4.46–31.91 mg/g) contents were found to increase while there were reductions in tannin (0.88–1.64 mg/g) and phytate (7.00–17.72 mg/g) content of the flour. For finger millet, DPPH ranged from 70.00% to 72.14% and pearl millet (49.95%–64.01%), while for FRAP, pearl millet (53.69–53.76 mg/g) demonstrated better activity compared to finger millet (46.91–53.54 mg/g). Findings from this work may suggest that further studies should be carried out on germinated finger and pearl millets to examine their abilities to serve as functional food (Himanshu *et al*., 2018).

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Finger millet contains carbohydrate 81.5%, dietary fibre 18% to 20%, starch 65% to 75%, protein 9.8%, fat 1% to 1.7%, minerals 2.7% and crude fibre 4.3% that is equivalent to other millets and cereals. Its mineral content & crude fibre are specifically higher than that of rice (minerals 0.6%, fibre 0.2%) and wheat (minerals 1.5%, fibre 1.2%); its protein content is reasonably well balanced; additionally, it also contains lysine, valine and threonine than other millet (Rathore *et al.*, 2019).

Edible mushrooms are rich sources of protein (3.27±0.12), fibre (1.87±0.08), carbohydrates (2.66±0.61), fats (0.22±0.05) and energy (28.50±1.22 Kcal) g/100g fresh weight basis respectively. In addition to these, white button mushrooms also contain the highest content of potassium (3560±153.33) and sulfur (2195.59±1405.60) mg/kg fresh weight basis mineral elements (Sathish *et al.*, 2020).

Carbohydrate:

Finger millet like any other cereal is a source of dietary carbohydrates which comprise of starch as the main constituent with non-starchy polysaccharide at levels of (15-20%) as unavailable carbohydrates. Millets have a larger proportion of non-starchy polysaccharides and dietary fibres compared to staple cereals and comprise (65–75%) carbohydrates. Finger millet contains carbohydrate (81.5%) (Rathore *et al.*, 2019). Button mushroom has 52 per cent to 53 percent of carbohydrate.

Protein:

Protein content of finger millet is roughly around (7 %) but a large variation in this nutrient, from 5 to 13 per cent (Kumar, 2016). The protein found in finger millet is considered as superior as it encompasses vital amino acids such as lysine, threonine, and valine**.** Among the millets, finger millet protein was relatively better balanced; it contained more lysine, threonine and valine (Hever and Cronise, 2017). Protein per cent in mushroom ranges from 1.8 to 5.9 (Wang *et al.*, 2018)

Fat:

Finger millet contains essential fatty acids such as linoenic and palmitic acids which are essential for the development of brain and neural tissue (Muthamilarasan *et al.*, 2016).

Button mushroom has very low fat percent. Its fat per cent is 0.22. ±0.05 (Sathish *et al.,* 2020).

Fibre:

The dietary fiber and polyphenols in finger millet are known to offer several health benefits such as antidiabetic, antioxidant, hypocholesterolaemic, antimicrobial effects and protection from diet related chronic diseases to its regular consumers. Finger millet contain 18 per cent dietary fibre (Chandra *et al*., 2016).Button mushroom contain 56.6 g/100 g dietary fibre (i.e. 56.99 g/100 g total dietary fibre, 48.79 g/100 g insoluble dietary fibre, and 8.20 g/100 g soluble dietary fibre (Lee *et al., 2020*)

Calcium

Calcium (Ca^{2+}) is an essential signaling molecule that controls a wide range of biological functions. In the immune system, calcium signals play a central role in a variety of cellular functions such as proliferation, differentiation, apoptosis, and numerous gene transcriptions (Park, 2020). Finger millet is one of the calcium dense foods, with three times the level of calcium than milk, and the only cereal that contains high calcium content which is consistent across different varieties 364 ± 58 mg/100 g (Wafula *et al* ., 2018).

Phosphorous:

Grain protein increases with increase in phosphorous rate, this is due to the significance of phosphorus in the synthesis of protein and it acts as an important building block in amino acids and generation of energy for the uptake and transfer of nitrogen in finger millet grains (Nelson *et al* ., 2016).

Vitamin D:

Exposure to UV led to an increase in vitamin D_2 content by 31-fold, over a period of 48 h. The amount of vitamin D_2 observed after 48 h of UV-C exposure was found to be 364.2 ± 2.60 µg/g DW (Patil *et al* ., 2018). Sun exposure of *Agaricus bisporous* has 9 ± 0.8 µg/g dry (DW) Vitamin D (Urbain *et al*., 2016).

D. Products and supplementation developed for vitamin D

Effect of supplementation with vitamin D_2 enhanced mushrooms on vitamin D status in healthy adults: Vitamin D_2 from enhanced mushrooms was bio available and increased serum25 (OH) D_2concentration with no significant effect on 25(OH) D_3 or total 25(OH) D (Stephen *et al.*, 2013).

The use of a UV-radiated mushroom food ingredient was effective in maintaining 25(OH) D in healthy, recreationally active volunteers. Mushroom-derived vitamin D2 powder may offer an adjunct strategy as a more cost-effective and widely applicable food ingredient for populations, including vegans and vegetarians, with low vitamin D status in supporting their vitamin D intake. Further research is required to find optimal dosages for daily mushroom-derived vitamin D_2 supplementation (Pinto *et al.*, 2020)

Enhancement of foods with vitamin D can be one of the possible modes for improving the vitamin D status. Mushrooms are one of the few plant foods which contain ergosterol, a precursor to vitamin D_2. The amount of vitamin D2 in mushrooms can be significantly increased by exposing mushrooms to ultraviolet (UV) light or in sunny areas. The mushroom cookies were prepared in two different concentrations (15% and 25%).Sensory evaluation was done by hedonic rating scale. The study concluded that mushroom cookies with (15% incorporation of mushroom powder) was highly acceptable and could be beneficial in increasing the vitamin D status of the population (Cardwell, 2018).

In another study , High sun dried mushroom powder concentration in mushroom cookies (25% mushroom powder) proved to be a high vitamin D rich product but the acceptability of the product contain 15% of sun dried mushroom powder was more as compared to 25% of sun dried mushroom powder. The study concluded that mushroom cookies with 15% incorporation of mushroom powder was highly acceptable and had vitamin D content of 26.15 mcg/100g which could be full fill the ½ of RDA of vitamin D content of the adult by just consuming 4-5 cookies per day (Prathiba and Mahak , 2016)

A study by Limcy (2018) study was to develop a low cost calcium and vitamin D supplement from the combination of chicken eggshell and sun-dried mushrooms (*Agaricusbisporus*). The supplement prepared was subjected to sensory analysis to check its acceptability by incorporating it to six commonly consumed Indian food preparations items (dal curry, chapatti, parantha, curd, nankhatai and milk) as per the dosage in one serving i.e. 1 g eggshell powder: 1 g sun-dried mushrooms, keeping in mind the upper limits of intake. Results infer that the prepared supplement can be best accepted in baked food products like nankhatai etc. The shelf life of the developed supplement was found out to be for 6 months. Developed value added product also be used during emergencies and disasters to meet nutritional requirements of the deficit population.

Methodology

III. METHODOLOGY

The methodology of study entitled **"Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products**" is described under the following headings:

PHASE I: Selection of foods rich in Vitamin D_2 and Calcium and its processing method

A. Selection of foods rich in Vitamin D_2 and Calcium

B. Processing of foods

PHASE II: Preliminary analysis of Vitamin D_2 and Calcium in mushroom and finger millet

A. Analysis of vitamin D_2 in mushroom

B. Analysis of Calcium in Finger millet

PHASE: III Formulation, standardisation and organoleptic evaluation of developed food products

A. Standardisation of Ready To Eat (RTE) and Ready To Cook (RTC) food products

B. Organoleptic evaluation of developed products

PHASE IV: Analysis of Physicochemical properties and Nutrients in developed food products

A. Physicochemical properties of the developed food products

B. Nutrient analysis of the developed food products

PHASE V: Shelf life of the developed Food products and Packaging

A. Shelf life assessment of the developed Food products and Packaging

Phase I: Selection of foods rich in Vitamin D_2 and Calcium

The study design is presented in Figure 1 and the ethical clearance obtained from the Institutional Human Ethical Committee approval no AUW/IHE/FSN-21-22/XPD-03.Ethical clearance certificate is given in Appendix I.

A. Selection of foods rich in Vitamin D_2 and Calcium

Finger millet and Button mushroom were chosen for this study as Calcium and Vitamin D_2 source.

Finger millet:

Finger millet (*Eluesine coracana)* is one of the best source of Calcium and vitamin D_2, with 36457.8 mg of calcium and 41.463.12μg of vitamin D_2 (Longvah *et al*, 2017). When compared to other millets, finger millet has good calcium bioavailability. It has three times the calcium found in milk and ten times the calcium found in brown rice. It is a great cereal that can be consumed on a regular basis. It also contains amino acids such as methionine and phytochemicals such as polyphenols, in addition to calcium. Finger millet has been shown to lower the risk of diabetes and gastrointestinal disorders (Kumar *et al*., 2016)

Mushroom

Mushroom, despite belonging to the fungal kingdom, is a vegetable in the culinary sense, distinguishing itself from plants and animals. There are various kinds of mushroom namely wild mushrooms, shiitake mushrooms, oyster mushrooms, Button mushrooms, and Italian mushrooms, and so on. The Button mushroom (*Agaricus bisporous*) is widely available in all seasons. Button mushroom (fresh) has around 20.54 μg vitamin D_2 per 100 g (Longvah *et al*, 2017).

Mushroom is a unique species which can synthesize ergosterol in their cell wall when exposed to UV radiation of sun. It has numerous health benefits such as reduction of cancer risk, aids in weight management, boosts immunity and increase in gut micro flora (Agarwal and Victor, 2021). Finger millet and Button mushroom were chosen as a base ingredient for the development of RTE and RTC food products.

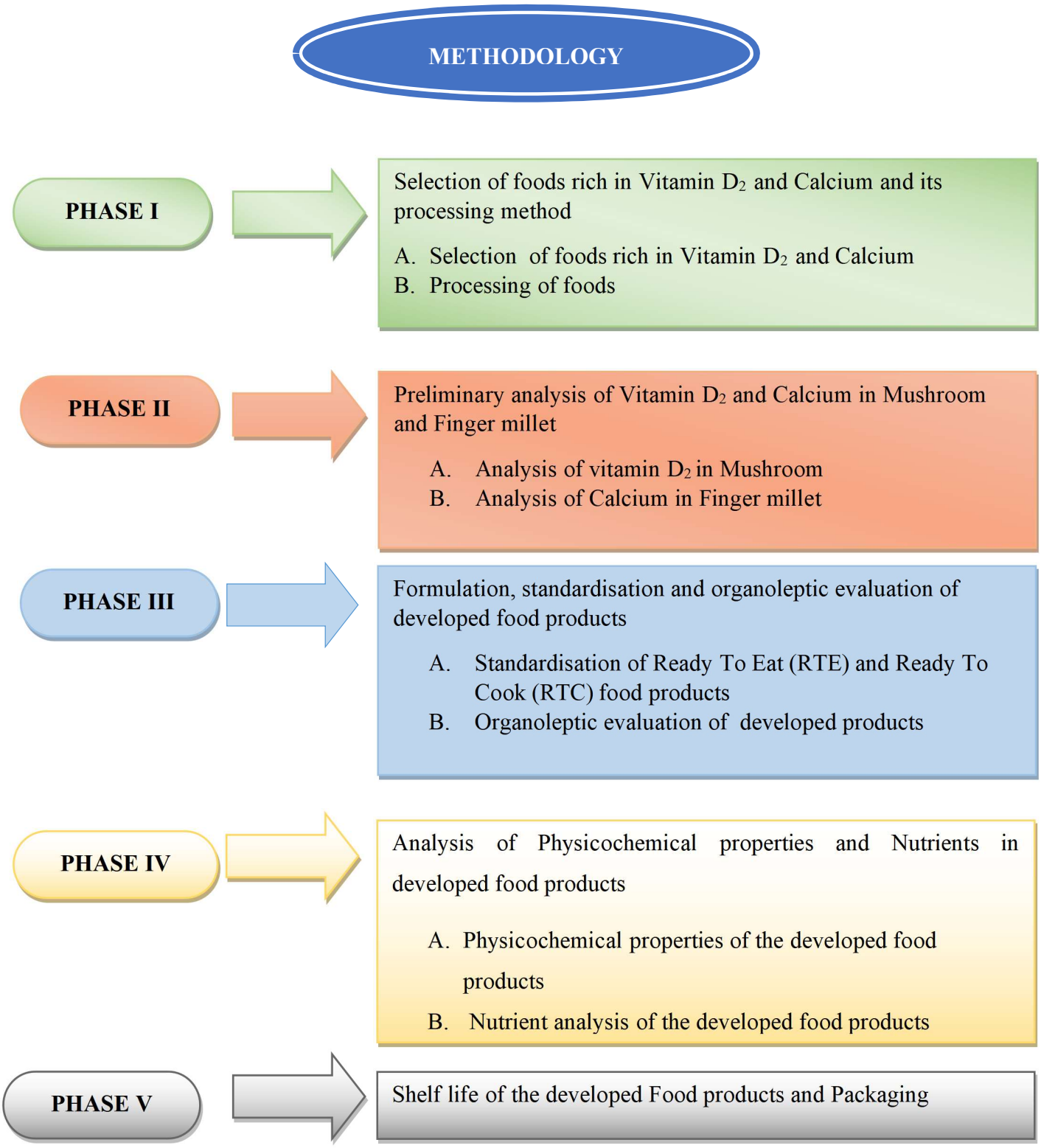


Figure 1

RESEARCH DESIGN

Wheat flour, refined wheat flour, butter, brown sugar, refined sugar onion powder, milk powder, garlic powder and pepper powder were also used to make RTC and RTE food products. Finger millet, button mushroom and other ingredients were purchased from the local market of Coimbatore City, Tamil Nadu. The ingredients were stored in the Foods lab of Department of Food Science and Nutrition at the Avinashilingam Institute for Home Science and Higher Education for Women.

A. Processing of foods :

Processing is essential as it enhances the bioavailability and Organoleptic property of foods. Different foods require different processing techniques. Decortication, grinding, soaking, heating, germination, and fermentation are some of the methods used to process millets. A Study by Najdi Hejazi *et al.,(* 2016) revealed that basic traditional food processing methods such as soaking and malting/germination can considerably reduce anti-nutrient content and boost nutrient bio-availability.

Processing of Finger Millet

Malting:

Malting is one of the traditional methods carried out to enhance the nutrient and physicochemical properties of the grain including their digestibility. The malting of finger millet was carried out with a slight modification in the procedure as suggested by Nirmala and Muralikrishna, (2002). Finger millet was washed in running tap water to remove sand, stones and dirt present in it. It was washed with water for 5 to 6 times and soaked in water for 24 hr. In between the soaking period the water was changed for every 6 hours to retard fermentation. After 24 hours of soaking excess water was drained, seeds were tied in a muslin cloth.

These seeds were germinated at 27± 3ºC for 24hr and shade dried for 2 days. The malted finger millet were grounded into flour then sieved. Finger millet flour was stored in an air tight container. It was analysed in the laboratory for calcium.A comparative study of analysis of millet revealed 24 hr soaking and 24 hr germination was found to be best for producing nutritionally enriched millet products. It reduces the anti-nutrition factor and improves the bio availability (Bhuvaneswari *et al.,* 2020). The detailed flow chart for the preparation of malted finger millet flour is given in Appendix II.

Processing of Mushroom

I) Cleaning, Pre-treatment and slicing

A combination of steam blanching, vinegar pre treatment and solar drying can minimize the antioxidant loss and mold infestation in mushroom (Mutukwa *et al*., 2019). Button mushrooms were washed, trimmed, and cut into slices of thickness about 5 mm. Then it was steam blanched for 3 min. This was followed by soaking in 500 ml of 0.5% chemical pre-treatment solution (Vinegar) for 10 minutes. Samples were then drained and dried.

2) Sun drying:

Sun drying is a traditional drying method for reducing the moisture content thereby it also enhances shelf life. The solar radiation heats up the surface as well as the surrounding air and thus increases the rate of water evaporating from the surface. The mushroom was weighed before and after drying. The drying rate depends on the time and day of the products exposed. After drying completely, it is stored in an air tight container and analysed in the laboratory for vitamin D_2. The detailed flow chart for the preparation of sun dried mushroom is given in Appendix II

Processing of other ingredients:

Garlic powder

Garlic was cleaned and sliced. It was kept in cabinet dryer for 14.5 hrs at 60 °C and was powdered, sieved and kept in a sealed polyethylene pouch. The detailed flow chart for the preparation of garlic powder is given in Appendix IV

Onion powder

Onion was cleaned and sliced. It was kept in cabinet dryer for 14.5 hrs at 60 °C and was powdered, sieved and kept in a sealed polyethylene pouch. The detailed flow chart for the preparation of onion powder is given in Appendix IV

Pepper powder:

Pepper was ground in to powder .It was stored in sealed polyethylene pouch.

PHASE : II Preliminary analysis of Vitamin $D_2$and Calcium:

Nutrient analysis is the process for determining the nutrient profile of foods and food products. It is a vital part of analytical chemistry that provides information about the chemical composition, processing, quality control and contamination of food. It ensures compliance with trade and food laws. It can be performed through a Laboratory analysis.

A . Analysis of Calcium in finger millet:

Calcium was estimated by using the procedure given by AOAC(1990) 5g of ignited sample was dissolved in hydrochloric acid and made up to 100 ml. 10 ml of the ash solution was pipette out in a conical flask and 90 ml of distilled water was added to it. Two drops of methyl red indicator were added. It was made strongly alkaline by adding ammonia and kept for boiling.20 ml of saturated ammonium oxalate was added to the solution, 10 ml each time to ensure complete precipitation directly. When it was hot, a few drops of acetic acid were added to render the medium acidic.

The precipitation was allowed to settle overnight. The next morning the solution was filtered with Whatman no.40 filter paper. The precipitate was washed first with ammonical water and then with hot water several times until it was free from chloride. To test it 5 ml of the washing was collected, in a test tube, and a drop of silver nitrate solution was added. The washing was continued till there was no precipitation with calcium chloride solution.

The filter paper was collected in a flask by making a hole in the filter paper. To this 2 ml of 2N sulphuric acid was added. This solution was heated to 60-80°C and when still hot was titrated against N/10 potassium permanganate solution. The volume of potassium permanganate solution uses up the milligrams of calcium present in 100 g of the sample was calculated. The analyses were performed in triplicates.

B. **Analysis of Vitamin D_2in Button mushroom:**

Vitamin D_2 determination was run by couple HPLC technique. Triplicate samples were taken from each one for the measurement of vitamin D_2. The extraction of vitamin D_2 was carried out based on the method of Keflie *et al*., (2019). Samples of pulverized and

homogenized powder (1 g) were mixed with 19 ml of ethanol (with a chemical purity of 99.7%), 4 ml of 50% potassium hydroxide (500 g KOH in 1 L of H2O), 1.333 ml of sodium ascorbate (1.75 g solved in 10 ml of 1 M sodium hydroxide) and 1 ml of vitamin D_3 (100 mg/L) as internal standard in a 50 ml falcon tube. The mixture was vortex mixed and subsequently saponified for 1 h in a water bath at 80 °C. The mixture was then cooled to ambient temperature in ice water. To promote a better separation of the layers, 10 ml of saturated sodium chloride solution was added. Subsequently, 15 ml of n-hexane was added, vortex mixed and centrifuged for 8 min at 4500g .The n-hexane layer was transferred into a new falcon tube and the extraction processes were repeated twice, one time with 15 ml of n-hexane and later with 10 ml of n-hexane. The pooled organic layers were washed three times with deionised water until neutralized. The organic layer was then transferred into a 100 ml round bottom flask and rotary evaporated to dryness. The flask was rinsed with 6 ml of n-hexane and transferred into 10 ml of round bottom flask and, rotary evaporated again. Once evaporated to dryness, the sample was immediately re-dissolved in 1 m of tetrahydrofuran and vortex mixed. Thereafter, the samples were centrifuged for 5 min at 1600g at 20 °C to remove impurities and then used for subsequent analyses by HPLC.

PHASE : III Formulation, standardisation and organoleptic evaluation of developed food products

New product development is a process which tries to develop a new product or tries to modify from the existing one. There are three crucial steps: creating new ideas, screening ideas for viability, and appealing to ideas. It is one of the innovative way to treat nutritional deficiency diseases like rickets. Convenience is a multifaceted term. Apart from cost, health, and sensory acceptability, and other concerns, it is one of the most important elements that influence food choice. When, where, what, and how we eat foods are all influenced by convenience.

As a result, demand for minimally processed ready-to-eat or ready-to-cook food has risen significantly in recent years. A study by Harinarayanan and Akhila (2019) revealed that calcium and vitamin D is a twin deficiency which was prevalent in India. Hence, Ready To Eat and Ready To Cook products which aids in prevention and treatment of nutritional deficiency disease were formulated. For this research Finger millet mushroom soup mix, finger millet mushroom cookie and finger millet mushroom flakes were developed and standardised.

A. Standardisation of Ready To Eat (RTE) and Ready To Cook (RTC) Food products

Finger millet mushroom cookies:

The methodology by Ikumola *et al.,* (2017) was followed with slight modification for finger millet mushroom cookies. Standardisation of product was carried out by formulating two trials (V_1 and V_2) with reference (V_0) standard recipe as given in (Table I) Butter and powdered sugar were creamed in mixer with a flat beater for 2 min at 5 rpm to obtain a homogenous cream, then dry raw materials were added and mixed for 5 min to form dough which was sheeted to thickness of about 0.25 cm using rolling pin and cut into a round shape (cookies) of 3 cm diameter then baked in an electric oven at 160°C for 15 min The detailed flow chart for preparation of Finger millet mushroom cookies is given in Appendix III

For standardisation of finger millet mushroom cookies, the standard recipe with wheat flour was adopted. The standard wheat flour cookie was prepared by 100 g wheat flour, 50 g butter, 50 g jaggery powder and a drop of vanilla essence. Keeping the other ingredients constant, variations replacing refined wheat flour with finger millet flour at $V_1$10 Per cent and $V_2$20 per cent. In both variations mushroom powder was 10 per cent. The cookies with these variations were prepared and subjected to descriptive sensory evaluation (Table I)

Table I

Composition of ingredients present in Finger millet mushroom cookies

Ingredients (g/ml)	Variation		
	V_0	V_1	V_2
Wheat flour	50	30	20
Malted finger millet flour	-	10	20
Mushroom	-	10	10
Butter	25	25	25
Brown sugar	25	25	25
Vanilla essence	0.4	0.4	0.4
Sodium bicarbonate	0.25	0.25	0.25

Instant Finger millet mushroom soup mix:

The soup mix was formulated by mixing the weighed ingredients in right proportion. Standardisation of product was carried out by formulating two trials (V_1 and V_2) with reference (V_0) standard recipe as given in Table II .The standard mushroom soup (V_0) was prepared by 25 g wheat flour, 20 g fat, 35 g skim milk powder, 5 g dried mushroom, 5 g mushroom powder, 5.5 g salt, 0.5 g pepper, 1.5 g sugar, 1.5 g garlic and onion powder. Keeping other ingredients constant, variations replacing refined wheat flour with finger millet flour at V_1 20 per cent and V_2 40 per cent (Table II). In both variations mushroom powder was 10 per cent. The soup with these variations were prepared and subjected to descriptive sensory evaluation. The detailed flow chart for preparation of Finger millet mushroom soup mix is given in Appendix IV.

Table II

Composition of Instant Finger millet mushroom soup mix

Ingredients (g/ml)	Variation		
	V_0	V_1	V_2
Wheat flour	25	20	15
Finger millet flour	-	5	10
Dehydrated mushroom	5	5	5
Dehydrated mushroom powder	5	5	5
Skim milk powder	35	35	35
Garlic powder	1.5	1.5	1.5
Onion powder	1.5	1.5	1.5
Sugar	1.5	1.5	1.5
Salt	5.5	5.5	5.5
Pepper	0.1	0.1	0.1

Finger millet mushroom flakes:

The finger millet mushroom flakes were formulated by the methodology of Attri *et al* , (2019) with slight modification. Standardisation of product was carried out by formulating two trials (V_1 and V_2) with reference (V_0) to the standard recipe as given in the (Table III).The Finger millet mushroom flakes was standardised by the standard recipe of wheat flakes(V_0). The variations are prepared with (V_1) 10 per cent and (V_2) 20 per cent by addition of finger millet flour. The other ingredients such as milk powder, sugar and refined wheat flour were kept constant. All the ingredients were measured and made in to dough .It was sheeted evenly using the roller and cut in to flakes. It were initially dehydrated in a

dehydrator at 60 ±2 ºC temperature followed by drying in OTG (Oven, toaster and griller) at 100 ºC for 15 min . The end product was left to cool before subjected to any sensory acceptance test. The detailed flow chart for preparation of flakes is given in Appendix V

Table III

Composition of Finger millet mushroom flakes

Ingredients (g/ml)	Variation		
	V_0	V_1	V_2
Wheat flour	50	30	20
Refined wheat flour	20	20	20
Malted finger millet flour	-	10	20
Dehydrated mushroom powder	-	10	10
Sugar	30	30	30
Salt	0.5	5.5	5.5
Vanilla essence	0.1	0.1	0.1

B .Organoleptic evaluation of developed products:

Sensory analysis or Organoleptic evaluation is the identification, scientific measurement, analysis and interpretation of the properties (attributes) of a product as they are perceived through the five senses of sight, smell, taste, touch, and hearing. In a product development it plays a vital role for the determination of product acceptability. The developed products were organoleptically evaluated by 30 semi trained panel members in Foods Sensory Laboratory of the Food Science and Nutrition Department at Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. PLATE I, II and III depicts the organoleptic evaluation of Finger millet mushroom Cookies, Finger millet mushroom soup and Finger millet mushroom flakes respectively.

Sensory attributes like colour & appearance, body or thickness, aroma, taste and overall acceptability for all samples were assessed using nine point hedonic scales. Hedonic scale was in the following sequence: like extremely - 9 like very much - 8, like moderately7, like slightly - 6, neither like nor dislike - 5, dislike slightly 4, dislike moderately - 3, dislike very much - 2, dislike extremely – 1 (BIS, 1971).The results were interpreted using one way ANOVA by the statistical software SPSS version 25 .From the results the best product is subjected to nutrient analysis.






ORGANOLEPTIC EVALUATION OF COOKIES

PLATE I





ORGANOLEPTIC EVALUATION OF SOUP

PLATE II





ORGANOLEPTIC EVALUATION OF FLAKES

PLATE III

PHASE IV: Analysis of Physicochemical properties and Nutrients in developed food products

A. Analysis of Physicochemical properties of the developed food products

Moisture content:

One gram food sample were accurately weighed in the Digital Moisture Analyzer (Model 120H, Shimadzu Corp, Japan) and dried at 200ºC for 20 minutes and 8 minutes until the weight was constant. The percentage loss on drying of the test sample was automatically recorded. The analyses were performed in triplicates.

Total ash content:

Total ash content was determined using the standard protocols given by Manual Methods (AOAC 1990) 5 g of sample was accurately weighed and placed in a tarred crucible, which was previously ignited, cooled, and weighed. The sample was charred carefully on a burner. The sample was incinerated by gradually increasing the temperature not exceeding 600 ºC in muffle furnace until free from carbon, then cooled in desiccators and weighed.

$$\text{Ash content (g/ 100g sample)} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

A. Analysing the nutrients present in developed products

1. Energy :

The energy values of the products were determined using advance research bomb calorimeter. One gram of food sample was taken and made in to pellets using pellet press (which consists of a mold into which the sample was placed and a pestle which is then pushed into the mold, crushing the sample material into a compact pellet).The pellet was placed in the sample holder of the crucible and a length of tungsten wire and cotton thread is tied onto the filament and its ends placed into the crucible so that it will act as a fuse. Pipette out 2 ml of distilled water in to the crucible. The sample holder is placed and crucible is closed .Through the valves the air is removed and oxygen is filled at 20 atm .It was kept inside the calorimeter vessel which contain 2000 g of water. Electrodes are fixed and the

calorimeter is closed .The thermocouple put in place in the top of the calorimeter. In the microprocessor based digital bomb calorimeter start button and stirrer was pressed .The change in temperature of the bomb is displayed in calorimeter. The difference in initial and final temperature was noted and calculation was carried out

2. **Carbohydrates**:

Carbohydrate content of the sample was analysed using the protocol given by AOAC (1990). Weighed 100 g of sample in a boiling tube and hydrolysed by keeping it in a boiling water bath. Boiled for 3 hours with 5 ml of 2N HCl and cooled to room temperature and neutralized it with solid sodium carbonate until effervescence ceased. Made up the volume to 100 ml and centrifuged. Collected the supernatant and 0.5 ml is taken for analysis. Prepared the standard, made up the volume to 1 ml in the entire test by adding water, and then add 4 ml of anthrone reagent and heated to 8 minutes. Cooled rapidly and colour was read at 630 nm. The analyses were performed in triplicates.

3. **Protein**:

Protein content of the sample was analysed using the protocol given by AOAC (1990). Determination of protein content was carried out by the Micro Kjeldahl method) which consists of wet digestion (digestion flask), distillation (distillation chamber), and titration. The protein content was determined by weighing 0.2g of sample and transferred to 250 ml Kjeldahl flask, care was taken to see that no portion of the sample clings to the neck of the flask. To this 1 to 2 g of catalyst mixture (potassium sulfate 100 g and copper sulfate 20 g) and 10 ml of concentrated H_2SO_4 were added. Flask was placed on the stand in the digestion chamber and continued the process of digestion until the colour of the digestible mixture is pale green. The digested mixture was cooled by adding 30 ml of water. After digestion, distillation was carried out with 40% NaOH and 20% boric acid using methyl orange as an indicator and titrated against 0.1N H_2SO_4. The protein content was calculated as follows:

$$\% \text{ Nitrogen} = \frac{14.01 \times \text{ml titre value of sample} \times \text{N } H_2SO_4 \text{ of} \times 100}{\text{Weight of the sample (g)} \times 1000}$$

4. **Fat**:

Crude fat was estimated by continuous Soxhlet extraction technique using petroleum ether (40-60°C B.P.) as a solvent. Two grams of Wet and Dry sample was placed in fat extraction thimble and placed in a clean, dry pre-weighed beaker to which 80 ml petroleum ether was added. This beaker was then placed in the Soxhlet apparatus for the extraction of fat for 2-3 hours. After extraction, the beaker was removed and kept in a hot air oven (100°C) to evaporate traces of solvent. It was then transferred to desiccators, cooled, and weighed. The difference between the weight of the beaker before and after gives the quantity of crude fat extracted from the unknown amount of the sample. The analyses were performed 2in triplicates

5. Crude Fibre

The crude fibre was estimated using Manual method of analysis of food (AOAC 1990).Five gram of the sample was weighed into a 500 ml beaker and 200 ml of boiling 0.255N sulphuric acid was added. The mixture was boiled for 30 minutes. Keeping the volume constant by adding water at frequent intervals (a glass rod inserted in the beaker helps smooth stirring and boiling). At the end of the period, the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. The mixture was then transferred to a beaker containing 200 ml of boiling 0.313N sodium hydroxide. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth. The residue was washed with hot water till free from alkali followed by washing with some alcohol and ether. It was then transferred into a crucible, dried overnight at 80-100°c and weighed. The crucible was heated in a muffle furnace at 600° c for 2-3 hours. Cooled and weighed again. The difference in the weight represents the weight of the fibre.

$$\text{Crude fibre (g/100g of the sample)} = \frac{W_1 - W_2 \times 100}{W}$$

W_1 -Weight of the bottle, lid and flour

W_2- Weight of the bottle, lid and flour after heating

W - Weight of the food sample taken

6. Calcium :

Calcium is determined by precipitation it as calcium oxalate and titration the oxalate solution in dilute sulphuric acid against standard potassium permanganate (AOAC 1990)

7. Phosphorus

When the ash solution is treated with ammonium molybdate, phosphomolybdic acid is formed. Phosphomolybdic acid is reduced by the addition of 1,2,4 amino naphtholsulphonic acid reagent to produce a blue colour which is apparently a mixture of oxides of molybdenum. The intensity of the colour developed is the measure of phosphorus present (AOAC 1990)

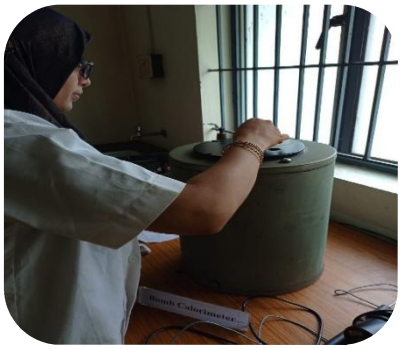
8. Vitamin D_2:

Vitamin D_2 determination was run by couple HPLC technique. Triplicate samples were taken from each one for the measurement of vitamin D_2. The extraction of vitamin D_2 was carried out based on the methodology Keflie *et al*., (2019).

Result from the nutrient analysis are analysed statistically by independent paired sample t test PLATE IV represents the nutrient analysis of the developed products

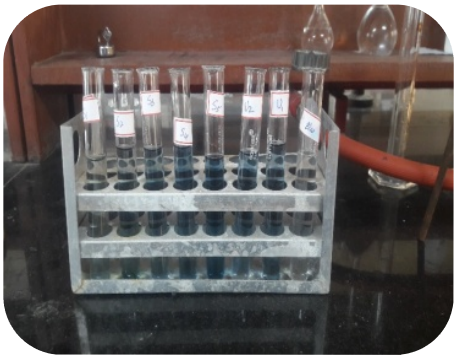
Determination of Moisture analysis

Determination of Ash



Determination of energy value using bomb calorimeter

Analysis of calcium



Determination of fat

Analysis of phosphorous

ANALYSIS OF NUTRIENTS

PLATE IV

PHASE V: Shelf life of the developed Food products and Packaging

A. Shelf life assessment of the developed Food products and Packaging

The storage studies were carried out for 15 days in the Zip lock heat resealable paper pouch lined with polyethylene packaging material for Flakes and Soup mix and metallised polyethylene pouches in cartons for cookies. By consideration of parameters like moisture content and weight of developed product was seen at room temperature. The observation was carried out after every 3 days from the initial day .Cost analysis also carried out for hundred gram of each product. The Label of choice is a pressure sensitive Label or a sticker label which is pre-coated with a permanent adhesive and has to be peeled off the label sheet and applied onto the object or package with a certain amount of pressure. The labelling was designed it includes the information such as Name of the Product, Brand name, Image of the product / key ingredients, Nutritional information, Basic Product description and Serving size .The cost of the product were also calculated. PLATE V represents the packaged and labelled RTE and RTC food product.

PACKAGING AND LABELLING

PLATE V

Results and Discussion

IV. RESULTS AND DISCUSSION

The results and discussion of the study entitled, "Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products" is given under the following headings.

A. Processing of finger millet , mushroom and other ingredients
B. Analysis of Vitamin D_2 and Calcium in mushroom and finger millet flour
C. Organoleptic evaluation of developed products
D. Physicochemical properties of the developed food products
E. Nutrient analysis of the developed food products
F. Shelf life of the RTE and RTC Food products

A. Processing of finger millet, mushroom and other ingredients

Finger millet and Button mushroom were chosen for this study as Calcium and Vitamin D_2 source. Garlic powder and onion powder were prepared for the formulation of RTE and RTE food products.

Thousand grams of Button mushroom was taken. Pre-treatment with vinegar was carried. Then it was shade dried for 2 days. Thousand grams of finger millet was taken. It was cleaned, soaked, sprouted, dried and powdered. The weight of the sample before and after drying was noted .The Table IV represents weight of the button mushroom and finger millet flour.

Table IV

Weight of finger millet flour and dried button mushroom

Sample	Weight (g)		
	Fresh	Dried	Powdered
Button mushroom	1000	100	100
Finger millet	1000	998	900

Sun dried Button mushrooms yielded 10 g. A supported study by Pratibha Sharma (2021) revealed that button mushroom contain (90%). Thousand grams of malted finger millet yielded 900 g of flour.

Hundred grams of small onion was dried in a cabinet dryer at 60 °C and powdered. 100 g of garlic was dried in a cabinet dryer at 60 °C and powdered. The weight of the sample before and after drying was noted. Table V gives details of weight of the Garlic powder and small onion powder used in the product

Table V

Weight of the Garlic powder and small onion powder

Sample	**Weight (g)**	
	Fresh	**Powdered**
Garlic (*Allium sativum*)	100	39
Small onion (*Allium cepa*)	100	12

Hundred grams of fresh garlic yielded 39 g of garlic powder .A study by Bondre *et al*.,(2016) reported that cabinet dried garlic powder showed the best results in physicochemical properties with high recovery per cent (39.00%) and registered high sensory score (7.3) than solar drying. However it took more time (14.7 hours) for drying up to (5%) moisture level. Hundred grams of fresh onion yielded 13 .5 g of onion powder it was supported by the study of Shalini Chakraborty *et al*.,(2018) which revealed the mean value of product recovery variedfrom13.8 to14.8 per cent in sample for the different drying techniques

B. Analysis of Vitamin D_2 and Calcium in mushroom and finger millet flour

Calcium in unmalted finger millet and malted finger millet was found to be 354±0.06mg and 452 ±0.02 mg respectively. A study by Anuradha *et al.*, (2010) revealed that calcium in unmalted finger millet and malted finger millet was 359±0.8 mg and 429±0.8 mg respectively.

A study by Phillips *et al.*, (2013) stated that the amount of vitamin D_2 generated depends on the time of day, season, latitude, weather conditions, and exposure time. Since these mushrooms have a higher surface area to volume (hence, more ergosterol is exposed),

sun-exposed sliced mushrooms produce more vitamin D_2 than whole mushrooms from the same amount of UV radiation exposure.

The mushroom are sliced and turned during drying. Results revealed that it had significantly increased the vitamin D_2 from 0.03±0.05µg to 36 µg ±0.02 µg study by Urbain *et al.*, (2015) revealed that midday in mid-summer in Germany, the vitamin D_2 content of sliced mushrooms was as high as 17.5 µg/100 g FW after 15 min of sun exposure and reached 32.5 µg/100 g FW after 60 min of sun exposure. Sun exposure to a single layer of small button mushrooms was sufficient to generate 10 µg /100 g FW after 1 hour, while large button mushrooms took 2 hr to generate the same amount of vitamin D_2.

C. Organoleptic evaluation of developed products

Organoleptic evaluation was carried out to evaluate the product by panel of members towards the satisfactory- using a hedonic rating scale. Ready To Eat (RTE) - Cookies and Millet flakes and Ready To Cook (RTC) – Soup mix with the incorporation of finger millet flour and mushroom powder are formulated. Standard and Variation of the recipes were prepared and it has been evaluated based on the nine point Hedonic rating Scale for Sensory attributes like Appearance, Colour, Flavour, Texture , Taste and Overall acceptability by 30 semi trained members for Successive trials. Table VI represents the data of mean acceptability scores for Finger millet mushroom cookies.

Table VI

Mean acceptability score for Finger millet mushroom Cookies

Sensory Characteristics	Mean± S.D			F Value	P Value
	Standard	Variation 1	Variation 2		
Appearance	8.33±0.884	7.16±0.985	8.50±0.776	20.766	<0.001
Colour	8.36±0.688	6.93±1.03	8.46±0.628	34.242	<0.001
Flavour	8.40±0.674	6.76±0.768	8.16±0.746	47.662	<0.001
Texture	8.50±0.572	7.20±0.886	8.20±0.924	21.171	<0.001
Taste	8.50±0.629	7.27±1.056	8.36±0.850	17.705	<0.001
Overall Acceptability	8.66±0.479	7.80±0.846	8.67±1.05	8.283	<0.001

Standard sample: Normal wheat flour cookies
Variation 1: Finger millet Mushroom cookies with incorporation of 10 percent each.
Variation 2: Finger millet Mushroom cookies with incorporation of 20 percent finger millet and 10 percent of mushroom powder
Significant at5 per cent level

Cookies were developed by adding 10 per cent and 20 per cent of malted finger millet flour and 10 per cent of dried mushroom powder .It was compared with the Control. Standard was labelled as S and cookie incorporated with mushroom powder and finger millet flour were labelled as V_1 and V_2.On considering the appearance, variation 1 obtained the highest score of 8.50±0.776 followed by control and standard with score of 8.33±0.884 and 7.16±0.985 respectively.

With regard to colour, standard obtained the highest score of 8.36±0.688 followed by variation 2 and variation 1 with score of 8.46±0.628 and 6.93±1.03 respectively .With regard to flavour, standard obtained the highest score of 8.40±0.67 followed by variation 2 and variation 1 with score of 8.8.16±0.74 and 6.76±0.768 respectively

With regard to texture, standard obtained the highest score of 8.50±0.57 followed by variation 2 and variation 1 with scores of 8.20±0.924 and 7.20±0.886 respectively. With regard to taste standard obtained the highest score of 8.50±0.629 followed by variation 2 and variation 1 with score of 8.36±0.850 and 7.27±1.056 respectively.

Data from the Table VI represents that V_2 have overall acceptability in terms of appearance ,colour ,flavour, texture and taste with statistically significant P value of <0.05 .A study by Sinha and Sharma (2017) revealed that Cookies prepared by incorporating Finger millet flour up to 50% level was found acceptable. Though colour and appearance of ragi incorporated cookies were significantly different from control, yet were acceptable and fell into the category of "moderately desirable"

Table VII

Mean acceptability score for Finger millet mushroom soup mix

Sensory Characteristics	Mean ± S.D			F value	P Value
	Standard	Variation 1	Variation 2		
Appearance	8.33±0.606	8.80±0.610	7.40±1.037	25.182	<0.001
Colour	8.16±0.647	8.76±0.430	6.83±0.949	58.487	<0.001
Flavour	8.03±0.556	8.83±0.379	6.63±0.889	89.651	<0.001
Texture	8.10±0.661	8.83±0.379	6.53±0.730	111.426	<0.001
Taste	8.06±0.639	8.83±0.379	7.10±0.884	50.837	<0.001
Overall Acceptability	8.13±0.571	8.80±0.406	6.60±0.674	120.927	<0.001

Standard sample: Mushroom soup mix
Variation 1: Finger millet mushroom soup mix with incorporation of 5 percent of malted finger millet flour
Variation 2: Finger millet Mushroom soup with incorporation of 10 percent malted finger millet and 10 percent of mushroom powder
Significant at 5 per cent level

Table VII represents Mean acceptability score for finger millet mushroom soup mix .With regard to appearance, variation 1obtained the highest score of 8.80±0.610 followed by standard and variation 2 with score of 8.33±0.606 and 7.40±1.037. With regard to colour, variation 1 obtained the highest score of 8.83±0.379 followed by standard and variation 2 with scores of 8.03±0.556 and 6.63±0.889 respectively.

With regard to flavour, variation 1obtained the highest score of 8.83±0.379 followed by Standard and variation 2 with score of 8.03±0.556 and 6.63±0.889 respectively .With regard to texture, variation 1 obtained the highest score of 8.83±0.379 followed by Standard

and variation 2 with score of 8.10±0.661and 6.53±0.730 respectively. Variation 1 have overall acceptability in terms of appearance, colour, flavour, texture and taste with statistically significant P value of <0.05. A study by Tulasi *et al*., (2020) revealed that soup prepared by incorporating finger millet flour up to (10%) level was found acceptable in instant millet base soup mix

Table VIII

Mean acceptability score for Finger millet mushroom flakes

Sensory Characteristics	Mean± S.D			F value	P Value
	Standard	Variation 1	Variation 2		
Appearance	8.33±0.606	6.90±0..758	8.53±0.628	53.345	<0.001
Colour	8.36±0.614	6.50±0.028	8.36±0.617	76.876	<0.001
Flavour	8.10±0.803	6.96±1.217	8.26±0.827	16.108	<0.001
Texture	8.13±0.776	6.63±0.808	8.46±0.571	54.230	<0.001
Taste	8.20±0.610	7.10±0.844	8.60±0.563	38.690	<0.001
Overall Acceptability	8.20±0.484	6.67±1.028	8.86±0.345	81.143	<0.001

Standard sample: wheat flakes
Variation 1: Finger millet Mushroom flakes with incorporation of 10 percent each
Variation 2: Finger millet Mushroom flakes with incorporation of 20 percent finger millet and 10 percent mushroom powder
Significant at 5 per cent

Table VIII represents mean acceptability score for Finger millet mushroom flakes. With regard to appearance ,variation 2 had obtained the highest score of 8.53±0.628 followed by Standard and variation 1 8.33±0.606 and 6.90±0.758 respectively. With regard to colour variation 2 and standard obtained the highest score of 8.36±0.614 and 8.36±0.617 respectively followed by standard and variation 1 with score of 6.50±0.028 respectively.

With regard to flavour ,variation 2 had obtained the highest score of 8.26±0.827 followed by standard and variation 1with the score of 8.10±0.803and 6.96±1.217 respectively. With regard to texture variation 2 had obtained the highest score of 8.60±0.563 followed by Standard and variation 1 with the score of 8.20±0.610 and 7.10±0.844 respectively. With regard to taste variation 2 had obtained the highest score of 8.60±0.563 followed by Standard and variation 1 with the score of 8.20±0.610 and 7.10±0.844 respectively.

Data from the Table VIII indicated that V_2 have overall acceptability in terms of appearance ,colour ,flavour, texture and taste with statistically significant P value of <0.05. A study by A study by Kavitha and Bharathi (2016) revealed that sensory characteristic of Ready To Eat millet Flakes was acceptable.

Table IX

Mean acceptability score for Finger millet mushroom Cookies, Finger millet mushroom Soup mix and Finger millet mushroom Flakes

Sensory Characteristics	Mean± S.D		
	Cookie	Soup	Flakes
Appearance	8.50±0.776	8.80±0.610	8.53±0.628
Colour	8.46±0.628	8.76±0.430	8.36±0.617
Flavour	8.16±0.746	8.83±0.379	8.26±0.827
Texture	8.20±0.924	8.83±0.379	8.46±0.571
Taste	8.36±0.850	8.83±0.379	8.60±0.563
Overall Acceptability	8.16±1.05	8.80±0.406	8.86±0.345

Significant at 5 per cent level

Table IX represents Difference in physicochemical values of standard and variation in Finger millet mushroom cookies**.** There was no significant difference in the acceptability of the product. All the developed products are equally acceptable. There is positive correlation among the products in terms of overall acceptability. A study by Tersoo Abiem *et al*., (2019) shows that nutritious and acceptable product can be produced from millet supplemented with 10% mushroom powder

D. Physicochemical properties of the developed food products

Table X

Difference in physicochemical values of standard and variation in Finger millet mushroom Cookies

Nutrient	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Moisture (%)	3.3±0.014	4.35±0.08	.001*	.001*
Ash (g)	1.4±0.007	2.4±0.5	.001*	.002*

Significant at 5 per cent level

Table X represents Difference in physicochemical values of standard and variation in Finger millet mushroom flakes. Moisture content increased from (3.3% – 4.3%) in cookies and ash content from (1.4 %-2.4%).Moisture content is of great significance in bakery products for acceptability and storage stability. A similar study by Rana *et al*., (2021) moisture content ranged from (2.90% to 3.80 %) with increased level of finger millet flour and findings have agreement with Amir *et al*., (2015)

Table XI

Difference in physicochemical values of standard and variation in Finger millet mushroom soup mix

Nutrient	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Moisture (%)	3.9±1.3	5.5±0.08	.007*	.013*
Ash (g)	1.3±0.05	1.6±0.08	.001*	.002*

Significant at 5 per cent level

Table XI represents the Difference in physicochemical values of standard and variation in Finger millet mushroom soup mix .Moisture content increased from (3.9 % – 5.5%) in soup mix and ash content from (1.3% -1.6%). The results of the present study corroborate with that of Sangwan and Dahiya (2013) who also reported higher ash content in sorghum incorporated biscuit.

Table XII

Difference in physicochemical values of standard and variation in Finger millet mushroom flakes

Nutrient	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Moisture (%)	3.5±0.1	4.2±0.1	0.001*	0.001*
Ash (g)	0.06±0.01	0.67±0.01	0.002*	0.001*

Significant at 5 per cent level

Table XII represents the Difference in physicochemical values of standard and variation in Finger millet mushroom flakes Finger millet mushroom flakes .Moisture content increased from (3.5% – 42%) in flakes and ash content increased from (0.06g –0.67 g). A Study by Tasnim Farzana (2019) analysed that the moisture were increased from (10.66 % to 10.13%) and ash (0.69 g to 1.26g) on incorporation of mushroom in blended wheat and oats flour Ash, protein contents were linearly increased with the increasing percentage of mushroom flour .These trends of increase are in agreement with the study of Genenu *et al.* 2017 .The highest ash and protein content was found for F_3 (1.26% and 16.49%) while least for control F_0 (0.69% and 14.04%).

A Study by Salehi (2019) revealed that addition of mushroom powder contributes to the higher content of vitamins, mineral (calcium, potassium, magnesium, phosphorus, iron, copper, zinc and manganese), polyphenols, crude fibre and protein content in the bakery products. A Study by Sona *et al.,* 2011 also revealed that total ash content significantly increased in the incorporation of buck wheat flour and millet flour.

E. Nutrient analysis of the developed food products

Table XIII

Proximate composition of Finger millet mushroom Cookies

Nutrients	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Energy (Kcal/100 g)	363.66±2.2	345.66±0.5	0.001*	0.004*
Carbohydrate (g/100 g)	55.2±1.41	53.8±0.08	0.001*	0.015*
Protein (g/100 g)	9.84±0.48	11.1±0.005	0.002*	0.004*
Fat (g/100 g)	32.45±7.4	24.05±0.01	0.031*	0.030*
Fibre (g/100 g)	1.33±0.014	4.37±4.58	0.001*	0.001*

Significant at 5 per cent level

Table XIII represents the proximate composition of Finger millet mushroom Cookies. Energy value of cookie is significantly decreased from 363.66±2.2 to345.66±0.5. Finger millet and button mushroom have low calories when compared to wheat flour. Mushroom carbohydrates are not a major source of energy for humans (Cheung *et al*., 2010).A similar study by Ranjeet *et al.*, (2016) revealed that energy value of malted finger millet incorporated cookie was 346.60 Kcal. There is a significant difference in carbohydrate .A study by Rahmaan *et al*.,(2016) revealed that decrease in carbohydrate content may be due to the dilution effect of mushroom flour since they are generally low in carbohydrate .A significant increase in protein was observed in cookies

Table XIV

Proximate composition of Finger millet mushroom soup mix

Nutrients	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Energy (Kcal/100 g)	370±0.63	357±1.69	.001*	.001*
Carbohydrate (g/100 g)	70.1±0.585	65 ±0.404	.020*	.001*
Protein (g/100 g)	10.7±0.05	11.6±0.05	.001*	.001*
Fat (g/100 g)	5.6±0.17	4.5±0.08	.001*	.001*
Fibre (g/100 g)	0.86±0.01	8.1±0.08	.003*	.006*

Significant at 5 per cent level

Table XIV represents the proximate composition of Finger millet mushroom soup mix .Energy and carbohydrate value had slightly decreased. This may be attributed to the addition of low Carbohydrate mushroom powder to it. There was a significant increase in protein content .Earlier study also reported an increase in moisture content of bakery products with increase in protein content (Anu *et al.,* 2007; Singh *et al,.* 2006). The fat content present in flakes was significantly decreased. Both finger millet and mushroom are low in fat (Longvah *et al.,* 2017) A study by Tersoo-Abiem (2019), also agreed that Significant ($P<0.05$) increment in protein (13.8% to 17.05%), moisture (4.32 to 4.92%), ash (2.06% to 7.96%) and fat (4.32 to 7.14%) content was observed with addition of mushroom flour. An increase in dietary fibre was observed. Protein content of the developed soup had significantly increased. As germination time increases, the protein content also increases. The protein content was in the range of 14% -17.5 % as germination time increase from 8 to 24 h .This is in agreement with reports of other researchers that edible mushrooms generally have high amounts of protein, ash and fat content (Ojo *et al.*, 2017).

Table XV

Proximate composition of Finger millet mushroom flakes

Nutrients	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Energy (Kcal/100 g)	352.6±0.5	335.8±0.7	.001*	.001*
Carbohydrate (g/100 g)	68.6±0.5	64.6±0.3	.002*	.014*
Protein (g/100 g)	6.6±0.1	8.6±0.25	.001*	.001*
Fat (g/100 g)	4.3±0.1	2.5±1.8	.003*	.001*
Fibre (g/100 g)	1.3±0.01	6. 6 ±0.05	.001*	.001*

Significant at 5 per cent level

Table XV represents the Proximate composition of Finger millet mushroom flakes. There is a significant decrease in energy and carbohydrate .A study by Okafor *et al*., (2012) also observed similar decrease in carbohydrate and crude fibre content of wheat-mushroom bread. There is a no significant decrease in dietary fibre was observed .Significantly lower crude fiber content observed in control as compared to experimental cookies might be attributed to the higher fiber content of finger millet (Gopalan *et al.* 2000). Similarly Sangwan and Dahiya (2013) reported significant increase in crude fiber content of biscuit after substitution of refined flour with sorghum and soybean.

Table XVI

Micronutrients in Finger millet mushroom Cookies

Nutrient	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Calcium (mg/100 g)	28.37±0.02	164±3.050	.001*	.001*
Phosphorus (mg/100g)	93.03±0.014	204±0.404	.001*	.001*
VitaminD_2 (mcg/100g)	0.064±0.007	8.13±0.404	.001*	.001*

Significant at 5 per cent level

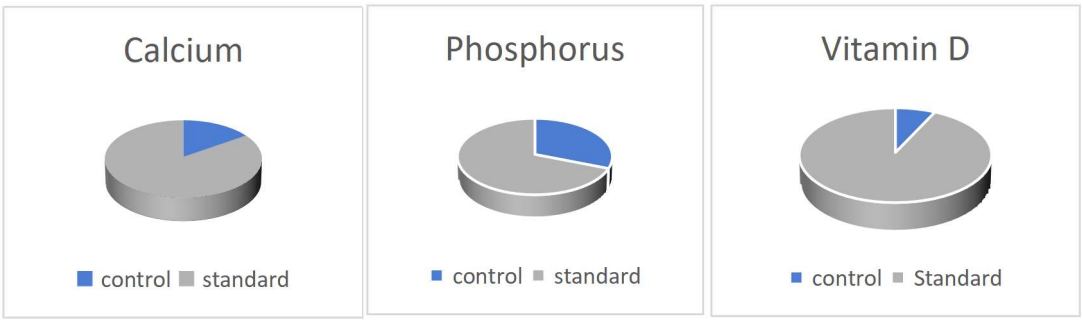


Figure 2

Micronutrients in Finger millet mushroom Cookie

Table XVI and Figure 2 represents the Micronutrients in cookies .Calcium content of cookies was significantly increased in cookie. A similar study by Sinha and Rekha, 2013 revealed that significantly higher calcium content observed in Type II as compared to Type 1 might be due to incorporation of higher percentage of finger millet. Phosphorus content of control cookies was 56.33+ 0.88mg/10g where as phosphorus content of finger millet incorporated cookies ranged from 98-100.34mg/10g.

Table XVII

Micronutrients in Finger millet mushroom soup mix

Nutrient	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Calcium (mg/100 g)	97±1	142±0.47	.001*	.001*
Phosphorus (mg/100g)	140±1	170±0.47	.001*	.001*
VitaminD_2 (mcg/100g)	3.95±0.01	5.57±0.03	.001*	.001*

Significant at 5 per cent level

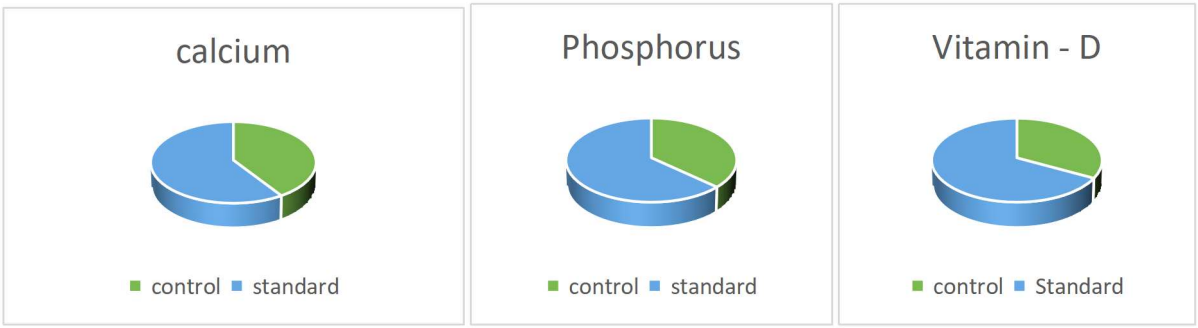


Figure 3

Micronutrients in Finger millet mushroom soup mix

Table XVII and Figure 3 represents the Micronutrients in Finger millet mushroom soup mix. There is a significant increase in calcium , phosphorus and Vitamin D_2. A study by Zachariya *et al., (*2020) revealed that calcium content in ragi flakes ranged from 19.29 mg/100 g to 222 mg/100 g due to germination**.**

Table XVIII

Micronutrients in Finger millet mushroom flakes

Nutrient	Mean±SD		P Value	
	Standard	Variation	One sided	Two sided
Calcium (mg/100 g)	47±0.984	98±0.23	.001*	.001*
Phosphorus (mg/100g)	97±0.23	182±2	.001*	.001*
VitaminD_2 (mcg/100g)	0.01±0.003	3.57±0.03	.001*	.001*

Significant at 5 per cent level

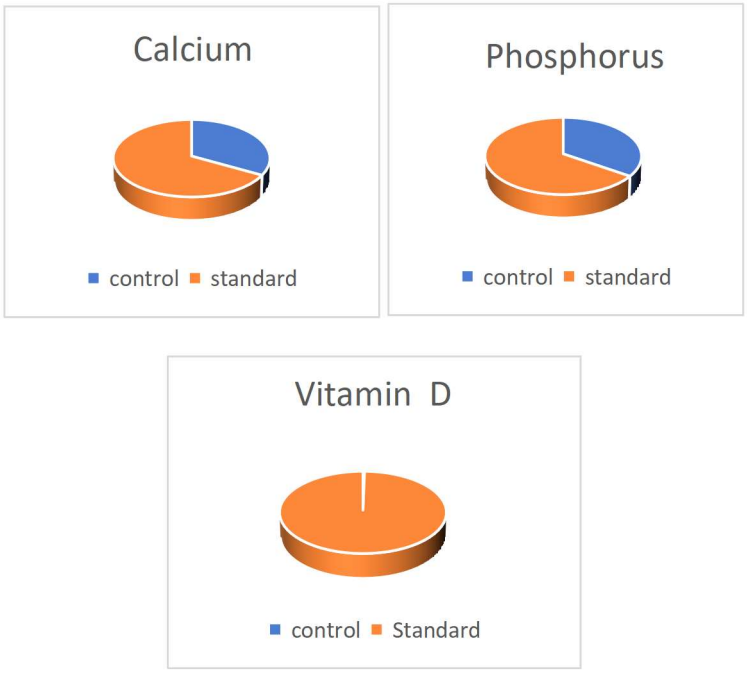


Figure 4

Micronutrients in Finger millet mushroom flakes

Table XVIII and Figure 4 represents the Micronutrients present in Finger millet mushroom flakes. There was significant increase calcium, phosphorus and Vitamin D_2.The results with respect to ash content were in strong agreement with the findings of Subastri *et al.*,(2015) who prepared koozh (water based finger millet porridge) by using germinated and non-germinated ragi flour. They observed that the germination of finger millet increased the mineral content

F. Shelf life assessment of the RTE and RTC Food products

Storage studies of RTE and RTC Food products was carried out. A storage study of developed products was conducted for 15 days in packaged condition. There was no significant difference in the sensory and physicochemical characteristics for the storage period of 15 days in all the products.Ready To Cook Soup mix and Ready To Eat Flakes were packed in heat resealable zip lock paper pouch laminated with LDPE. As LDPE pouches have high moisture and water vapour barrier properties, minor moisture absorption is seen without any colour change in it. A study by Marsh and Bugusu, (2007), Paper can be laminated with polyethylene to make it heat sealable and to improve gas and moisture barrier properties. Laminated paper is used to package dried products such as soups, herbs, and spices (Marsh and Bugusu, 2007). Cost analysis for 100 g of soup mix and flakes was found to be Rs .50 and Rs. 25 respectively .Cost analysis for 100g of cookie was found to be Rs. 35.Ready To Eat Cookie was packed in metalized polythene cover in a carton. Hundred grams of packet contains eight cookies .Hundred grams of soup has five serving. Hundred grams of flakes serves one.

Summary and Conclusion

V. Summary and Conclusion

The present study entitled "Formulation and Evaluation of Vitamin D_2 and Calcium rich Ready To Eat (RTE) and Ready To Cook (RTC) Food products" represents the work in development of RTE and RTC food products rich in calcium and Vitamin D which is needed in this era to combat the twin burden of Vitamin D and Calcium deficiency.

Vitamin D and Calcium deficiency is prevalent all over the world. They are inseparable nutrients needed to maintain the bone health. Vitamin D plays an important role in the metabolism of calcium. Supplements for calcium deficiency contain Calcium and Vitamin D to enhance the bioavailability. Therefore the present study focused to develop food products rich in Vitamin D and Calcium.

Ready To Eat and Ready to Cook products are gaining familiarity among the consumers as convenience is the multidimensional concept which attracts the consumer towards it. Instead of developing recipe from Vitamin D and Calcium rich foodstuff RTE and RTC products are developed from Button mushroom and finger millet .

Button mushroom (*Agaricus bisporous*) when exposed to sunlight will generate Vitamin D_2.Vitamin D enhanced mushrooms contain high concentrations of vitamin D_2, which are relatively stable during storage and cooking. Consumption of vitamin D-enhanced mushrooms could substantially contribute to alleviating the global public health issue of vitamin D deficiency.

Finger millet (*Eleusine coracona*) is one of the ancient millets in India. Of all the cereals and millets, finger millet has the highest amount of calcium (344 mg) and potassium (408 mg). It has higher dietary fibre, minerals, and sulfur containing amino acids compared to white rice, the current major staple in India. Depending on the type of processing, calcium bioavailability either increased or decreased. Finger millets contribute to high calcium retention and extremely high bioavailable calcium.

The present investigation was therefore undertaken to develop the RTE and RTC food product and to assess their Organoleptic and shelf life assessment and to analyse the physicochemical and nutrients in the developed product .The results obtained during the analysis have been briefly summarized and concluded. The results obtained from the formulation and evaluation RTE and RTC products have been briefly summarized and concluded.

Salient findings of the study:

- Malting of finger millet had significantly increased the calcium content from 359 mg to 429 mg and in sun dried mushroom Vitamin D_2 increased significantly P <0.05 from 0.03 mcg to 36 mcg .
- Organoleptic evaluation RTE Finger millet mushroom Cookie with Incorporation of (20%) malted finger millet flour and (10%) mushroom powder was found to have an overall acceptability in terms of appearance ,colour ,flavour, texture and taste with statistically significant P value (<0.05) .
- Organoleptic evaluation of Finger millet mushroom Soup mix powder with (5 %) incorporation of malted finger millet flour was found to be have overall acceptability in terms of appearance ,colour ,flavour, texture and taste with statistically significant P value (<0.05) .
- Organoleptic evaluation of RTE Finger millet mushroom flakes revealed (20%) addition of finger millet flour and (10 % addition of mushroom powder was found to be have overall acceptability in terms of appearance ,colour ,flavour, texture and taste with statistically significant (P <0.05) .
- Moisture content of the RTE and RTC product have been increased significantly (P<0.05) compared to standard due to addition of malted finger millet flour and mushroom powder to it. Moisture content was increased from (3.3%) to (4.4%) in Finger millet mushroom cookies, (3.9 %) to (5.5 %) in Finger millet mushroom soup mix powder and (3.5%) to (4.2 %) in Finger millet mushroom flakes.
- Ash content of the RTE and RTC product have been increased significantly (P>0.05) compared to standard due to addition of malted finger millet flour and mushroom powder to it .Ash content was increased from 1.4g to 2.4 g in Finger millet mushroom cookies, 1.3 g to 1.6 g in Finger millet mushroom soup mix powder and 0.06 g to 0.67 g in Finger millet mushroom flakes.
- Energy value (Kcal) of Finger millet mushroom Cookie was significantly decreased from 363.66±2.2 to 345.66±0.5 compared to standard.
- Energy value (Kcal) of Finger millet mushroom Soup powder was significantly decreased from 370±0.63 to 357±1.69 compared to standard

- Energy value (Kcal) of Finger millet mushroom flakes powder was significantly decreased from 352. 6±0.5 to 335.8±0.7 compared to standard
- Carbohydrate content of Finger millet mushroom cookies , Finger millet mushroom soup powder and Finger millet mushroom flakes were decreased significantly as incorporation per cent of finger millet flour and mushroom powder increased
- Protein content of the developed product had increased significantly ($P<0.05$) from 10.2 g to 11.2 g in Finger millet mushroom cookies , 10.8 g to 11.7 g Finger millet mushroom soup powder and 6.5g to 8.4 g in Finger millet mushroom flakes.
- Fat content of the developed product had decreased significantly ($P<0.05$)from 29.86 g to 24.05 g in Finger millet mushroom cookies , 5.8 g to 4.5 g in Finger millet mushroom soup powder and 4.3 g to 2.5 g in Finger millet mushroom flakes
- Fibre content of the product was increased significantly ($P<0.05$) from 1.3 to 4.3 in Finger millet mushroom cookies ,6.6 to 8.6 in Finger millet mushroom soup ,1.3 to 6.6 in Finger millet mushroom flakes
- Calcium content of the product was increased significantly ($P<0.05$) from 28 mg to 164 mg in co Finger millet mushroom cookies , 97mg to 142 mg in Finger millet mushroom soup and 47 mg to 98 mg in Finger millet mushroom flakes .
- Phosphorus content of product was significantly increased ($P<0.05$) from 93 mg to 204 mg in Finger millet mushroom cookie , 140 mg to 170 mg in Finger millet mushroom soup and 97mg to 182mg in Finger millet mushroom flakes .
- Vitamin D_2 content of the developed product increased significantly ($P<0.05$) from 0.064 mcg to 8.13 mcg in Finger millet mushroom cookies, 3.95 mcg to 5.57 mcg in Finger millet mushroom soup mix and 0.01 mcg to 3.57 mcg in Finger millet mushroom flakes .
- Storage studies of RTE and RTC food product was for 15 days in packaged condition revealed that there was no significant difference in the sensory and physicochemical characteristics of the RTE and RTC food product
- Cost for 100 g Finger millet mushroom Cookies is Rs 35. Hundred grams of packet contain eight cookies
- Cost for 100 g Finger millet mushroom Soup mix is Rs.50. Hundred grams of packet serves five.
- Cost for 100 g Finger millet mushroom flakes is Rs. 25. Hundred grams of packet serves one.

Conclusion:

The results from the study revealed that developed RTE and RTC food products is rich in Vitamin D_2 and calcium. Sun drying had significantly increased the Vitamin D_2 in mushroom. Malting had significantly increased the calcium in finger millet. Sun dried button mushroom (10 %) and malted finger millet flour (5% - 20 %) can be incorporated to Finger millet mushroom cookies, Finger millet mushroom flakes and Finger millet mushroom soup mix . The study concluded that Button mushroom and Finger millet can be incorporated to enhance Vitamin D_2 and Calcium in RTE and RTC food products.

Recommendation for further study:

- Assessment of Vitamin D_2 and Calcium loss before and after cooking.
- Storage stability of Vitamin D_2 and Calcium in the product.
- *In vivo* bioavailability of calcium and vitamin D from the developed product

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Appendices

APPENDIX -I

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam
Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A++' Grade by NAAC
Recognised by UGC Under Section 12 B
Coimbatore-641 043, Tamil Nadu, India

26th February 2022

To
Ms.Asma.K
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Asma.K,

Ref: Your proposal No. IHEC/21-22/FSN-03 entitled "Formulation and Evaluation of Vitamin D_2 and Calcium Rich Ready to Eat (RTE) and Ready to Cook (RTC) Food Products " submitted for approval of IHEC on 23.11.2021.

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/21-22/ FSN-03 entitled "Formulation and Evaluation of Vitamin D_2 and Calcium Rich Ready to Eat (RTE) and Ready to Cook (RTC) Food Products" submitted by you. The Approval number for the same is AUW/IHEC/ FSN-21-22/XPD-03.

We wish you all the best in your research endeavours.

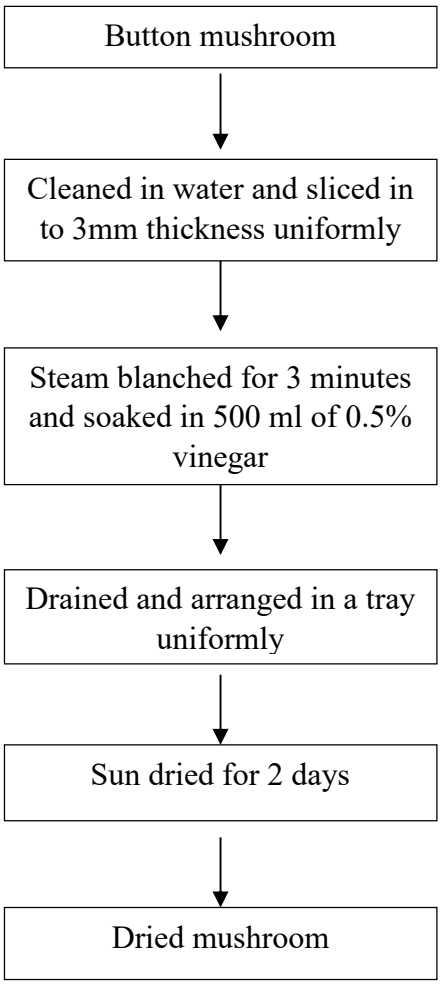
Regards,

Dr.S.Uma Mageshwari
Member Secretary

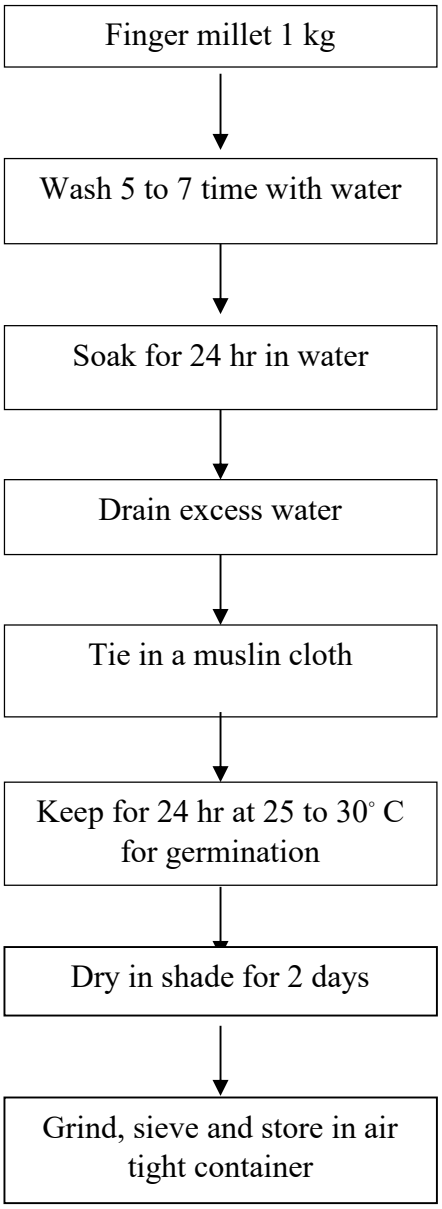
Institutional Human Ethics Committee

APPENDIX II

FLOW CHART FOR PROCESSING OF MUSHROOM

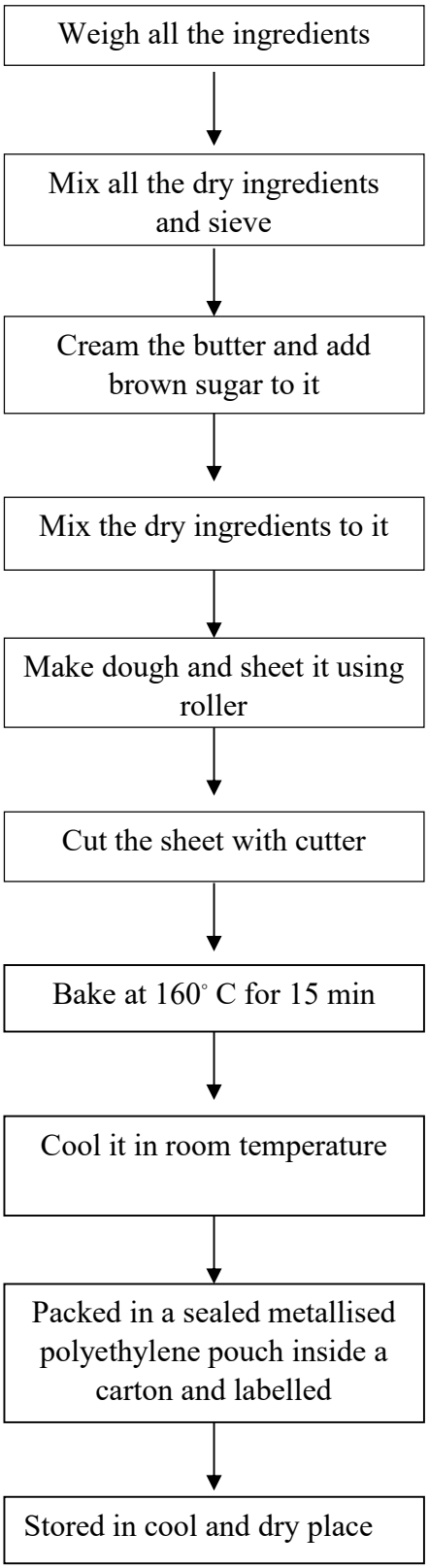


FLOW CHART FOR PROCESSING OF FINGER MILLET FLOUR



APPENDIX III

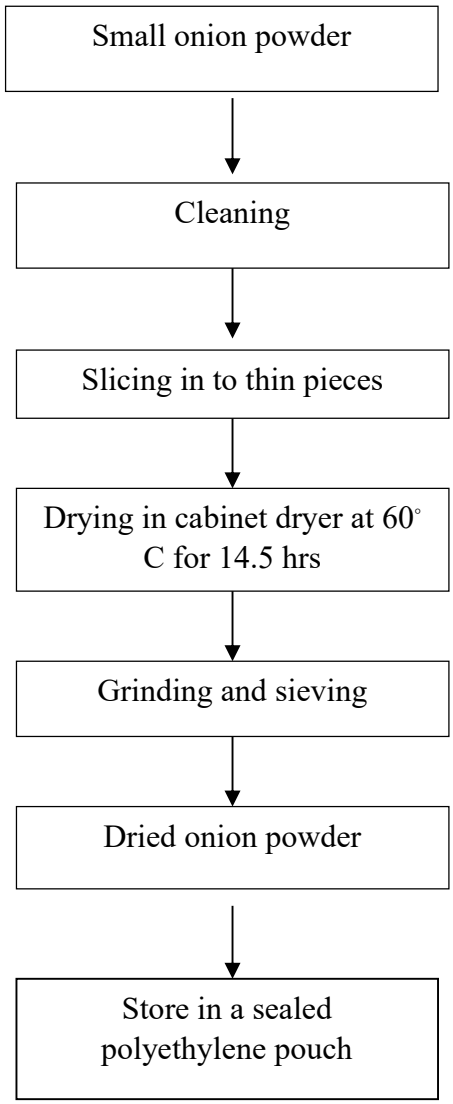
FLOW CHART FOR PROCESSING OF FINGER MILLET MUSHROOM COOKIE



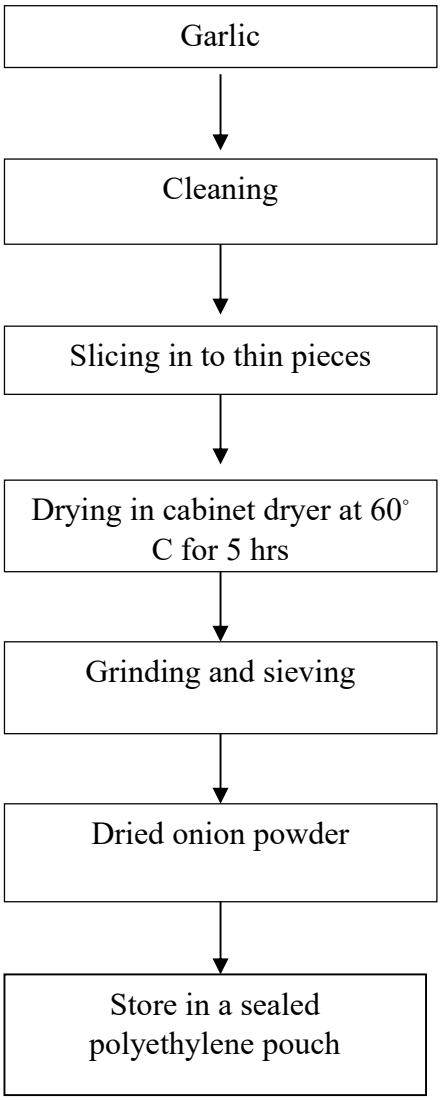
APPENDIX IV

FLOW CHART FOR PROCESSING OF FINGER MILLET MUSHROOM SOUP MIX

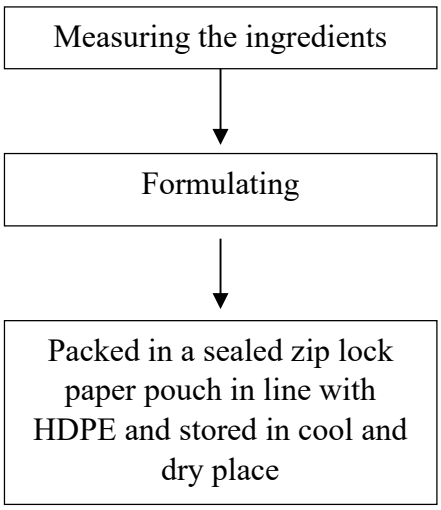
Small onion powder:



Garlic powder:



Finger millet mushroom soup



APPENDIX V

FLOW CHART FOR PROCESSING OF FINGER MILLET MUSHROOM FLAKES