

**FORMULATION AND EVALUATION OF VALUE ADDED
SNACK (BISCUITS) SUITABLE
FOR BONE HEALTH**

**Thesis Submitted In
Partial Fulfilment Of The
Degree of Master of Philosophy (M.Phil.)**

By

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July, 2019

DECLARATION

I declare that the dissertation entitled "**Formulation and Evaluation of Value Added Snack (Biscuits) Suitable for Bone Health**" submitted by me for the **Degree of Master of Philosophy (M.Phil.)** is the record of research work carried out by me during the period from 2018-2019 under the guidance of **Dr. A Thirumani Devi**, Professor and Head of the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and not formed the basis for the award of any Degree, Diploma, Associateship and Fellowship, Title in this university or any other University or institute of Higher learning.

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Signature of the Candidate

CERTIFICATE FROM THE SUPERVISOR

I certify that the dissertation entitled "**Formulation and Evaluation of Value Added Snack (Biscuits) Suitable for Bone Health**" submitted for the degree of **Master of Philosophy (M.Phil.)** by Manu Devi is the record of research work carried out by her during the period from 2018-2019 under my guidance and supervision and that this work has not formed the basis for the award of any Degree, Diploma, Associateship and Fellowship, Title in this university or any other University or institute of Higher learning.



31/7/19

**Signature of the
Head of the Department**



31/7/19

**Signature of the Supervisor
with designation**

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1. INTRODUCTION

Health and well-being is the primary concern of every nation and their health is not only an indicator of socio-economic status and standard of living in the country, but it also reflects the values and beliefs of society. A healthy citizen is a happiness to the family and society. To lead a healthy and happy life in energy stages of life, good health is the strong base which is the real reflection of the optimum nutrition. Among several factors, which influence the quality of life, nutrition with the combination of lifestyle patterns is the essential factor in the promotion and maintenance of the health status of an individual.

Proper nutrition enables one to lead a physically, mentally, socially and economically active life and it improves the quality of life as evidenced through the enhanced nutritional status, among the group of the population, better work efficiency rate, and reduced mortality and morbidity rate by raising the standard of living. Life expectancy is also increasing in most of the countries. The life span of the larger population is also lengthened due to remarkable technological advancements in food production and availability of adequate medical facilities, good hygiene and a high standard of living.

A well-balanced diet provides adequate energy, macro, and micronutrients to keep the body and mind healthy. Eating the right foods at the right time can also help to prevent a variety of health problems by maintaining healthy body weight, structure, and posture and promoting a general feeling of well-being. To meet the body's adequate nutritional needs, it is necessary to include a good supply of whole grains, pulses, vegetables, fruits, nuts and oilseeds with suitable quantity and quality of protein-rich animal food like milk and milk products, flesh foods including different kinds of seafood egg and so on.

A balanced diet has become less important over the last 50 years, at least following a balanced diet alone is no longer a route to good health. However, a balanced diet in natural means is healthy and prevents diseases. A person should aim at three main meal and healthy snacks to have a balanced diet. Balanced diet today must focus on not only macronutrients but also on the types of foods eaten within those groups. A healthy diet and lifestyle pattern are the best weapons against lifestyle diseases. Meanwhile, a considerable number of

people succumbed too many health issues because of communicable and non-communicable diseases. Including unrefined whole grains or germinated grains, fruits vegetables, nuts and oilseeds, and fiber-rich foods help to promote health status and prevent diseases and reduces the risk of participating in many lifestyles related diseases including bone diseases.

In earlier of the 20th century, the major nutritional problems among populations have shifted from nutritional deficiency diseases to concern today about overweight and obesity mainly due to overconsumption, poor dietary intake. Skipping one or another part of a day's meal and faulty dietary intake. This overweight and obesity are considered as the main causative factor, highly responsible to invite much non-communicable and lifestyle related to health problems. The prevalence of non-communicable diseases has shot up and burgeoning both developed and developing countries among the other nutritional problems and metabolic disorders including bone-related disorders and diseases. The disease conditions including bone health problems are multifactor disorders, consisting of physiological, biochemical, metabolic, anatomical, psychological and social alternatives and its development is due to an interaction between genes and environment.

The primary cause for this non communicable disease conditions is unlimited access to food reduction in physical activity as well as the fulfilment of genetic response's that the body stores up fat reserves during times of abundance for leaner periods later on the causes of the occurrence of overweight and obesity which is the complex condition, with severe social and psychosocial dimensions, affecting virtually all ages and all socio-economic groups worldwide. And also invites all types of critical health problems and one such problem is bone disorder.

Semple *et al.* (2014) stated that shifting demographic profile and industrial advancements have to lead to numerous health problems in combination with inactive lifestyle and unsuitable diets have paved way for chronic degenerative diseases like osteoporosis, diabetes mellitus, cardiovascular disease, and other metabolic disorders. Obesity is a recognized risk factor for osteoarthritis and was significantly common among obese women. Research findings show that the

prevalence of osteoarthritis was 49 percent among the study groups by Mandal *et al* (2009) and 57 percent in the study conducted by Lena *et al* (2009).

Obese women lead to a decrease in their weight by many methods like dieting, exercise, and medicinal intervention. Few women practice the habits of chronic diet controlling to reduce their body weight. This, in turn, leads to the deprivation of essential vitamins and minerals. A study was conducted to evaluate the diet pattern and bone mass density among chronic dietary obese women. It was observed that 33 percent of chronic dietary obese women tend to have low bone mass disorders. These methods of dieting have ill effects and need to be avoided. In turn, a balanced diet and physical exercise should be practiced regularly and also systematically.

Food and Drug Administration concluded that maintenance of an adequate protein and calcium intake throughout life may optimize peak bone mass at skeletal maturity and help to slow the rate of bone loss later in life and may help to reduce the risk of osteoporosis. Protein is not only essential for the muscle formation and also essential for the formation of bone mass and density in which protein acts as a matrix where mineral mixtures are embedded and facilitated for bone mass and health. Calcium is the major element essential for optimum bone health. Calcium is an excellent foundation for consolidating bone and health. Maintenance of an adequate calcium intake throughout life may optimize peak bone mass at skeletal maturity and helps to slow the rate of bone loss in later life and may help to reduce the risk of bone problems especially osteoporosis.

Functional foods play a positive role in maintaining bone mass and density, enhancing health and modulating immune functions to prevent specific diseases. They also hold great promise in clinical therapy due to their potential to reduce side effects and significant advantages in reducing health care costs. Dietary patterns may influence bone turnover and bone mass density. A healthy dietary pattern with a high intake of functional foods including whole and germinated grains, nuts and oilseeds with adequate quantity of fat may lead to less bone resorption and poor dietary pattern rich in processed foods is associated with a decrease in bone mineral density. So a healthy diet is required for strong bones.

A study showed that dietary supplementation with flax oil seeds, chia seeds, germinated ragi flour, soy protein isolate, whole wheat flour etc. can help to reduce the level of deoxypyridinoline in the urine and normalize bone density. The researchers also suggested that the presence of omega 3 fatty acids in flax seeds or chia seeds plays an important role in promoting bone health and protecting the process involved in bone formation and mineralization. Thereby helps to offset the negative effects of menopause and diabetes and can have on bone health (www.nutripeople.com).

Dietary supplementation is also known as a food supplement or nutritional supplement is a preparation intended to provide nutrients that are missing or are not consumed insufficient in quantity in a person's diet. Some countries define dietary supplements as foods, while in others, they are defined as drugs (Ziari, 2017). Epidemiological studies and clinical trials suggested that soybean especially its protein and isoflavones have beneficial effects on bone mineral density bone turn over markers and bone mechanical strength in postmenopausal women. However, there are conflicting results related to estrogen status of the body, metabolism of isoflavones among the individuals and other dietary factors. The long term safety of soy and its products supplements remain to be demonstrated.

According to cross *et al* (2014), flax seeds and chia seeds are very beneficial in resolving the symptoms of bone problems especially osteoporosis. These oilseeds are rich sources of calcium, magnesium, phosphorus zinc, etc. and help to regularize the hormonal cycle indirectly, reducing the occurrence of bone health problems and increase the bone mineral density. Priyaka (2013), found that foods developed on basis of ragi, wheat mix contribute an appreciable amount of protein calcium and dietary fiber. The sensory evaluation of the products was found to be acceptable and low cost and these grains based health mix can be suggested for domestic cooking purposes to enhance the quality and quantity of the daily dietaries. Wheat products also an excellent source of zinc and zinc has a positive influence on bone mass. In general, zinc is necessary to activate genes involved with protein synthesis and wellness of bones.

Research also indicated that calcium-rich foods and other bone boosting minerals are tremendously defended against bone health problems. The research also pointed out that dietary fiber like oats straw triggers the release of the luteinizing hormones leading to an overall hormone flow that stimulates cell growth and makes oat straw a great supplement for building bone mass and strength (<http://undergroundhealthreporter.com/oatstrawbenefits-longevity>). Eating healthy is critical whether our goal is weight loss, fitness or a healthier lifestyle in general. However if one does not have to meticulously count calories or starve our self to be healthy rather it needs to provide our body with ample nourishment, while junk foods and sugary treats might present challenges and temptations, adopting correct eating ideas and strategies will help to make healthy eating a natural food habits (Karan 2013).

Physical growth and development consist of four important main components of the fluid compartment, adipose tissue muscle mass and bone mass. Hence, the body requires all nutrients in correct quantum and quality to cater to the different needs of these four components. These can be achieved by including a variety of nutrients obtained from various food sources. Diet should also have low sodium content, so care should be taken to include low sodium foods and drinks with adequate water. This aids in digestion flush out toxin and keeping bowels clean that ultimately gear healthy eating habit and sense of well-being.

Calcium is required for bone devolvment. Protein is also binding to the blocks of cells. Our meal should be comprised of a wide variety of foods that will give us our daily dose of nutrients for physical and mental growth and development. With these backdrops, a variety of food items are available and used for the preparation of a variety of food products. The most important, commonly used food products irrespective of age and gender are biscuits.

Biscuits are the popular foodstuff consumed by a wide range of population due to their varied taste, long shelf life, and relatively low cost. The nutrient content is also increased by adding a variety of functional foods, which are valuable for its nutritional importance and health benefits. Because of the competition in the market and increased demand for healthy, natural and

functional foods products, attempts are being made to improve the nutritive value of biscuits and functionality by modifying their nutritive composition. Such effects are very often achieved by increasing the ratio of whole grains, raw materials, other ingredients having different types of dietary fiber in basic recipes with the attempts to increase the nutrient content of the biscuits especially proteins. Minerals like calcium, phosphorus, Fe, and zinc for proper quality and easy availability using value addition in terms of inclusion of functional foods valuable contribution of protein, minerals, and vitamins. And phytochemicals which also have nutraceutical effects to promote health and prevent diseases.

The value addition biscuits are rich in bone forming nutrients especially calcium, phosphorus, iron, and Protein. This value-added snack (biscuits) and highly nutritional and value for its minerals content especially the mineral's involved in bone formulation and synthesis. In this value addition snacks (biscuits) carbohydrates are the main energy source and are advised to include into our daily dietaries especially snacks (biscuits) for our young children, adolescents, adults, and elderly.

For the best results choose complex and germinated cereal, grains, protein from soy protein isolates, phytochemicals rich flax seeds and chia seeds. Addition of butter and sugar valuable for flavor, taste, texture and nutrient content including energy content. Value-added snacks are foods for health and hygiene and it fulfills the nutritional requirement of an individual. Based on age, sex, physical activities, and physiological condition, the quantum of biscuit consumption is varied and suggested to have biscuits are based on these four criteria.

Based on 2015-2016, National Health and Nutrition Examination Survey data, At least 80% of the people with osteoporosis are women. For females, the peak bone mass is achieved, usually by the age of 20, to assure optimal bone mass throughout adult life. The decline in bone mass in the aging population increases the risk of osteoporosis. This decline in women usually begins at the time of menopause and once was a reason for treating women with hormone replacement therapy (estrogen with or without progesterone).

Adequate dietary intake of several micronutrients is critical, especially calcium and vitamin D to maintain optimal bone mass in the postmenopausal population, the RDA for calcium in the 41-60 years old female population is 1200mg/day. For men of the same population, the RDA is 1000mg/day. Of concern is that females more than males do not consistently meet the RDA for calcium. This may be a reason why females rely heavily on calcium supplements. Many of the calcium supplements have been targeted toward the female population. For a woman who is counting her calories, it may be a lot more desirable to slowly enjoy the yummy taste of 20-30Kcal chocolate-flavored candy-like calcium supplement with added vitamin D and K than to get the equivalent amount of calcium from milk and milk products.

These value-added healthy biscuits help to provide the nutrients required for bone growth and health by satisfying the nutritional needs of a healthy population. With these backdrops, value-added biscuits are prepared which is suitable for all ages groups, wheat flour germinated ragi flour, soy protein isolates, functional foods contributing relatively rich in protein, fat, vitamins and minerals.

This biscuit is healthy suitable for bone health. The present study entitled **“Formulation and Evaluation of Value Added Snack Biscuits Suitable for Bone Health”** was designed with the following objectives:

1. To formulate and evaluate value added snack biscuits.
2. To determine the physical and chemical properties of value added biscuits.
3. To calculate and analyze the nutrient content of value added biscuits.
4. To analysis, the storage stability and cost effectiveness of the value added food product of biscuits.

2. REVIEW OF LITERATURE

The Review of literature on the study entitled “**Formulation and Evaluation of Value Added Snack (Biscuits) Suitable for Bone Health**” is discussed under the following headings:

2.1 Nutritional significance and health benefits of ingredients used in value added snack Biscuits.

2.2 Health benefits of value added Biscuits.

2.3 Physiochemical, microbiological and sensory characteristics of value added Biscuits.

2.4. Common bone health problems among middle aged women.

2.5. Value added Biscuits for promoting Bone Health.

2.1 Nutritional Significance and Health Benefits of Ingredients Used In Value-Added Snack Biscuits.

Sukeerthi, *et.al.* (2017), recommended a new approach to achieve optimal health status by promoting the state of well-being and possibly eliminating the risk of disease resulted in the development of various functional foods. With a change in time Food industry has been striving to cater to the diverse range of the population. They did a study by utilizing the underutilized, more nutritious ancient grains like Quinoa, Jowar, and Flaxseed in preparing biscuits of exceptional high nutritive value. Biscuits were prepared by adding quinoa and Jowar at different levels and keeping flaxseed constant (20%) and were compared. These were assessed for their nutritional composition as well as daily values. Biscuits prepared with quinoa, flaxseed and Jowar in 40:40:20 was superior in protein (12.14%) dietary fiber (10.64%) Potassium (299.72mg), calcium (74.20mg), Magnesium (123.98mg), Phosphorous (248.88mg) Iron (6.84mg) and Vitamin c (11.86mg).

Suzanne *et.al.* (2018), find out that Menopause is a physiological phenomenon in women's life. The decrease of estrogen hormone level leads to the appearance of uncomfortable physical and psychological symptoms harm

all aspects of female's daily life. This study aimed to help the female's body to adapt to diminish estrogen hormone around the time of the menopause by cookies, prepared from whole meal wheat flour, soybean flour, anise seeds, and flaxseed. Chemical analysis of the cookies showed that the supplement was enriched in protein, total phenols, and fiber and minerals contents.

Hussein (2006) did a study to evaluate the effect of the functional diet on bone health for elder women with osteoporosis and to elucidate its effect on the bone turn- over. The diet is in the form of biscuits contain whey protein and soybean. Fourteen osteoporotic elder women were recruited in the study and given 60 g biscuits daily for one month. Serum calcium and osteocalcin (OC), urinary deoxypyridinoline (U-Dpd) and Cr were measured before and after the ingestion of biscuits formula. Serum calcium level was changed after one month the ingestion of the biscuits formula. Serum OC concentration has increased significantly after one month of ingestion, while U-Dpd excretion had decreased significantly after one month of ingestion. The study revealed that there was a positive significant correlation between serum osteocalcin (OC) and urinary deoxypyridinoline U-Dpd before and after the ingestion of the biscuits.

Enrichment of cereal-based foods with other protein sources such as oilseeds and legumes has received considerable attention (Ayo and Olawale, 2013). Grains, legumes, like Soybean (*Glycine max*) is an excellent source of protein (40-45%). Hence the seeds are the richest in food value of all plant foods consumed in the world (Kure *et al.* 1998).

AL-Ansi, *et al.* (2017), found that the composite flour includes a higher content of protein, ash, Zn, Cu, K, Mg, Ca, and Amino Acids. The sensory evaluation for the composite flour biscuit showed the highest hardness compared to the mixture flour. Wheat flour both fermentation and malting significantly ($p < 0.05$) increased swelling capacity, oil, and water absorption capacity, with a decrease in the bulk density of the flours. The diffraction pattern on X-ray diffraction (XRD) showed a slight increase in the crystallinity of the fermented and malted millet flours, while

baking resulted in a reduced crystallinity of the biscuit samples (Adebiyi, *et al* 2016).

Farzana, and Mohajan (2015), studied the quality characteristics of soy-mushroom enriched biscuits which could be used as a protein supplemented cereal snack food. In this study, wheat flour was replaced with soy flour at different levels that are 20% (T3), 15% (T2), and 10% (T1) and without soy flour was kept as control (To). The mushroom was added in both biscuits. Biscuits were analyzed for chemical and sensory parameters. Protein content of soy flour-supplemented biscuits increased from 11.07% to 17.86% as compared to control along with a significant increased in fat (17.36–20.89%), fiber (0.48– 0.92%), iron (1.56–1.99 mg/100 g), and energy value (463–485 Kcal/g). Ash content also increased but not significantly. Results from chemical analyses and organoleptic evaluation indicate that good quality biscuits can be prepared by substituting wheat flour with 15% soy flour and the addition of mushroom powders may affect the backing quality.

Gull *et.al.* (2016), Finger millet (*Eleusine Coracana* L.) also known as African millet and is commonly called “ragi” in India. It is having excellent nutritional value and even superior to other common cereals. It is the richest source of calcium (344 mg) and magnesium (408 mg) than other millets. Predominant fatty acids of this millet are Oleic (49%), linoleic (25%) and palmitic acids (25%). Finger millet contains both water-soluble and liposoluble vitamins. Emerging products prepared from this millet are pasta, noodles, vermicelli, and bread. Being gluten-free is suitable for individuals suffering from celiac disease. It is also a rich source of several phytochemicals. Commonly used processing techniques for this millet are milling, malting, popping and decortications.

According to Ullah, *et.al* (2016), Chia seeds, the Ancient Grain is becoming enormously popular in modern food regiment in many countries; the higher proportion of α linolenic acid makes chia the superb source of omega- 3 fatty acid (about 65% of the oil content). Omega -3 fatty acid has been associated with a large number of physiochemical functions in the human body. Chia seed was characterized by a rich fatty acid composition and a higher level of phenolic compounds. Most importantly, the results showed that the substitution of wheat

flour with chia seeds up to 6% did not negatively affect the final product acceptance (Romankiewicz, *et al*, 2017).

Chen, *et.al.* (2003), conducted a study to evaluate the consumer acceptability of cookies prepared using four different soy ingredients including two types of extruded and expelled soy flours (INTSOY and Insta-Pro), a soy protein isolate and a soy protein concentrate. The study result of the study revealed that Women liked isolate-containing cookie colour better than men, No gender difference existed between other treatments. Consumers over 41 years of age gave Insta-Pro containing cookies higher overall liking scores than other cookies while no difference existed between cookie treatments for consumers between 26 and 40 years of age. Flaxseed, soy, and corn flour showed significant effects on the hardness, cohesiveness, springiness, gumminess, fracture force and stiffness values ($P < 0.05$). Added to a typical snack formulation up to levels of 10% with a reasonable acceptance offering a promising nutritious and healthy alternatives to consumers (Alpaslan, and Hayta, 2006).

Ganorkar and Jain (2013), Flaxseed is mainly considered an oilseed crop. Moreover, the other nutritional parameters than its oil content, make it a more favorable choice for food technologists to develop functional foods. Flaxseed contains a good amount of α -Linolenic Acid (ALA), an omega-3 fatty acid, protein, dietary fiber, lignan, specifically Secoisolariciresinol diglucoside (SDG). Flax is rich in fat, protein, and dietary fiber. An analysis of brown Canadian flax averaged 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash, which is the mineral-rich residue left after samples are burned (Morris, 2013).

The composition of flaxseed is varied with genetics, growing environment, seed processing and method of analysis (Daun *et al.*, 2003). The protein content of the seed decreases as the oil content increases (Daun and DeClercq, 1994).

According to Rubilar, *et.al* (2010), scientific evidence supported that flaxseed consumption; however, a large sector of the population is still unaware of the benefits associated with its consumption and its possible applications

as functional food ingredients in foodstuffs. Flaxseed is mainly known by its high alpha-linolenic acid content, but it is also a lignan source, soluble fiber, and protein, compounds that are biologically active in the prevention of some non-transmissible chronic diseases. Southern Chile has comparative advantages for the cultivation of this crop. Together with its full processing, this crop could strengthen the regional industry. The purpose of the present review is to highlight the nutritional properties of flaxseed.

2.2 Health Benefits of Value Added Biscuits.

According to the studies carried out by (Geervani, et.al; 1996) to analyze the effect of the combination of millet and legume and processing on digestibility, biological value and net protein utilization of the study were evaluated using albino rats. The millets and legumes selected for the study include sorghum, pearl millet, finger millet, chickpea and green gram (*Pradiatus*). The processes tested include dehulling, boiling, roasting, malting and baking. Among the combinations tested, the sorghum-chickpea combination had significantly ($p < 0.05$) higher digestibility. Between the processes tested, roasting resulted in significantly higher net protein utilization. Results of biological study on biscuits prepared by using millet and legume combination flours indicated the biscuits to be of good protein quality.

Finger millet is a rich source of calcium and other minerals. The presence of high amounts of phytic acid, polyphenols and dietary fiber in the finger millets may lower the bio accessibility of minerals. The study aimed to determine the influence of decortication, popping and malting on bioaccessibility of calcium, iron, and zinc in finger millet. The seed coat fraction of the millet was also included in the studies the study revealed that no significant increase in bioaccessibility of zinc was observed on popping the decorticated millet but native millet showed an increase of 18 g/100 g. Malted millet showed higher values of bioaccessibility for all the minerals while seed coat fractions exhibited comparatively lower values, because of the high proportion of inhibitory factors. Total phytic acid, polyphenols, and dietary fiber decreased significantly on decortication and malting (Krishnan, *et.al*; 2012).

Analyzed the flaxseeds as an essential source of high-quality protein and soluble fiber and has considerable potential as a source of phenolic compounds. Flaxseed is emerging as an important functional food ingredient because of its rich contents of α -linolenic acid (ALA), lignins, and fiber. Lignans appear to be anti-carcinogenic compounds. The omega-3s and lignan phytoestrogens of flaxseed are in focus for their benefits for a wide range of health conditions and may possess chemo-protective properties in animals and humans (Chishty and Bissu 2016).

According to the Nilesh *et.al*, (2018), flaxseed can be added to baked products as a whole seed, imparting a healthy appearance and increased texture quality. Flaxseed is emerging as one of the key sources of phytochemicals in the functional food arena. In addition to being one of the richest sources of alpha-linolenic acid oil and lignans, in the present study, the efforts are made towards the nourishment of cookies with flaxseed flour. So three different samples were taken for recipe standardization and the ratios are made with 5%, 10% and 15% wheat flour replacement with flaxseed flour. Finally, prepared cookies were analyzed for physical properties, chemical properties, and sensory attributes. By all means, the sample T2 with 10% flaxseed flour incorporation was found suitable and sample T2 found the best one. At and above 15% addition structure of cookies and other factors found decreased in terms of many attributes. Flaxseed proteins are potent multi-functional ingredients for food formulation owing to their techno functionalities, food preservation capacity, and health benefits. A possible synergistic effect with mucilage on their functionalities could be valuable even though this co-product in flaxseed may limit the protein yield during their production processes. Their techno-functional properties could also be considered in mixture with other flax bioactive components such as lignans and fibre to enhance the value of the flaxseed meal (Rabetafika, *et.al*; 2011).

The effects of the fortification of flaxseed at different levels were assessed on the nutritional and sensory quality of biscuit. Supplementation of wheat flour with flaxseed powder was done at different levels of 0-43%. A novel fortified biscuit was successfully produced by the authors and it was observed that the concentration of flaxseed increased the moisture, fat, ash, and protein showed a

gradual increase whereas dietary fiber showed a rapid increase and however, carbohydrate content was decreased (Masoodi and Bashir 2012).

From the Nutritional and Epidemiological point of view, it can be seen an occurrence increase of Chronic Non-Communicable Diseases, as well as the inflammatory ones, ordinarily associated with a wrong feed, poor in fibers and rich in fats and simple and refined carbohydrates (Rolim, 2015). Awareness of phosphorus intake is important because both phosphorus deficiency and overloading impair bone health and quality of life. Phosphorus consumption is increasing in many countries. Most dietary phosphorus is contained in protein-rich foods such as meat, milk, cheese, poultry, fish, and processed foods that contain phosphate-based additives to improve their consistency and appearance (Takeda, *et.al.*; 2012).

2.3 Physiochemical, microbiological and sensory characteristics of value-added Biscuits.

“Biscuit is a very delicious item that everybody loves to munch and enjoy as a snack. Today several companies produce biscuits with various brands, tastes, and ingredients. Biscuits may be cookies, crunches, and biscuits produced by companies” (Swati, 2011).

According to (Manley, 2011), Biscuits “can be stapled foods snacks and luxury gifts, and dietary products, infants foods, dog and cat foods, and with additions of chocolate and cream, etc. they borderline with confectionary. They are all made with flour, (wheat flour and all have low moisture content and thereby long shelf life if protected from, moisture and oxygen in the atmosphere. They are the original convenience manufactured foods. “Sensory scores indicated high acceptability for treated biscuits samples”. Protein and fat contents of biscuits increased with increasing soy fortifications. (Awasthi, *et.al.* 2012).

According to Devi, and Haripriya, (2014), the input of protein pasting behavior may contribute with the starch pasting property in the quality hitherto expanding the scope of partial substitution for wheat Maida with soy flour in the preparation of biscuits. Nutritional evaluation of best rated supplemented biscuit was protein (15.7%), fat (19.5%), fiber (2.2%), and moisture (3.6%). Thus supplementation of

soy flour and rice bran at 15% level each, would improve the nutritional quality without adversely affecting the sensory parameters. (Mishra, and Chandra, 2012)

According to Agu and Okoli, (2014), found that there was no significant difference ($P < 0.05$) in taste, crispness, flavor, and texture of the biscuits while significant differences ($P < 0.05$) existed in color and overall acceptability. The proximate composition of the biscuits ranged from 1.84% to 2.55% for moisture, protein 8.03–9.26%, fat 30.07–35.81%, ash 2.94–3.68%, crude fiber 0.47–0.80%, carbohydrate 48.74–55.96%, and energy 526.53–554.21 kcal/100 g. The microbial count of the best biscuit after 20 days of storage was 4.0×10^3 cfu/g for bacteria and mold contained 5.0×10^4 cfu/g.

According to Ndife, *et.al.* (2011), there was no significant difference observed between the whole wheat bread and the soy bread samples in the sensory attributes of crust colour and crumb appearance, While significant difference ($p < 0.05$) was observed in texture, flavour, and overall preference respectively. It was concluded that substitution of 10% soy flour into wheat flour gave the bread with the best overall quality acceptability. Sometimes it is necessary to look to alternative crops, 1 of which is millet. The results of their study indicated that as the amount of millet in the biscuits and extruded snacks increased, the liking of the flavour, texture and overall liking decreased. Millet contributed to a bitter taste and bitter aftertaste and resulted in gritty and dry food products. Further work is required to refine the products tested as well as to identify further products that can be added to the diet in to take advantage of the health benefits that millet provides (McSweeney, *et al*; 2016).

According to (Doxastakis, *et.al*; 2002) did a study with the following ingredients full- fat lupin, soya, and triticale flour were added to a medium-strength wheat flour. The added flour was used to replace 5 and 10% w/w of wheat flour. The effects of lupin, soya and triticale flour supplementation on physical dough properties, such as water absorption capacity, dough development time, dough stability, and crumb, porosity, and bread structure and quality characteristics were studied. Lupin and soya flour, at 5 and 10% substitution levels, increased the stability and the tolerance index of the dough.

According to (Gjore, 2017), Biscuits are products that are consumed by all age groups. The physical properties of the dough and the type of biscuits depend on the method of biscuit production. During the process of baking, the biscuits take place processes that cause changes in the dough, which lead to getting a porous, fragile and friable structure of biscuits. It reduces the amount of moisture and changes the color. Flaxseed has many health benefits and is considered a functional food ingredient. Flaxseed flour (0–18%) was used to partially replace wheat flour in cookies and its effects on the physical and sensory characteristics of the cookies were investigated. A correlation analysis was conducted between the instrumental and sensory data. Results indicated that flaxseed flour can be incorporated in cookies as a partial replacement of up to 12% of wheat flour without negatively affecting the physical and sensory quality. The correlation results suggest that the flaxseed flavour attribute best predict consumer preference for overall acceptability, though texture and colour attribute also contribute (Khouryieh and Aramouni, 2012).

According to (Mishra and Chandra 2012), Supplementation of Wheat flour with soya and rice bran was tried at 10 %, 15%, 20%, 25% level each. Prepared Biscuit is subjected to physical, Sensory and nutritional analysis to evaluate the suitability of biscuit for consumption. Thus supplementation of soy flour and rice bran at a 15% level each, would improve the nutritional quality without adversely affecting the sensory parameters. The designed to be designed to carried out to formulate calcium and iron-enriched cookies. The objective of this research was to prepare the nutritionally enriched cookies developed by incorporating ragi. The Chemical composition of finger millet revealed that the total carbohydrate content of finger millet has been reported to be 73.3mg/100 gm. of product. Finger millet has nearly 6.2mg/100gm of protein. Total ash content is higher in finger millet than in commonly consumed cereal grains. The ash content is nearly 1.5mg/100 g in finger millet. Calcium content of ragi was found to be 320 mg/100g, Finger millet is the richest source of calcium and iron. The iron content was 3.8+0.1mg/100 g of ragi. Calcium deficiency leading to bone and teeth disorder, iron deficiency leading to anemia can be overcome by introducing finger millet in our daily diet. It was observed that cookies prepared with 30% of ragi was highly acceptable. The iron and calcium content was found to be 9.5+ 0.1&152 +

0.1mg/100 g of product. The use of 30% of ragi in the preparation of cookies is a useful strategy to optimize the consumption of food rich in functional ingredients (Bhite, *et.al*; 2018).

According to (Kulthe, *et.al*; 2014), cookies high in proteins and low in calories were prepared by substituting wheat Maida with defatted soy flour (DSF) at 0, 10, 15, 20 and 25% levels and sugar with stevia leaves powder (SLP) at 0, 15, 20, 25 and 30% levels using traditional creamery method. Cookies were evaluated for physicochemical and sensory quality parameters. The thickness and hardness of cookies increased; weight, diameter, spread ratio, and spread factor decreased with increasing levels of DSF while there was an increase in protein, crude fiber, and ash content and a decrease in fat and carbohydrate contents. Cookies are good carriers of nutrients like carbohydrate and fat which can be enriched with protein by partially replacing refined wheat flour with protein-rich flour up to an acceptable level. The research study was conducted to identify the most suitable blend of pulses flour for partial replacement of wheat flour. Protein-energy malnutrition can be combated with such high protein cookies. Soybean, Moth bean, and Chickpea did a study were replaced with the base flour i.e. refined wheat flour at different replacement level (10, 15, 20 & 25 percent) and cookies of the above blend were developed. These cookies were studied concerning physical, chemical, textural and sensory analysis and Cookies with 20% Chickpea and 80% refined wheat flour was finalized and protein content increased from 2.9% to 6.8%. (Soni, Kulkarni and Patel 2018).

2.4. Common Bone Health Problems among Middle-Aged Women.

Thokchom and Chhugani (2014), conducted a Cross-sectional Study to screen the urban population for osteoporosis in the study. The results show that there was no significant association between osteoporosis, osteopenia with a sedentary lifestyle, family history, thyroid problems, and low calcium diet. The prevalence of osteoporosis was found at 48.5% in postmenopausal women. The study reveals that there was a significant correlation between low dietary intake of calcium and vitamin D, body mass index, early menopause, parity occupation, education, socio-economic condition, and addictions (Sanga, *et.al*.2018).

Osteoporosis is a major public health problem, affecting millions of individuals. Dietary intake is an important modifiable factor for bone health. Inadequate intake of nutrients leads to the risk of bone loss and subsequent osteoporosis. The process of bone formation requires an adequate and constant supply of nutrients, such as calcium, protein, magnesium, phosphorus, vitamin D, potassium, and fluoride (Palacios, 2006).

Yakub *et al*; (2018), to assess the prevalence of osteoporosis in ambulatory postmenopausal Indian women as measured by Calcaneal Quantitative ultrasound (QUS) and to study dietary calcium intake and vitamin D status and their influence on bone mineral density (BMD). The prevalence of osteoporosis was found 48.5% in postmenopausal women. There was a significant correlation between low dietary intake of calcium and vitamin D, body mass index, early menopause, parity, occupation, education, socio-economic condition, and addictions.

According to (Thulkar and Singh 2015), Osteoporosis is a multifactorial and slowly emerging global health problem. The lifetime risk of dying from a hip fracture is the same as that of breast cancer. One out of three women between the age group of 50-60 years in India suffers from osteoporosis. Indian women have an early age of onset of osteoporosis as compared to western counterparts. There is a need for early diagnosis, identification of high-risk groups and prevention and treatment of osteoporosis in the Indian context.

Agrawal and Gupta, (2013) studied that Osteoporosis is a condition of low bone mass that predisposes to fractures. This silently progressive metabolic bone disease is widely prevalent in India in both sexes and occurs at a younger age than in the western population. Rapid bone loss occurs in postmenopausal women due to hormonal factors. Indians have low bone density compared to Caucasians. High prevalence of vitamin D deficiency is a major factor for poor bone health in India. The bone health of the population can be improved by encouraging children to drink milk and take enough exercise. Results of randomized trials have revolutionized treatments and several effective therapeutic drugs are available. Despite new treatments many patients with fractures still do not receive appropriate management. Early detection and the

management of this condition can avoid the risk of fractures and associated morbidity and mortality.

Sudhaa *et.al.* (2006), was screened a substantial female population from Osteoporosis and Osteopenia using the calcaneal QUS method utilizing the same WHO T score criteria that otherwise shall remain undiagnosed and face the complications. The quality of life for women after menopause is one of the key health issues today, and osteoporosis is a silently progressing metabolic bone disease widely prevalent in post-menopausal women in India. Rapid bone loss occurs in post-menopausal women due to hormonal factors that lead to an increased risk of fractures. Thus, the present study was undertaken to observe the serum calcium and alkaline phosphatase (ALP) levels in post-menopausal women as these substances are biochemical markers of bone metabolism. In this small-scale cross-sectional study, 100 samples were taken. Of these, 50 were taken from post-menopausal women (experimental group) and 50 were taken from pre-menopausal women (control group). Serum calcium and serum ALP were measured in the blood samples of both groups. The findings demonstrated that the serum calcium level was significantly lower in the post-menopausal group than in the pre-menopausal group, while the ALP level was slightly higher. Therefore, an increase in bone turnover accelerates the bone mass reduction in post-menopausal women, whereas a decrease in bone turnover is associated with the preservation of bone mass (Bhattarai, *et.al.* 2014).

Jhaveri, *et al;* (2015), revealed that the developed a questionnaire that was filled by a total of 84 orthopedics throughout India. The prevalence of osteoporosis in India according to the orthopedic surgeons was 38.4% and there was a female preponderance. Most of the respondents felt out of every 100 osteoporosis patients in India, less than 20 patients are diagnosed and treated for osteoporosis. Osteoporosis is a growing health problem recognized in both developed and developing countries. It is associated with substantial morbidity and socioeconomic burden worldwide. 30% to 50% of women and 15%-30% of men suffer from osteoporosis-related fractures in their lifetime (Prasad *et.al;* 2010).

Numerous clinical studies have shown bisphosphonates (BPs) to be useful and cost-effective options for fractures prevention and postmenopausal bone loss. The use of oral bisphosphonates is an established option for management of osteoporosis in postmenopausal women, but many of them complain from gastrointestinal side effects or frequently dosed oral regimens. To improve upon the suboptimal therapeutic compliance in postmenopausal women, newer, longer-acting intravenous formulations of BPs have been approved for intermittent administration in postmenopausal women. These preparations would become an option for patients who cannot tolerate oral BPs or it was ineffective in increasing their bone density. This article proposed to review the effectiveness and tolerability of intravenous BPs in postmenopausal women with osteoporosis (Mottaghi, 2010).

2.5 Value-Added Biscuits for Promoting Bone Health.

According to Cashman (2007), Osteoporosis is a debilitating disease that affects many middle-aged and older people. Fragility fractures are the hallmark of osteoporosis. Although nutrition is only 1 of many factors that influence bone mass and fragility fractures, there is an urgent need to develop and implement nutritional approaches and policies for the prevention and treatment of osteoporosis that could, with time, offer a foundation for population-based preventive strategies.

Osteoporosis poses a significant public health issue, causing significant morbidity and mortality. Calcium and vitamin D utilization in the optimization of bone health is often overlooked by patients and health care providers. Besides the optimal standard of care for osteoporosis should encompass adequate calcium and vitamin D intake. Dietary intake or supplementation with calcium and vitamin D will be reviewed, including recent recommendations for increased vitamin D intake. Compliance with calcium and vitamin D therapy is paramount to the effective prevention of osteoporotic fractures. A recently released algorithm (FRAX) estimating absolute fracture risk allows the health care provider to decide when pharmacologic therapy is warranted in addition to calcium and vitamin D (Sunyecz, 2008).

3. METHODOLOGY

Methodology dedicated in the bearing of the present study on **“Formulation and Evaluation of Value Added Snack Biscuits Suitable for Bone Health”** is deliberated underneath behind the titles.

3.1 Selection and Procurement of Ingredients.

3.2 Preparation, Standardization Value Added Snack Biscuits Suitable For Bone Health.

3.3 Sensory Evaluation of Value Added Snack Biscuits Suitable For Bone Health.

3.4 Physical Characteristics of Value Added Biscuits.

3.5 Analysis of Proximate Composition of Value Added Biscuits.

3.6 Assessing The Microbial Count And Cost Effectiveness of the Value Added Biscuits.

3.7 Packaging and Labelling of the Value Added Biscuits.

3.8 Statistical Analysis and Interpretation of the Data.

3.1 Selection and Procurement of Ingredients

Proper nutrition is the most important part of a balanced diet. It is important to maintain health and a sensible body weight in terms of water content, muscle mass, fat deposition, and bone mass. No single food provides all the essential nutrients that the body requires to be healthy and function efficiently. All the macro and micro nutrients and also phytochemicals are essential to promote the health and to prevent disease conditions.

The value addition of ingredients enrich the nutrient content in terms macro and micro nutrients including minerals and vitamins. Germinated ragi, wheat flour, soy protein isolate, flax seeds, chia seeds, brown sugar and butter were selected for the preparation of value added health mix incorporated biscuits suitable for bone health. This value added health mix incorporated biscuits combined with normal food intake helped to improve bone mass and density, thereby bone health.

Health mix incorporated snack was prepared with the targeted nutrients for the optimum bone synthesis and was suggested to include in the form of snack (biscuits) regularly. In the present study, ingredients were selected carefully to formulate value added biscuits, considered as the snack. The nutritional significance and health benefits of the selected ingredients are discussed in the following pages.

3.1.1 Wheat Flour

Whole wheat flour is valuable for its nutritional significance and health benefits. Regular consumption of whole wheat grains and its products are associated with the reduced risk of various types of chronic life style diseases. Whole wheat grain is rich source of protein in the form of gluten, dietary fibre, vitamins and minerals and also phytochemicals for promoting health status especially bone health. Wheat is the most effectively consumed food crop across the globe and considered are the excellent source of protein, minerals B complex vitamins as the dietary fibre. Each portion of wheat, e.g. grain germ, flakes etc. has nutrients and rich content of protein in which helps to build strong body and used as the matrix for minerals deposition, hence wheat was used in the preparation of value added health mix.

3.1.2 Soy Protein Isolate

Soy beans play an integral part in Asian culture, both as a food and as a medicine, for many centuries. Existing database on the health effects of soy food intake is quite extensive and clearly warrants greater recognition by the research and clinical communications. Given the ease with which soy foods can be incorporated in to the diet, be it via the traditional soy foods such as tofu and soy milk or the more modern soy protein products like soy isolate, concentrates and flours, soy foods may be able to have a significant beneficial impact on health status of an individual. Generally soy protein isolate and soy protein concentrates are used in the food industry for the preparation of health mix to supplement or enrich the quality and quantity of nutrients especially protein in the diet.

3.1.3 Flax Seeds

Flax seeds contain one of the richest plant sources of omega 3 fatty polyunsaturated fatty acids, α -linolenic acid (ALA). ALA is believed to be one of the dietary factors responsible for providing significant protective effects against the incidence of musculoskeletal myocardial complications. The three main classes of phytoestrogens are isoflavones, lignans and coumenstans. Oilseeds are a good source of lignans with flax seeds being particularly rich.

Flax seeds consumption has previously been associated with changes in bone turnover markers in post-menopausal women. Many studies were designed to test the hypothesis that consumption of a proper quality and quantity of flax seeds achievable in an individual habitual diet (40g) will induce improvements in bone turnover markers, mediated through the increased circulation of phytoestrogens, in the menopausal women and revealed that the phytoestrogen rich flax seeds decreased bone turnover in post-menopausal women.

Flax seeds can be added to baked products as a whole seed, imparting a healthy appearance and increased texture quality. However, flax seeds can be ground (in milled), prior to consumption to obtain the potentials health benefits from the omega 3 fatty acids and lignans. Flax seeds is high in mucilage (gums) that can increase the water absorption properties of the dough, which have impact on mixing time and dough handling characteristics. Flax seeds is an economically important oil seeds crop essential to meet the nutritional requirement of an individual.

3.1.4 Chia Seeds (*Salvia Hispanica*)

The demand for chia seeds is sky rocking and with good reason. When prepared properly they are easy to digest, loaded with an array of nutrients and pack a powerful energy boost. The benefits of chia seeds have been widely researched and include improving digestive health, promoting heart, muscle and bone health boasting energy and metabolism and can even help to treat diabetes, obesity and so on Chia seeds are valuable for its high antioxidants contents, high in vitamin and minerals includes calcium, potassium and magnesium, contains 37 percent dietary fibre 20 percent protein and also high in omega 3 fats (20 percent omega

3 ALA). Calcium is a mineral that can get in abundance from plant based sources. In fact, one ounce of chia seeds will give 18 percent of daily recommended intake of calcium.

3.1.5 Ragi (*Eleusine Coracana*)

Ragi is also known as finger millet, ranks sixth among the cereals grown in India. It has extremely good shelf life and conserved as an important stable foods among Indian especially the lower income groups. It's highly valuable for its nutrient content especially calcium, potassium and dietary fibre. Ragi consumption helps in developing bones in growing children and maintaining the bone health of various groups of population. Knowing the nutritional significance and strength benefits of these ingredients, the investigator selected all these ingredients for the preparation of health mix highly suitable to strengthen bone mass of the body.

3.2 PREPARATION, STANDARDIZATION AND EVALUATION OF VALUE ADDED SNACK BISCUITS SUITABLE FOR BONE HEALTH.

Functional foods play an important role in maintaining wellbeing by enhancing health and modulatory immune function to prevent specific disease, they also hold great promise in clinical therapy due to their potential to reduce side effects and significant advantages in reducing the health care cost.

In the first steps, the selected ingredients such as grams and legumes were thoroughly cleaned washed and dried properly. Basically ragi is germinated easily and enhance the nutrients content. To enhance the nutrients content including phytochemicals, care was taken to include chia seeds, flax seeds, wheat flour, germinated ragi flour, and soy protein isolate with butter four variations were formulated with suitable quantity. The able over acceptability scores which included flavour, taste, texture and over all baking quality of the biscuits. Four different variations of value added biscuits were prepared along with the standard products of biscuit termed as the control. Table I highlights the quantity of ingredients used in value added snack in terms of biscuits.

TABLE- I**QUANTITY OF INGREDIENTS USED IN VALUE ADDED BISCUITS**

Variations	(WF) (g)	(GRF) (g)	(SPIF) (g)	(CSF) (g)	(FSF) (g)	Sugar (g)	Butter (g)
Standard	100	-	-	-	-	60	60
Variation A	75	25	15	5	5	55	50
Variation B	50	50	10	7.5	7.5	55	50
Variation C	25	75	15	5	5	55	50
Variation D	20	80	15	5	5	55	50

Four variations were prepared by mixing refined wheat flour with germinated ragi flour and soy protein isolates in different quantity. The quantity of Sugar standard (60g), butter (60g) and Variations (55g), butter (50g) and a pinch of baking powder were used in all the four variations and control to enhance the taste and texture of the value added biscuits. All the ingredients were added systematically to prepare for dough preparation. The dough was allowed to stand 15 minutes until a uniform smooth texture was obtained. Then the dough was rolled on a flat rolling board sprinkled with some wheat flour to a uniform thickness using wooden hand roller. Circular biscuits cutter was used to cut the flatten dough, placed on a greased baking tray. The baking of four blends were baked in an oven at a temperature of 160° for 10 minutes. When a brown colour was developed, then the biscuits were removed and allowed to cool and packed in air tight container box and stored. The control biscuits were prepared using refined wheat flour with the same procedure adopted in the method and stored at ambient temperature. The fresh biscuits samples are used for sensory evaluation and analysis the physiochemical properties, nutrients content, microbial count and cost effectiveness of the sample of control and four variations.

Sensory evaluation is defined as a scientific method used to evoke measure, analyze and interpret those responses to products as perceived through the sense of sight, smell, touch, taste, and hearing (Stone and Sidel, 2014).

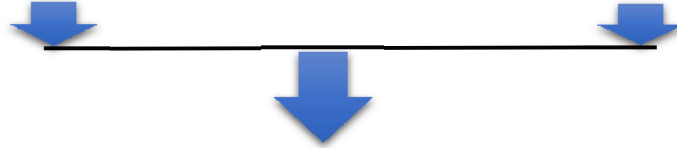
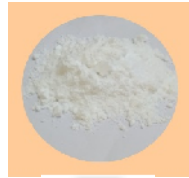
FIGURE-1
INGREDIENTS USED IN VALUE ADDED BISCUITS



PLATE- 1

BISCUITS PREPARATION FOR STANDARD

Whole wheat flour + Sugar + Milk + Baking powder + Butter
(100g) (60gm) (4tsp) (a pinch) (60g)



Creaming (Butter + Sugar, 6-7 minutes)

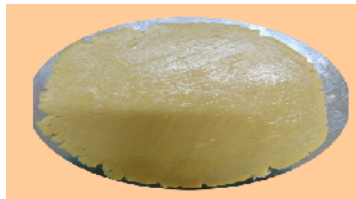


Dough preparation using

all ingredients



Kneading and sheeting



Moulding



Baking



Cooling



Packaging & Labelling



PLATE- 2

PREPARATION OF GERMINATED RAGI FLOUR



FIGURE-2
STEPS INVOLVED IN PRE- PREPARATION OF VALUE ADDED BISCUITS

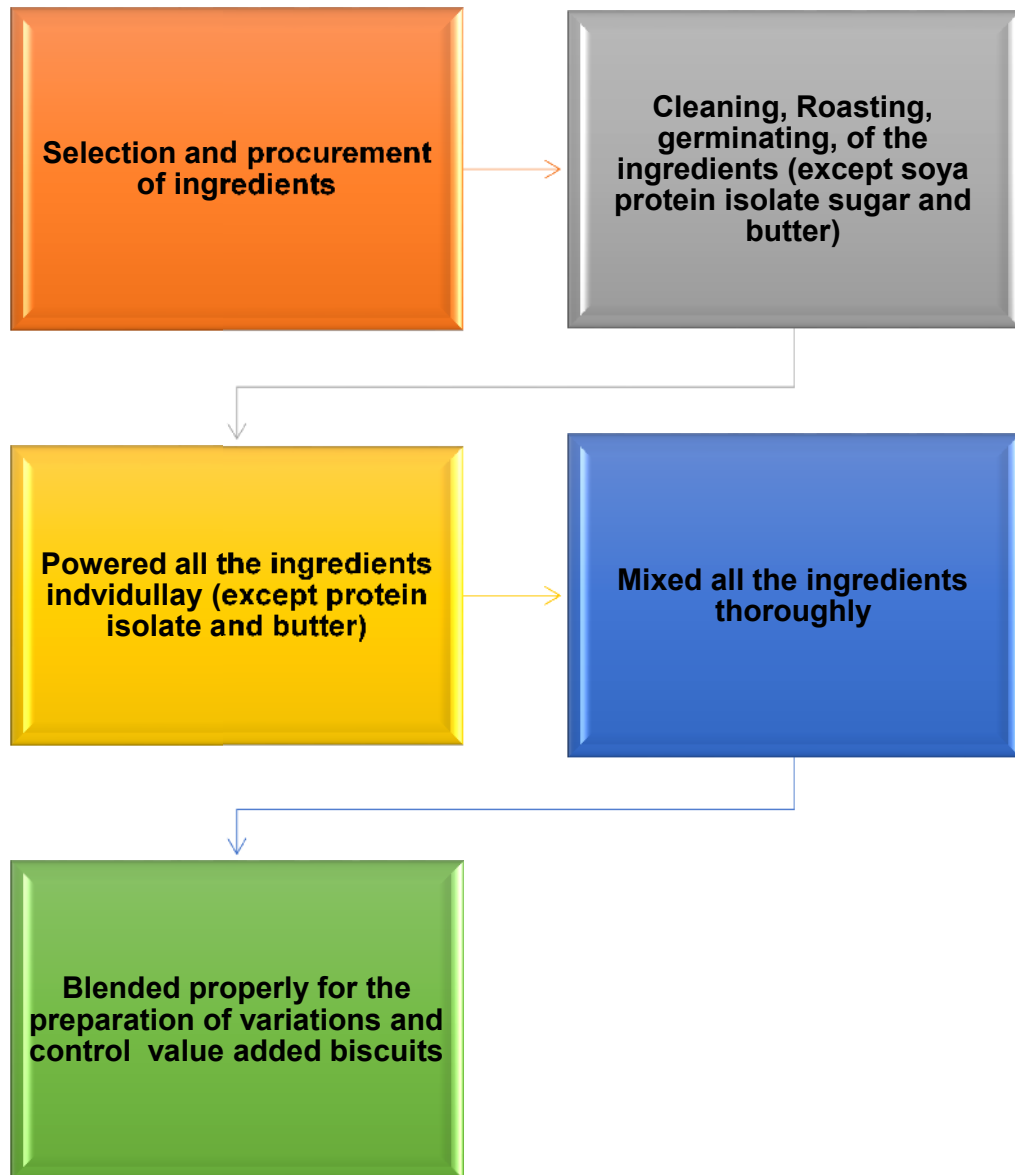


FIGURE-3
PREPARTION OF VALUE ADDED BISCUITS



PLATE- 3

BISCUITS PREPARATION OF VARIATION A, B, C, D

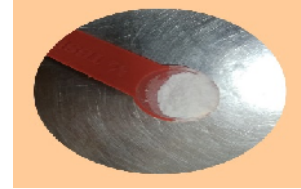
Whole wheat flour + Germinated ragi flour + Soy protein isolate powder



Chia seed powder +

Flax seed powder +

Baking Powder



Butter

Sugar



↓
Creaming and Dough Preparation

↓
Kneading



↓
Cutting

↓
Baking

↓
Cooling

↓
Packaging and labeling



PLATE- 4
VALUE ADDED STANDARD AND BISCUITS



STANDARD



VARIATION - A



VARIATION - B



VARIATION - C



VARIATION - D

PLATE - 5
SENSORY EVALUATION OF BISCUITS



Standard



Variation A



Variation B



Variation C



Variation D



3.3 SENSORY EVALUATION OF VALUE ADDED BISCUITS

Sensory evaluation was carried out in the following steps.

3.3. 1. Selection of Venue

Foods Laboratory of Food Science and Nutrition Department in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore was selected as the venue for the preparation, standardization, sensory evaluation and storage of the value added snacks in terms of biscuits.

3.3. 2. Selection of Taste Panel Members

Twenty Post Graduate Students of Food Science and Nutrition were selected on the basis of their health, co-operation willingness and knowledge of sensory analysis and also the ability to discriminate the various criteria for sensory evaluation.

3.3.3. Formulation of Score Card

Hedonic scale having 9 points was used to evaluate for the acceptance of appearance, colour, texture, flavour, taste, and overall acceptability of the products. Scores were calculated on the basis of points given to the degree of acceptance of the team of taste panel members.

3.3.4 Conducting Sensory Evaluation

Four different types of variations of value added biscuits were prepared and presented to panel members for sensory evaluation at different point of time. The product which obtained the highest scores through sensory evaluation by the panel members were considered as the highly acceptable products. Evaluation was carried out thrice to obtain more reliable results. The scores obtained were analysed to find out the best acceptable snack of biscuits for further analysis.

3.4. PHYSICAL CHARACTERISTICS OF VALUE ADDED BISCUITS

3.4.1 Bulk Density (g/cm³)

The bulk density was determined according to the methods described by Okaka and Potter (1977). 50g of sample was put into 100ml. granulated measuring cylinder and tapped 20-30 times. The bulk density was calculated as weight per unit volume of sample.

$$\text{Bulk Density} = \frac{\text{Weight of the value added flour (g)}}{\text{Volume of the value added flour (ml)}}$$

3.4.2 Spread Ratio

Spread ratio was determined using Ayo *et al* (2017) method. Three rows of the five well-formed biscuits were made and the height measured as well as arranging the same biscuits horizontally edge and sum of the diameter measured.

$$\text{Spread ratio} = \frac{\text{Diameter of Biscuits (cm)}}{\text{Height of Biscuits (cm)}}$$

3.4.3 Diameter

Diameter of biscuits was determined by placing six biscuits edge to edge. The total diameter of the six biscuits was measured in mm. by using a calliper. The biscuits were rotated at an angle of 90 degree for duplicating reading. This action was repeated twice and average diameter was recorded in millimeter.

3.4.4 Thickness

Thickness of the biscuits was determined by placing six biscuits on top of one another. The total height of biscuits was measured in millimeter with the help of ruler. This process was repeated thrice to get an average value and result were reported in mm. (AACC, 2000).

3.4.5 Volume

Submerge the biscuits into a jug of water with a scale on the side the water on 100ml and biscuits were put in to the jug volume of the biscuits is 20ml. This is 20 cubic an as $1 \text{ ml} = 1\text{cm}^3$ (to the power of 3) (AOAC, 2000).



3.4.6 Water Absorption Capacity

Ground biscuits sample was suspended in 30ml of water at 30°C. In a 50 ml pre weighed centrifuge tube was centrifuged at 300 rpm for 10 minutes. The supernatant was poured into dish. The remaining gel was weighed and the water absorption capacity was recorded (Nawabuege, 2006).

$$\text{Water Absorption Capacity} = \frac{\text{Gel weight (g)}}{\text{Dry sample weight (g)}}$$



3.3.7 Ash Content

Ash content of sample 2 g was weighed and kept in a crucible which was heated at the low flame till all the material was completely charred and cooled. Then it was kept in muffle furnace for about two hours at 600 °C. It was again and cooled weighed the crucible. Then ash weight was measured. Percentage of ash was calculated by significant the change among initial and final weight (AOAC, 1998).

$$\text{Weight of Ash} = \frac{W_3 - W_1}{2} \times 100$$



3.3.8 Moisture Content

Moisture was assessed by weight of 2 g of the value added biscuits and using the moisture Shimadzu (MOC-120H). Kept the two gram biscuits sample and then moisture content was analysed. The temperature was set at 120°C. It was again weighed after finished the process (AOAC, 1998).

3.3.9 Texture Analysis

The textural properties of the value added biscuits were analysed using texture analyzer Shimadzu EZ-XS. The value added biscuits were valuated based on textural characteristics such as hardness cutting stroke chewiness, brittleness and adhesive force (Nath and Chattopadhyaya 2007). The test was achieved under the behind conditions the test speed is 5mm/sec the value added biscuits the compressed the five main methods is hardness, cutting stroke, chewiness, brittleness and adhesive force and the test type is three type bend. The relates the textural things. The highest of the force-distance plot of the texture analyzer was recorded as cutting strength, expressed in Newtons (N) (Chakraborty et al. 2009).



3.5 ANALYSIS OF PROXIMATE COMPOSITION OF VALUE ADDED BISCUITS

Analysis of chemical contents of biscuits is an important aspect of value added food products. The chemical components of biscuits such as ash, moisture, energy, protein, and fat and minerals like calcium, iron, phosphorus and dietary fibre were analysed using the standard procedures. Reagents used calculation followed were given in annexure. The methods used for analysis of the nutrients are discussed in the following pages.

3.5.1 Estimation of Energy Content

Calorie content of value added biscuits was estimated using bomb calorimeter. Calorie content conversion information on the label for fat, carbohydrates and protein is optimal. Calorie can be expressed in number of ways. A calorie which is the standard for the measurements of energy value of substances and to express the body energy requirements, is the amount of heat required to raise the temperature of 1g of water to 1 degree (one calorie is equal; to 4.18 and joules). With this principle, energy content of the biscuits was estimated.

3.5.2. Estimation of Protein by Lowry's Methods

Extraction of Protein from Sample: Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500 mg of the sample and grinded well with a pestle and mortar in 5-10 ml of the buffer. Centrifuged and used the supernatant for protein estimation. Pipette 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes .Pipette 0.1 ml and 0.2 ml of the sample extract in two other test tubes. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of water serves as the blank. Add 5 ml of reagent C to each tube including the blank. Mix well and allow standing for 10 minutes. Then add 0.5 ml of reagent D, mix well and incubated at room temperature in the dark for 30 minutes. Blue colour is developed. Take the readings at 660 nm. Draw a standard graph and calculate the amount of protein in the sample and express the amount of protein in mg/g or 100 g sample.

3.5.3 Determination of Total Carbohydrate by Anthrone

Reagents

Weigh 100 mg of the sample in to a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cool to room temperature. Neutralize with solid Sodium carbonate until the effervescence ceases. Make up the volume to 100 ml and centrifuge. Collect the supernatant and take 0.5 and 1 ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, '0' serves as blank. Make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water. Then add 4 ml of Anthrone reagent. Heat for 8 minutes in a boiling water bath. Cool rapidly and read the green to dark green colour at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube

3.5.4. Estimation of fat by Soxhelt Methods

The soxhelt method is an example of the semi continuous extraction method for demi continuous solvent extraction, the solvent builds up in the extraction chamber for 5-10 minutes.

3.5.5 Estimation of Dietary Fibre

Following enzymatic breakdown heat 220 ml ethanol 95 % to 60 °C and add to the contents of each beaker. Allow the precipitate to settle for at least 1 h at room temperature and then, decanting under slight vacuum, filter through the prepared glass filter crucibles (wash residues remaining in the beaker into the crucible with small amounts of ethanol 78 %). Prepare the glass filter crucibles used as follows: Heat the thoroughly cleaned glass crucibles for 1 h at 525 °C, add approximately 1 g Celite® 545 (ignited overnight at 525 °C, cooled, and stored in a stoppered container), dry overnight at 105 °C, cool and weigh to the nearest 0.1 mg. In order to avoid negative blank values, new crucibles will need to be prepared repeatedly as described. Crucibles should be cooled and stored in a desiccator until required. Wash the residues with 3 × 15 ml ethanol 78 %, then rewash with 2 × 10 ml ethanol 95 % and 3 × 10 ml acetone Dry overnight at 105

°C. Cool and weigh to the nearest 0.1 mg. When determining protein and ash, use separate filter crucibles for sample and blank.

3.5.6. Estimation of Vitamins and Minerals

Vitamin like Vitamin C and Minerals like calcium, phosphorus, iron, were estimated using the standard produce given in NIN laboratory Technique manual.

3.5.7 Vitamin C

Pipette a 20 mL aliquot of the sample solution into a 250 mL conical flask and add about 150 mL of distilled water and 1 mL of starch indicator solution. Titrate the sample with 0.005 mol L⁻¹ iodine solution. The endpoint of the titration is identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex. Repeat the titration with further aliquots of sample solution until you obtain concordant results (titers agreeing within 0.1 mL).

3.5.8. Procedure for the Estimation of Calcium

The fruit sample was dried in an air oven at 105°C for 3 hours. The dried sample was next charred. The charred sample was ashed in a muffle furnace at 550°C until the whitish or greyish ash was obtained. The ash was treated with concentrated hydrochloric acid, transferred to a volumetric flask and made up to 100 ml. Take above 100ml in conical flask. Add 2-3 drops of sodium hydroxide 1N solution (4.1) and to raise the pH 12 -13. Add a pinch of Patton &Reeder (4.3) indicator and stir well. Titrated against the solution with 0.01M EDTA (4.4). The end point is appearance of blue colour.

3.5.9 Standard Operating Procedure for Testing of Phosphorous

Weigh 1gm exactly of sample into the crucible or porcelain dish, and ignite. Allow to cool, then weigh, to within 1 mg about 0.1 to 10g of fat, according to its presumed phosphorus content. Burn of the fat. Ignite to a white ash in the furnace at 800 to 900°C.

Dissolve the magnesium containing ash in exactly 5 ml of the aqueous nitric acid solution with the aid of a 5 ml pipette. Add exactly 20 ml of a mixture of 10 ml of

the aqueous ammonium molybdate solution and 10 ml of the acid aqueous ammonium vanadate solution.

Mix and allow to stand for 20min. prepare a blank test, not containing fat, under exactly the same conditions. Transfer the test solution into the cell of the apparatus, Measure the extinction at 460 nm against the blank solution. Read the absorbance.

3.5.10 Estimation of Iron Sample

Take 100 ml standard flask. Prepare Iron standards (*Nist traceable*) to 0.05, 0.1, 0.125, 0.15, 0.20&0.25 mg/l in nitric acid (1:499) from 1000 ppm solution. Prepare a blank solution in 100ml distilled water. Pipette out 100 ml of sample in a beaker and digest with 0.5 ml. of conc. Nitric acid and add 25 ml CaCl_2 till the volume reduced to three fourth. Make up to 100 ml. with distilled water. Process the blank also in the above manner. Set the AAS as per the specific work instruction. Aspirate the blank, standards and DIGESTED FOOD SAMPLE solutions. Measure the absorbance of the iron at 248.3nm.

3.6 ASSESSING THE MICROBIAL CONTENT AND COST EFFECTIVENESS OF THE VALUE ADDED BISCUITS

3.6.1. Microbial Count

Peroxide value is an indicator of rancidity development during storage. Peroxide value of fresh as well as seven and 30 days old biscuits sample were determined as per the methods described by Sadasivam and Manickam (1992).

Microbial population like bacteria and fungus were estimated by serial dilution followed by solidification in petriplate using nutrients agar and rose bengel agar respectively. After solidification both bacteria and fungus along continuums plate were incubated at room temperature for 24-48 hours. Then formed colonies were counted and converted as the number of colony forming units (CFU) per grams of sample (Aneja, 1996). Microbial load estimation for the biscuits samples were carried for the fresh, 30 and 60 days old sample.

3.6.2 Cost Effectiveness

The cost of 100g of value added biscuits was calculated by compulsory the cost of raw ingredients purchased from the department stores at the time of products formulation for evaluation of the products of biscuits.

3.7 PACKAGING AND LABELLING OF VALUE ADDED BISCUITS

3.7.1 Packaging of the Value Added Biscuits

Shelf life of food is the time which can be stored before quality of food becomes stale and it included the time to give food to retailers and stored it by the consumer. It also necessary to note that selection of packaging material for a particular food depends not only on its technical suitability but it also on the availability and its cost in a particular area and one of the marketing considerations flavour to choosing a kind type of packaging.

The value added biscuits were packed in butter paper and sealed with properly. It was then kept in air tight container to retain its shelf life. Air tight containers were readily available in the market as it cost effective and acts as a barrier against moisture and gases. The value added biscuits were kept intact for a month in the same air tight container for shelf life study.

3.7.2 Labelling of Value Added Biscuits

The amount of biscuits which are purchased on impulse tends to be expensive. It's necessary to ensure that packing has on attractive colour and design and it sufficient to describe the nutrients contents. Many of the markets demands the manufacture date or best before which are printed onto each pack as a way of altering customers that the biscuits or cookies are safe and good for customers.

The value added biscuits were packed in two air tight container along with a computerized label, mentioning "Mix flour Biscuits Control" and "Variation A". The date of packaging, nutrient content, and procedure used, best use before, or date of date of expiry was mentioned on the label. The label would be improvised later and will be commercialized for health benefits of the population.

3.8 STATISTICAL ANALYSIS AND INTERPRETATION OF THE DATA

The data was compiled and analysed by using statistical methods. Descriptive statistics mean, standard deviation, variation and ANOVA test were computed using MS-Excel. The multiple ranges of tests were applied to determine the significant differences between samples. The data collected and analysed are given systemically.

4. RESULTS AND DISCUSSION

The findings of the present study on “**Formulation and Evaluation of Value Added Snack Biscuits Suitable for Bone Health**” are discussed under the following headings.

4.1 Acceptability Trial of the Value Added Biscuits

4.2 Physical Properties of the Value Added Biscuits

4.3 Proximate Composition of Value Added Flour Biscuits

4.4 Nutrients Analysis of Value Added Snack Biscuits.

4.5 Microbial and Peroxide Analysis of Value Added Biscuits And

4.6 Cost Effectiveness of Value Added Biscuits

4.7 Packaging and Labeling Of Value Added Snack Biscuits

4.1 Acceptability of Value Added Biscuits

The produced biscuits were standardized and also evaluated for its acceptability by experts as outlined in the methodology.

4.1.1 Sensory Evaluation and Standard deviation of Value Added Biscuits

Sensory evaluation was carried out by a panel of specialists using 9 points hedonic scale .This test describes the degree of consumer acceptance and satisfaction regarding product attributes. The scores were assumed, for all the types of biscuits for its colour, appearance, texture, flavour, taste and overall acceptability. The data in the table below, signifies the mean score of the various types of biscuits on formulated and evaluated the basis of sensory evaluation scores.

Table I
Mean Sensory Scores of Value Added Biscuits
Mean ± SD

Variations	Standard	Variation A	Variation B	Variation C	Variation D	One way ANOVA (p=0.05)
Colour	8.45±0.51	8.25±0.1	7.8± 76	7.5±0.94	7.9± 0.85	0.01
Texture	7.75±1.02	8.5± 0.60	8.4±0.59	8.35±0.7	8.10±.85	0.42**
Flavour	8.15±.85	8.05±1.5	8± 0.97	8.05±1.4	8.05±1.3	0.430**
Taste	8.2 ±0.76	7.75±1.5	7.55±1.5	7.75±1.5	7.95±1.9	0.995**
Appearance	8.5± 0.68	8.15±0.4	7.85±1.3	7.75±1.20	7.7 ±1.30	0.084**
Overall acceptability	8.2± 0.61	8.4 ±0.59	7.9± 0.55	8.05±1.09	7.95±1.05	0.303**

Significant*, not significant**

The Table I depicted the secured scores of the biscuits for its sensory criteria such as like colour, texture, taste, flavour, appearance and overall acceptability. The obtained scores of the standard biscuits in terms of colour, texture, flavour, taste, appearance and overall acceptability were 8.4, 7.75, 8.15, 8.2, 8.5, and 8.2 respectively. Variation A biscuits secured scores of 8.25, 8.5, 8.05, 7.75, 8.15, and 8.4 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively. The Variation B secured scores of 7.8, 8.4, 8, 7.55, 8.15, and 7.9 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively. The Variation C secured scores of 7.5, 8.35, 8.05, 7.75, 7.75, and 8.05 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively while the Variation D secured scores of 7.9, 8.1, 8.05, 7.975, 7.7, and 7.95 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively. Among these variations, Variation A secured the maximum scores for

colour, texture, taste, flavour, appearance and overall acceptability were distinctly high. The overall acceptability score of the control biscuit was 8.2 ± 0.61 . Variation A obtained the overall acceptability score of 8.4 ± 0.59 while the Variation B obtained the score as 7.9 ± 0.55 and Variation C obtained the score as 8.05 ± 1.09 and variation D was 7.95 ± 1.05 . It indicated that there remains a substantial variance in terms of texture, flavour and overall acceptability amongst the different variations. The criteria for the taste and flavour is different and is less significant as compared to all the other variations.

4.2 PHYSICAL PROPERTIES OF THE STANDARD AND VALUE ADDED BISCUITS

Table II

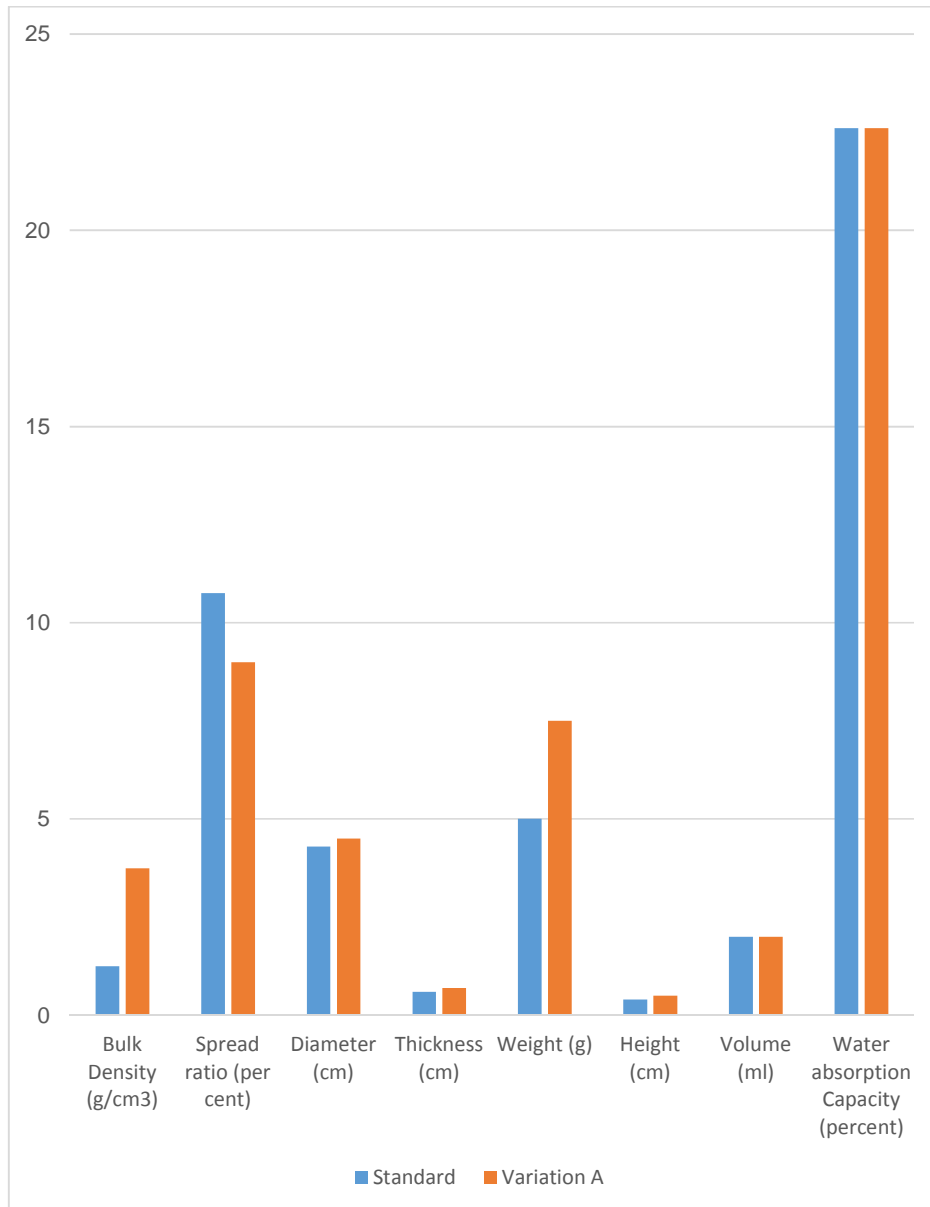
Physical properties of the value added biscuits and its control

Criteria	Standard	Variation A
Bulk Density (g/cm ³)	1.25	3.75
Spread ratio (per cent)	10.75	09
Diameter (cm)	4.3	4.5
Thickness (cm)	0.6	0.7
Weight (g)	5	7.5
Height (cm)	0.6	0.7
Volume (ml)	2	2.0
Water absorption Capacity (percent)	22.6	22.6

Table II shows the physical properties of value added biscuits. Standard biscuits of the Bulk Density 1.25 g/cm³, spread ratio is the 10.75 per cent, diameter is the 4.3 cm, thickness is the 0.6 cm and weight is the 5g, height of the standard biscuits 0.6 cm and volume is the 2 ml and the water absorption capacity is the 22.6 per cent. Variation A biscuits of the Bulk Density 3.75 g/cm³, spread ratio is the 9 per cent, diameter is the 4.5 cm, thickness is the 0.7 cm and weight is the 7.5g, height of the Variation A biscuits 0.7 cm and volume is the 2 ml and the water absorption capacity is the 22.6 per cent. The conclusion by the Standard and Variation A is the similar the volume and water absorption capacity and other physical properties is the different.

FIGURE- 1

Physical Properties of the Standard and Value Added Biscuits



The above Figure highlights the physiochemical properties of value added biscuits and its control biscuits

4.2.1 Determination of the Texture Analysis

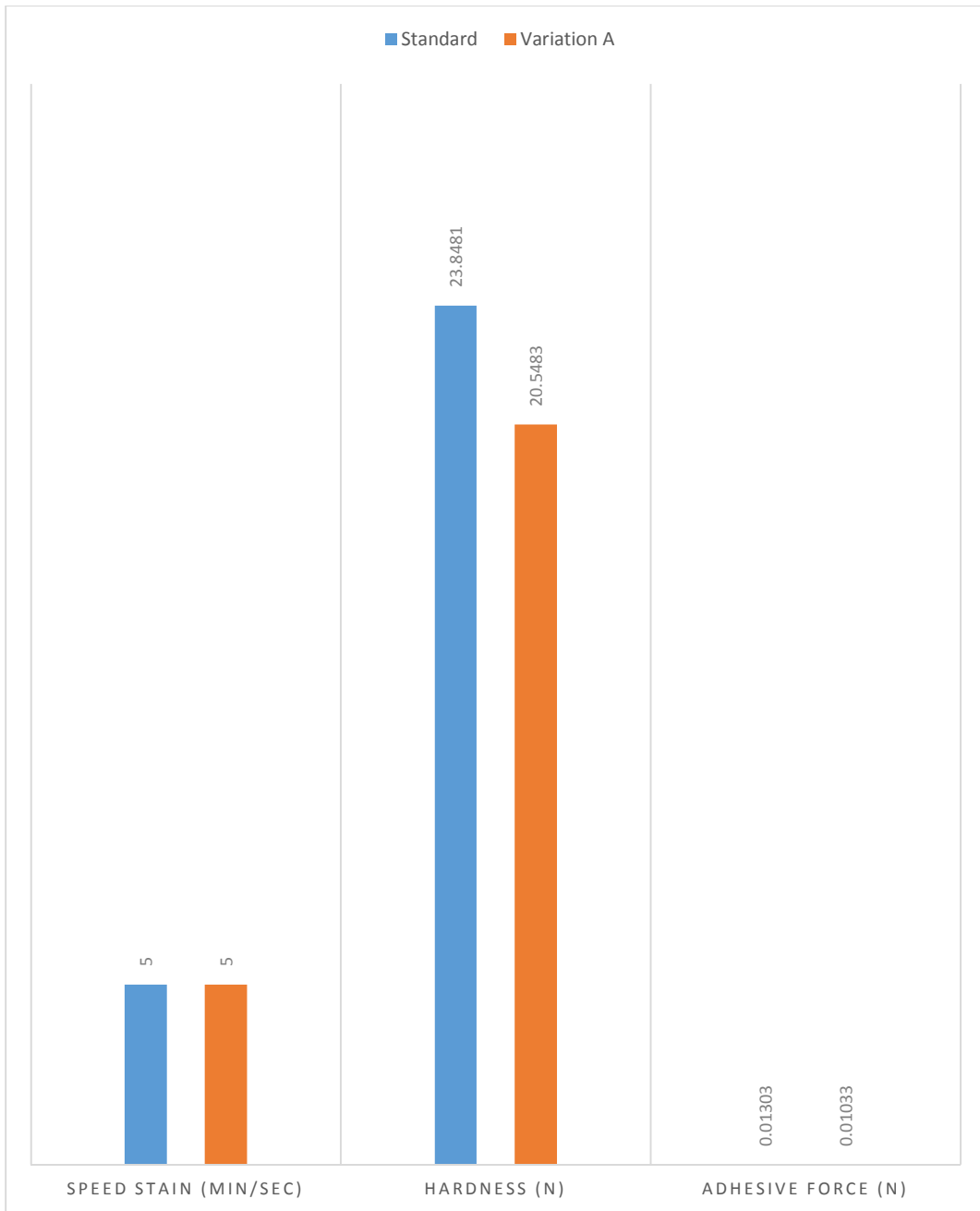
The texture analysis the value added biscuits were analyzer using the analyzer is shown the Table III.

Table III
Texture Analysis - 3 Point Bend Test

Criteria	Standard	Variation A
Speed Stain (min/sec)	5	5
Hardness (N)	23.8481	20.5483
Adhesive force (N)	0.01303	0.01033

FIGURE- 2

Determination of the Texture Analysis



4.3. PROXIMATE COMPOSITION OF VALUE ADDED FLOUR USED FOR THE PREPARATION OF BISCUITS

Flour appearances regulate baking appearance besides products value. The functionality of dusts of cereal grains, which would be subjected to an excessive level of fat, dietary fibre, carbohydrates and protein, phosphorus, calcium, gratified of flours, subsidies a lot to the preparation and baking value of the concluding products. Consequently, flour remained to study their proximate arrangement. Then it essential for the increase of value additional biscuits. Proximate composition of the value added flour was highlighted in the Table IV

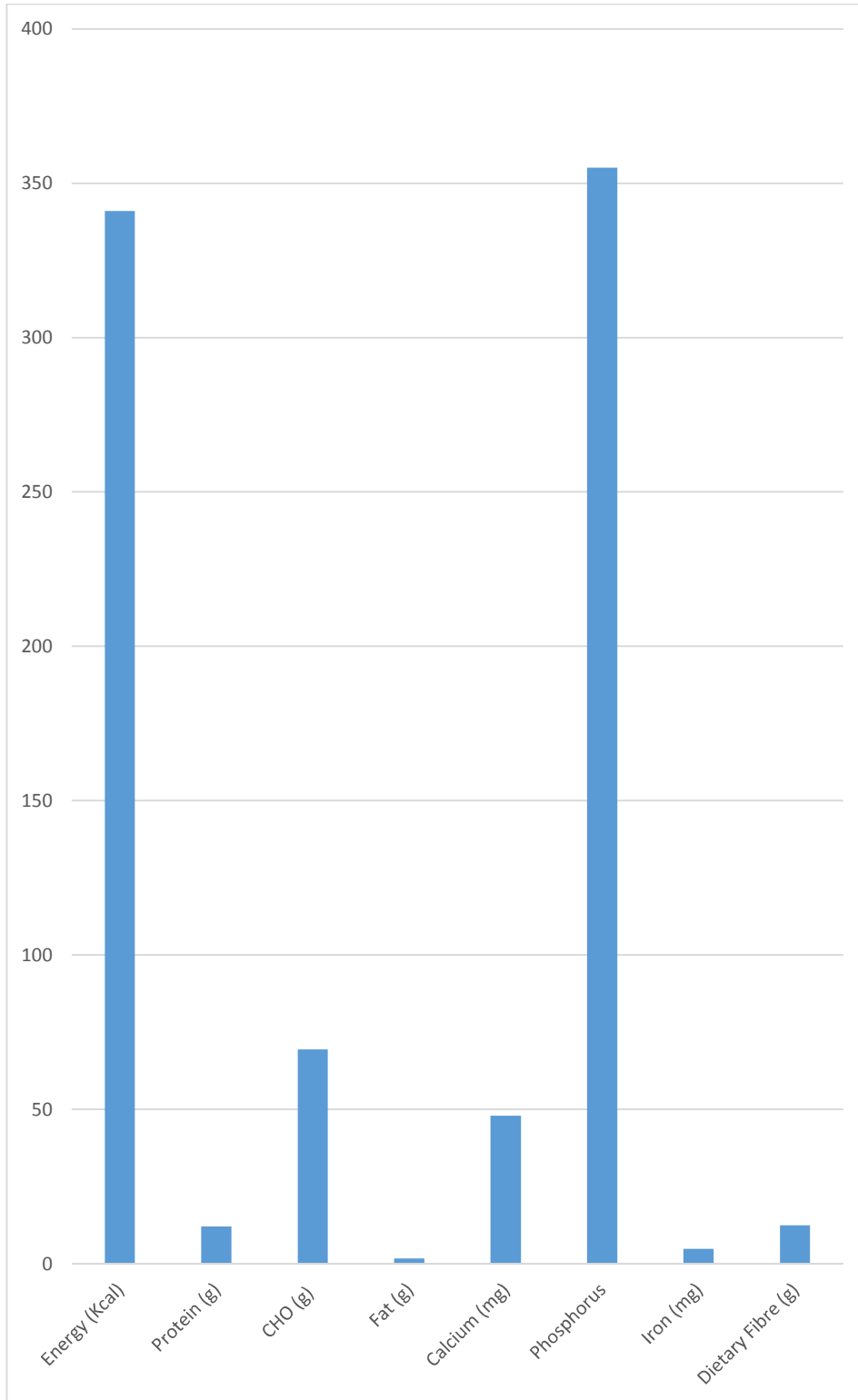
4.3.1 Table IV Proximate Composition of Value Added Flour Used in Standard Biscuit

Ingredients	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Dietary Fibre (g)
Whole Wheat Flour	341	12.1	69.4	1.7	48	355	4.9	12.5

Table IV Standard depicted the proximate composition of value added flour of energy, carbohydrates, protein, fat, calcium, phosphorus, iron and dietary fibre. The standard biscuit had the energy as 341kcal, protein 12.1g, carbohydrates 69.4 g, fat 1.7 g, phosphorus 355mg, calcium 48mg, iron 4.9mg and dietary fibre 12.5g.

FIGURE - 3

Proximate Composition of Value Added Flour Standard



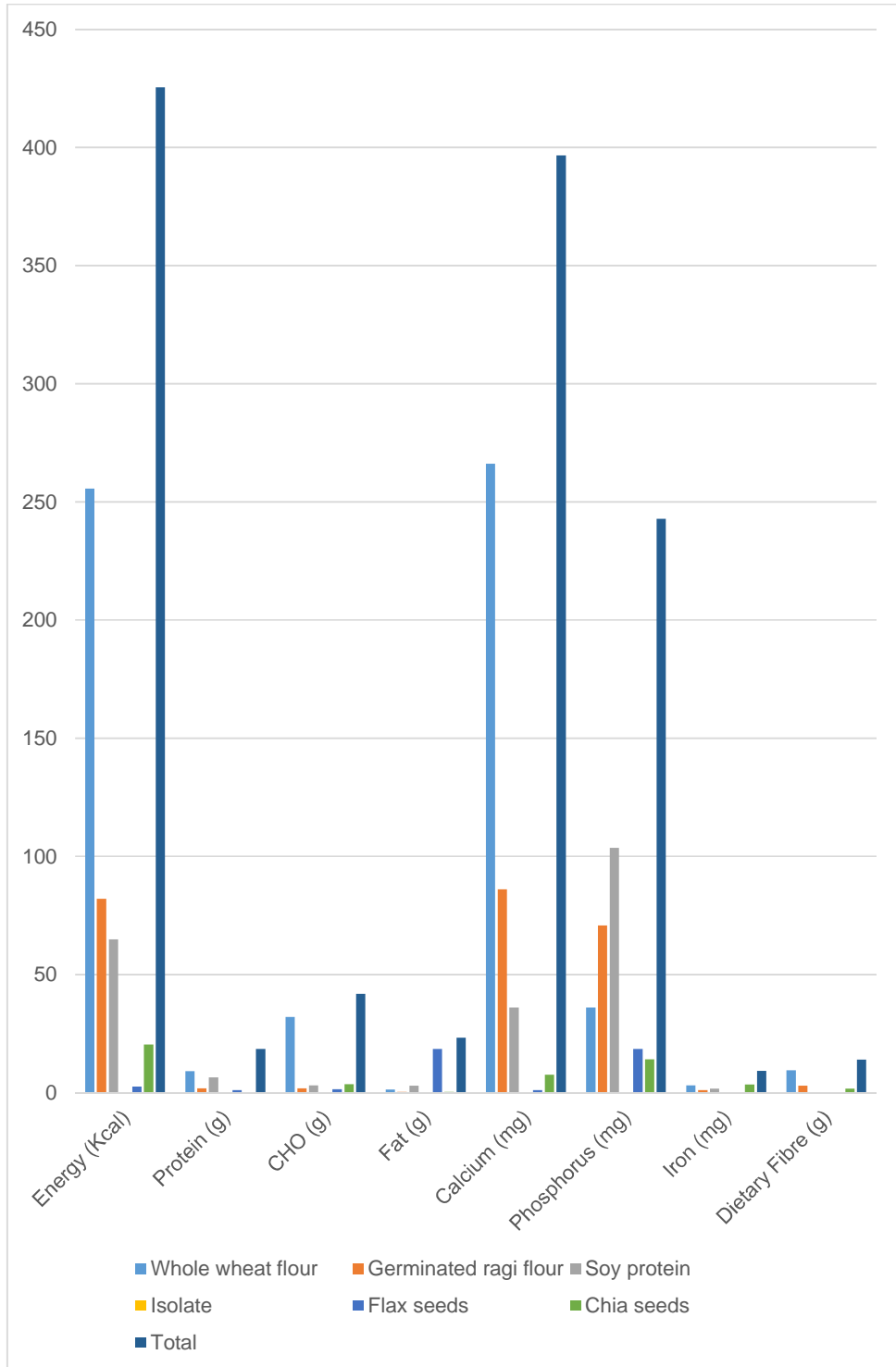
**4.3.2 Table V – Proximate Composition of Value Added Flours in Variation-A
Biscuits**

Ingredients	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Dietary Fibre (g)
Whole wheat flour	255.7	9.1	32.0	1.3	266.2	36	3.1	9.4
Germinated ragi flour	82	1.8	1.8	0.3	86	70.7	1.0	2.9
Soy protein Isolate	64.8	6.5	3.1	2.9	36	103.5	1.6	-
Flax seeds	2.6	1.0	1.4	18.5	1.0	18.5	0.1	-
Chia seeds	20.4	0.1	3.5	0.2	7.5	14.1	3.4	1.68
Total	425.5	18.5	41.8	23.2	396.7	242.8	9.2	13.98

Table V (Variation A) depicted the proximate composition of value added flour of whole wheat flour energy 255.7kcal, protein 9.1g, carbohydrates 32.0g, fat 1.3g, phosphorus 266.2mg, calcium 36mg, iron 3.1mg and dietary fibre 9.4g. The ragi flour energy level as 82kcal, protein 1.8g, carbohydrates 1.8g, fat 0.3g, phosphorus 70.7mg, calcium 86mg, iron 1.0mg and dietary fibre 2.9g. The soy protein isolate powder's energy level was 64.8kcal, protein 6.5g, carbohydrates 3.1g, fat 2.9g, phosphorus 103.5mg, calcium 36mg, iron 1.6mg and dietary fibre was nil. Flax seeds energy level is 2.6kcal, protein 1.0g, carbohydrates 1.4g, fat 18.5g, phosphorus 18.5mg, calcium 1.0mg, iron 0.1mg and dietary fibre contains meagre amount chia seeds contributed energy of 20.4kcal, protein 0.1g, carbohydrates 3.5g, fat 0.2g, phosphorus 14.1mg, calcium 7.5mg, iron 3.4mg and dietary fibre is 1.68g. The total nutrient contribution of value added snacks are expressed in terms, of 425.5 Kcal of energy, 18.5g of protein, 41.8g of carbohydrates, 23.2g of fat, 396.7mg of calcium, 242.8mg of phosphorous, 9.2mg of iron and 13.98g of dietary fibre.

FIGURE - 4

Proximate Composition of Value Added Flour Variation A



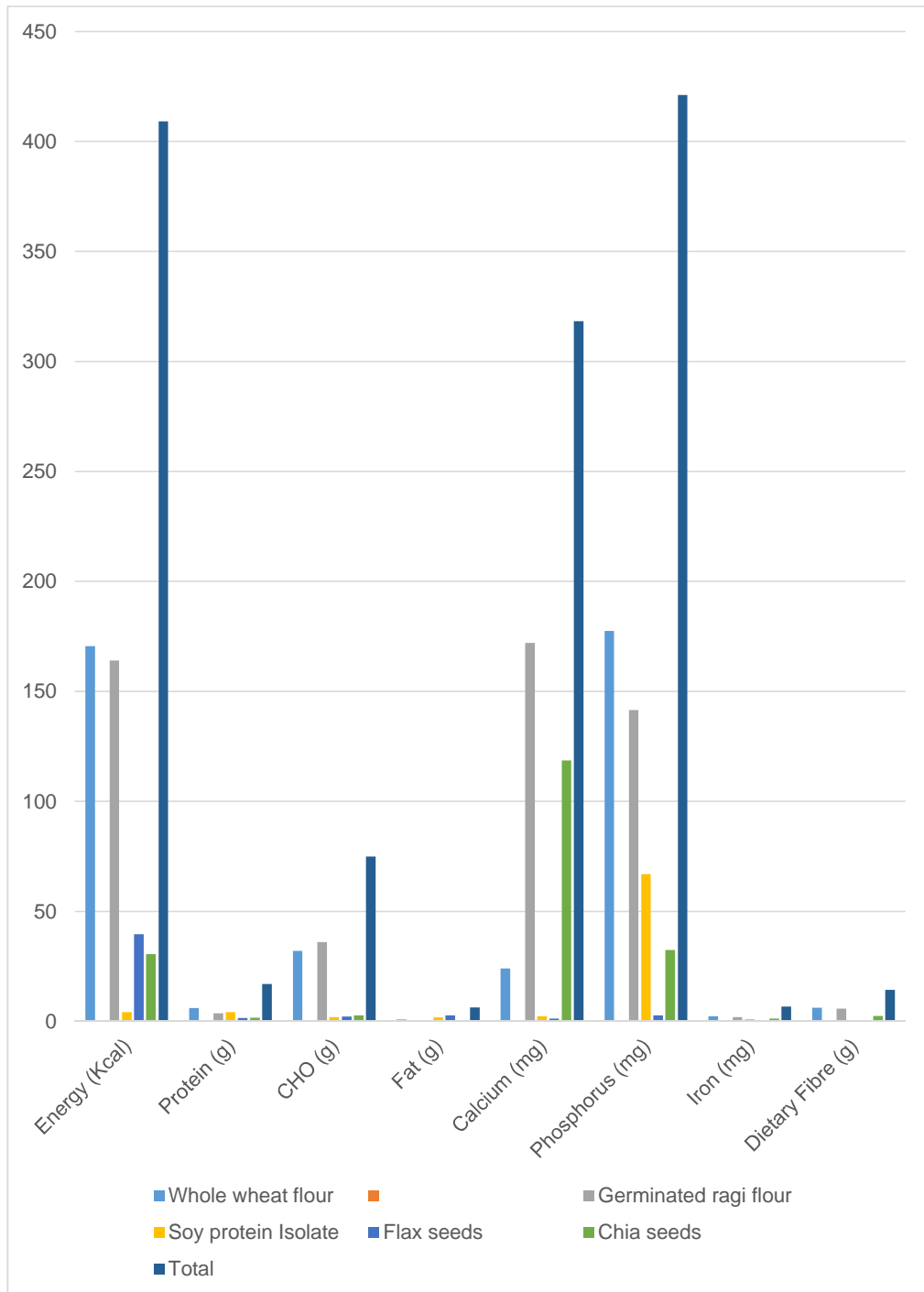
4.3.3. Table VI – Proximate Composition of Value Added Flours in Variation-B Biscuits

Ingredients	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Dietary Fibre (g)
Whole wheat flour	170.5	6.0	32	0.85	24	177.5	2.4	6.2
Germinated ragi flour	164	3.6	36	0.6	172	141.5	2.0	5.7
Soy protein Isolate	4.3	4.3	2.0	1.9	2.4	67	1.0	-
Flax seeds	39.7	1.5	2.2	2.8	1.3	2.8	0.2	-
Chia seeds	30.6	1.6	2.8	0.2	118.5	32.4	1.2	2.52
Total	409.1	17	75	6.35	318.2	421.2	6.8	14.42

Table VI (Variation B) depicted that the proximate composition of value added flour of Variation B whole wheat flour energy was 170.5kcal, protein 6.0g, carbohydrates 32g, fat 0.85g, phosphorus 177.5mg, calcium 24mg, iron 2.4mg and dietary fibre 6.2g. Ragi flour had the energy level as 164kcal, protein 3.6g, carbohydrates 36g, fat 0.6g, phosphorus 141.5mg, calcium 172mg, iron 2.0mg and dietary fibre 5.7g while the soy protein isolate powder's energy level was 4.3kcal, protein 4.3g, carbohydrates 2g, fat 1.9g, phosphorus 2.4mg, calcium 2.4mg, iron 1.0mg and dietary fibre as nil. Flax seeds energy level was 39.7kcal, protein 1.5g, carbohydrates 2.2g, fat 2.8g, phosphorus 2.8mg, calcium 1.3mg, iron 0.2mg and dietary fibre was nil and the last flour which was chia seeds had its energy level as 30.6kcal, protein 1.6g, carbohydrates 2.8g, fat 0.2g, phosphorus 32.4mg, calcium 118.5mg, iron 1.2mg and dietary fibre as 2.52g. The total nutrients contribution of value added snacks are expressed in terms of 409.1Kcal of energy, 17gms of protein, 75 g of carbohydrates, 6.35 g of fat, 318.2mg of calcium, 421.2mg of phosphorus, 6.8 mg of iron and 14.42g of dietary fibre.

FIGURE - 5

Proximate Composition of Value Added Flour Variation B

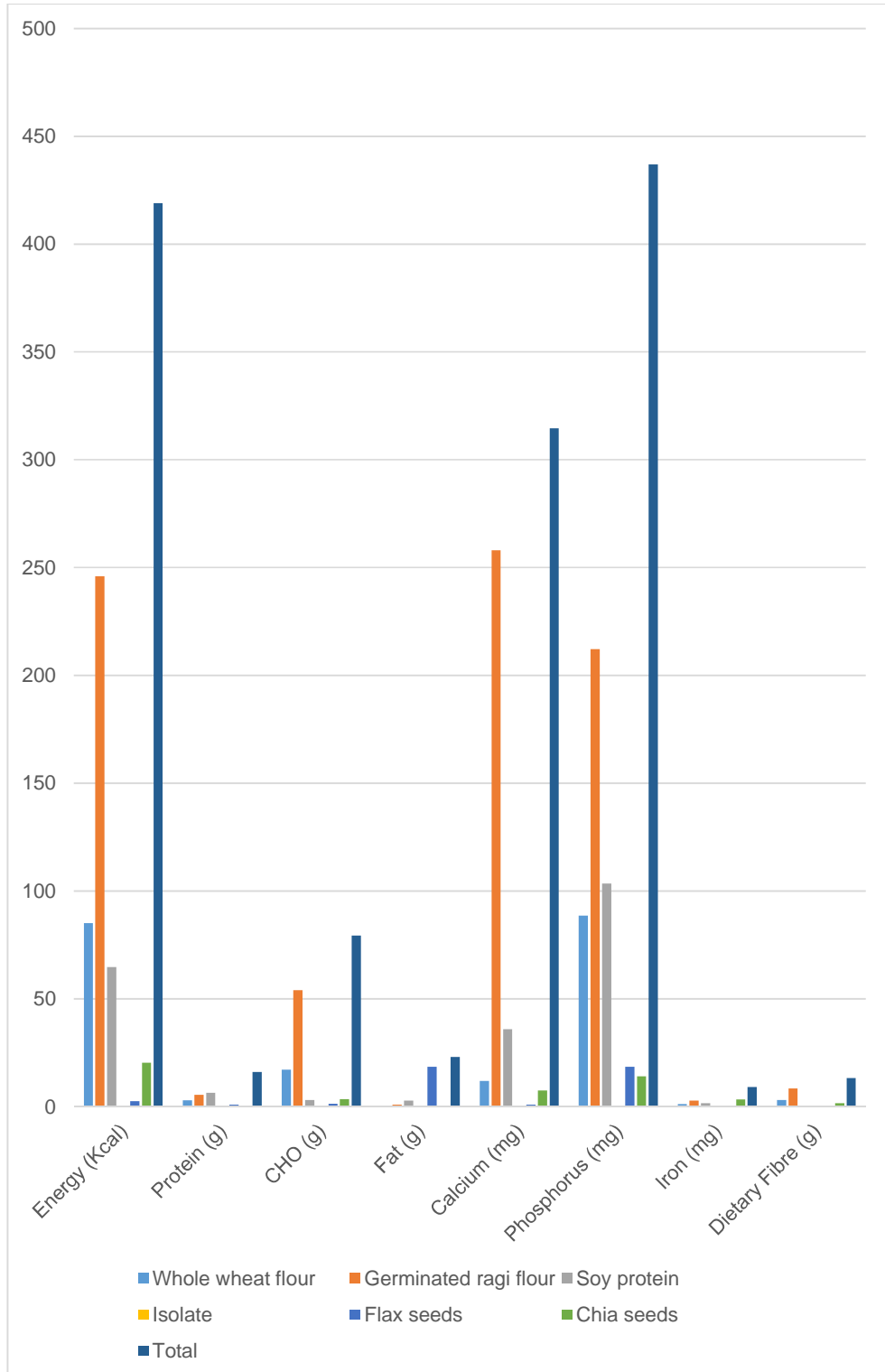


**4.3.4 Table VII – Proximate Composition of Value Added Flours
in Variation-C Biscuits**

Ingredients	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Dietary Fibre (g)
Whole wheat flour	85.2	3.0	17.3	0.4	12	88.7	1.2	3.1
Germinated ragi flour	246	5.5	54	1.00	258	212.2	2.9	8.6
Soy protein Isolate	64.8	6.5	3.1	2.9	36	103.5	1.6	-
Flax seeds	2.6	1.0	1.4	18.5	1.0	18.5	0.1	-
Chia seeds	20.4	0.1	3.5	0.2	7.5	14.1	3.4	1.68
Total	419	16.1	79.3	23	314.5	437	9.2	13.38

Table VII (Variation C) depicted that the proximate composition of value added flour of whole wheat flour had the energy of 85.2kcal, protein 3.0g, carbohydrates 17.3g, fat 0.4g, phosphorus 88.7mg, calcium 12mg, iron 1.2mg and dietary fibre 3.1g. The energy level of Ragi flour was 246kcal, protein 5.5g, carbohydrates 54g, fat 1.00g, phosphorus 212.2mg, calcium 258mg, iron 2.9mg and dietary fibre 8.6g while the soy protein isolate powder's energy level was 64.8kcal, protein 6.5g, carbohydrates 3.1g, fat 2.9g, phosphorus 103.5mg, calcium 136mg, iron 1.6mg and dietary fibre was nil. Flax seeds energy level was 2.6kcal, protein 1.0g, carbohydrates 1.4g, fat 18.5g, phosphorus 18.5mg, calcium 1.0mg, iron 0.1mg and dietary fibre is nil. Chia seeds whose energy level was 20.4kcal, protein 0.1g, carbohydrates 3.5g, fat 0.2g, phosphorus 14.1mg, calcium 7.5mg, iron 3.4mg and dietary fibre was 1.68g. The total nutrients contribution of value added snacks are expressed in terms of 419.1Kcal of energy, 16.1g of protein, 79.3g of carbohydrates, 23g of fat, 314.5 mg of calcium 437mg of phosphorus, 9.2mg of iron and 13.38g of dietary fibre. In the above table the overall nutrient content of the value added flour used in biscuit preparation is clearly explained.

FIGURE – 6
Proximate Composition of Value Added Flour Variation C

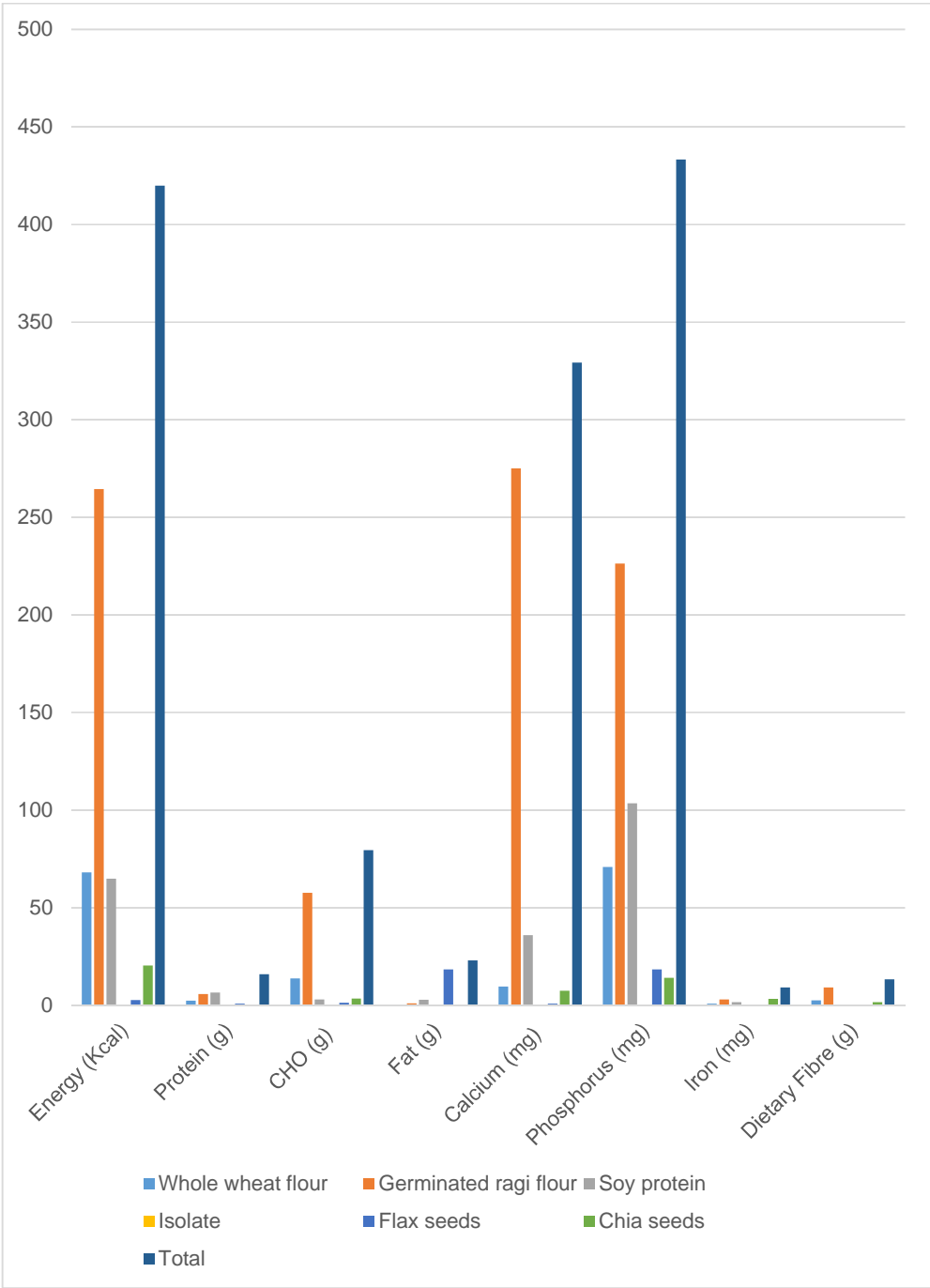


4.3.5 Table VIII – Proximate Composition of Value Added Flours in Variation-D Biscuits

Ingredients	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Dietary Fibre (g)
Whole wheat flour	68.2	2.4	13.8	0.3	9.6	70.9	1.0	2.5
Germinated ragi flour	264.4	5.9	57.6	1.1	275.2	226.3	3.1	9.2
Soy protein Isolate	64.8	6.5	3.1	2.9	36	103.5	1.6	-
Flax seeds	2.6	1.0	1.4	18.5	1.0	18.5	0.1	-
Chia seeds	20.4	0.1	3.5	0.2	7.5	14.1	3.4	1.68
Total	420	15.9	79.4	23	329.3	433.3	9.2	13.38

Table VIII (Variation D) depicted that the proximate composition of value added flour of the whole wheat flour's energy content was 68.2kcal, protein 2.4g, carbohydrates 13.8g, fat 0.3g, phosphorus 70.9mg, calcium 9.6mg, iron 1.0mg and dietary fibre as 2.5g, ragi flour energy level was 262.4kcal, protein 5.9g, carbohydrates 57.6g, fat 1.1g, phosphorus 226.3mg, calcium 275.2mg, iron 3.1mg and dietary fibre 9.2g, and soy protein isolate powder energy level was 64.8kcal, protein 6.5g, carbohydrates 3.1g, fat 2.9g, phosphorus 103.5mg, calcium 136mg, iron 1.6mg and dietary fibre content is very meagre. Flax seeds energy was 2.6kcal, protein 1.0g, carbohydrates 1.4g, fat 18.5g, phosphorus 18.5mg, calcium 1.0mg, iron 0.1mg and reliable dietary fibre was present in it and chia seeds contribute energy of 20.4kcal, protein 0.1g, carbohydrates 3.5g, fat 0.2g, phosphorus 14.1mg, calcium 7.5mg, iron 3.4mg and dietary fibre was 1.68g. The total nutrients contribution of value added snacks are expressed in terms of 420Kcal of energy, 15.9gms of protein 79.4gms of carbohydrates 23gms of fat 329.3mg of calcium, 433.3mg of phosphorus 9.2mg of iron and 13.38gms of dietary fibre. In the figure 5 the overall nutritional content of the value added flour of variation D was expressed clearly.

FIGURE – 7
Proximate Composition of Value Added Flour
Variation D



4.4. NUTRIENTS ANALYSIS OF VALUE ADDED BISCUITS

Table IX – The Nutrient Content of Value Added Biscuits

Parameters	Standard Value Analysed Value	Variation A Value Analysed Value
Energy (Kcal)	420	436
Protein (g)	8.0	9.4
Fat (g)	15.2	17.2
Carbohydrates (g)	63	61
Dietary Fibre (g)	12.5	13.98
Calcium (mg)	98	115
Phosphorus (mg)	110	206
Iron (mg)	1.0	5.2

This Table IX depicts the analysed nutrient content of energy, protein, fat, carbohydrates, dietary fibre, calcium, phosphorus, and iron of the value added biscuits. The values obtained for the standard biscuits is given as follows – energy content was as 420 kcal, protein was 8.0g, fat was 15.2g, carbohydrates was 63g, dietary fibre was 12.5g, calcium was 98mg, phosphorus was 110mg and iron was 1.0mg. Variation A had its energy content of 436 kcal, protein as 9.4g, fat as 17.2g, carbohydrates as 61g, dietary fibre as 13.98g, calcium as 115mg, phosphorus as 206mg and iron 5.2mg.

4.4.1 Ash and Moisture Content of Value Added Ingredients and Biscuits

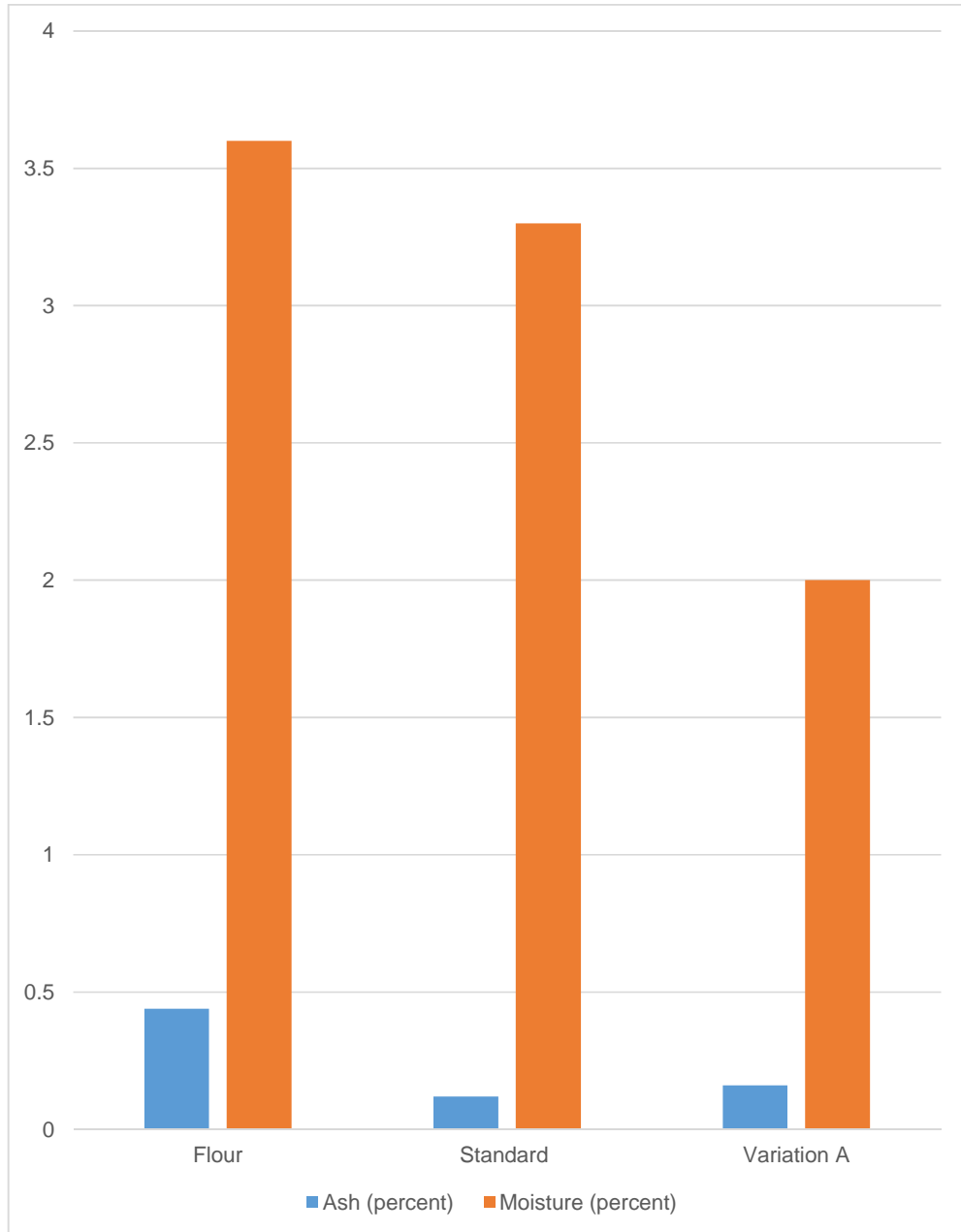
Table- X Ash and Moisture Content of Value Added Biscuits

Criteria	Flour	Standard	Variation A
Ash (percent)	0.44	0.12	0.16
Moisture (percent)	3.6	3.3	2

Table X specifies that the value added biscuits the ash and moisture content. The ash of the flour is the 0.44 per cent and the moisture is the 3.6, standard biscuits is the ash content is the 0.12 per cent and the moisture content is the 3.3 percent. Variation A is the ash content is the 0.16 and moisture content 2 percent.

FIGURE- 8

Ash and Moisture Standard Value Added Ingredients and Biscuits



4.5 MICROBIAL LOAD AND PEROXIDE ANALYSIS OF VALUE ADDED BISCUITS

Microbial load of value added biscuits remained inside the acceptable limit for a period of seven days from the day of production. The microbial content of the biscuits were counted and it was found that in the variation biscuits was lesser than the adequate limit. This is represented in Table.

4.5.1 Microbial Load and Peroxide Analysis Value Added Biscuits

Table- XI

Bacterial Count	Standard	Variation A
One day	Nil	Nil
Seven Days	18×10^2 cfu/g	15×10^2 cfu/g

This table X nine represents that the total bacterial count of Standard and variation were within the adequate limit. At the time of preparation of biscuits there was no or nil bacterial count. But after seven days, the colony forming units in the control biscuits were 18×10^2 cfu/g and the Variation A had the total bacterial count as 15×10^2 cfu/g. Peroxide value is an indicator of rancidity development which is observed during storage time. No rancidity development was observed in formulated value added. The studied conducted by Aruna (2000) also indicated that no rancidity development was observed during storage.

4.5.2 Microbial Load and Peroxide Analysis After 30th day Standard and Value Added Biscuits

Table- XII

Bacterial Count	Standard	Variation A
One Day	Nil	Nil
One month	20×10^2 cfu/g	18×10^2 cfu/g

This table XIV represents that the total bacterial count of standard and variation were within the adequate limit. At the time of preparation of biscuits there was no or nil bacterial count. But after seven days, the colony forming units in the control biscuits were 20×10^2 cfu/g and the Variation A had the total bacterial count as 18×10^2 cfu/g.

4.6 COST EFFECTIVENESS

4.6.1 Cost Effectiveness of Standard Biscuits

Table XIII

Ingredients	Amount Used (g)	Cost (Rs)
Whole Wheat Flour	100	5.00
Butter	60	30.55
Sugar	60	2.275
Milk	8ml	0.36
Total	228	38.18

4.6.2 Cost Effectiveness of Value Added Biscuits

Table- XIV

VARIATION A –BISCUITS

Ingredients	Amount used (g)	Cost (Rs.)
Whole wheat flour	75	2.5
Germinated ragi flour	25	0.90
Soy protein isolate	15	1.00
Flax seeds flour	5	2.00
Chia seeds Flour	5	4.1
Butter	50	23.5
Sugar	55	1.75
Milk	8ml	0.36
Total	243	36.11

Above tables highlight the cost effectiveness of value added biscuits (variation) and the sample (control)

4.7 NUTRIENTS ANALYSIS OF VALUE ADDED BISCUITS AFTER 30th DAY

Table XV

Nutrients	Standard Value	Variation A Value
Energy (Kcal)	422	436.6
Protein (g)	8.2	9.1
Fat (g)	15.1	17
Carbohydrates (g)	63.5	61.8
Dietary Fibre (g)	7.6	8.0
Calcium (mg)	97	116
Phosphorus (mg)	110	204
Iron (mg)	1.1	5.1

This Table XIV depicts the nutrient analysis of the sample stored for one month is expressed in terms of nutrients such as energy, protein, fat, carbohydrates, dietary fibre, calcium, phosphorus, and iron of the samples stored for one month is expressed in term if nutrients such as value added biscuits. The values obtained for the control biscuits is as follows – energy value as 422 kcal, protein as 8.2g, fat as 15.1g, carbohydrates as 63.5g, dietary fibre as 7.6g, calcium as 97mg, phosphorus as 110mg and iron as 1.1mg. Variation A had its energy value as 436.6 kcal, protein as 9.1g, fat as 17.0g, carbohydrates as 61.8g, dietary fibre as 8.0g, calcium as 116mg, phosphorus as 204mg and iron 5.1mg.

5. SUMMARY AND CONCLUSION

Biscuits are the popular foodstuff consumed by a wide range of population due to their varied taste, long shelf life, and relatively low cost. The nutrient content is also increased by adding a variety of functional foods, which are valuable for its nutritional importance and health benefits. Because of the competition in the market and increased demand for healthy, natural and functional foods products, attempts are being made to improve the nutritive value of biscuits and functionality by modifying their nutritive composition. Such effects are very often achieved by increasing the ratio of whole grains, raw materials, other ingredients having different types of dietary fiber in basic recipes with the attempts to increase the nutrient content of the biscuits especially proteins. Minerals like calcium, phosphorus, Fe, and zinc for proper quality and easy availability using value addition in terms of inclusion of functional foods valuable contribution of protein, minerals, and vitamins. And also phytochemicals which have nutraceutical effects to promote health and prevent diseases.

The value addition biscuits are rich in bone-forming nutrients especially calcium, phosphorus, iron, and protein. This value-added snack (biscuits) and highly nutritional and value for its minerals content especially the mineral's involved in bone formulation and synthesis. In this value addition snack (biscuits) carbohydrates are the main energy source and are advised to include into our daily dietaries especially snack (biscuits) for our young children, adolescents, adults and elderly.

This value-added healthy biscuits help to provide the nutrients required for bone growth and health by satisfying the nutritional needs of a healthy population. With these backdrops, value-added biscuits are prepared which is suitable for all age groups. Wheat flour germinated ragi flour, soy protein isolates and functional foods used in the value added biscuits and contributing relatively high in protein, fat, vitamins and minerals.

This biscuit is nutritious suitable for bone health. The present study entitled **“Formulation and Evaluation of Value-Added Snack (Biscuits) Suitable for Bone Health”** was designed with the following objectives:

1. To formulate and evaluate value-added biscuits.
2. To determine the physical and chemical properties of value-added biscuits.
3. To calculate and analyze the nutrient content of value-added biscuits and
4. To analysis, the storage stability and cost-effectiveness of the value-added biscuits.

Methodology adopted for the present study is discussed in the following paragraphs.

The first step of the study was the selection and procurement of ingredients which are rich in macro, micro nutrients and phytochemicals. These nutrients are essential to promote and preserve health and also prevent ill health conditions. Ingredients were carefully selected to formulate value added biscuits considered as snack. In the next steps the selected ingredients such as grains (ragi, wheat) seeds like chia seeds, flax seeds were thoroughly cleaned, washed and dried properly. Ragi was allowed to germinate to increase nutrient content. Care was taken to include chia seeds, flax seeds, germinated seeds, wheat flour, soy protein isolate, butter and brown sugar to enhance the nutrient content of the formulated value added biscuits.

Using these ingredients in different quantity four variations were formulated and termed as variation A, B, C and D. Standard was prepared and termed as control.

Including these ingredients control and variations were prepared by using the standard procedure in terms of cleaning and mixing for dough preparation. Then kneading, sheeting, moulding were adopted for baking. Then baked biscuits were allowed to have a process of cooling, packing and labelling. The value added biscuits variations A, B, C, D and Control were subjected to have a sensory evaluation using nine point hedonic scale. Scores were calculated on the basis of the points given to the degree of acceptance of the team of taste panel members. Evaluation was carried out thrice to obtain more reliable results. The

score obtained were analysed to find out the best acceptable snack for further analysis.

In the next steps proximate nutrients including energy content, protein, total carbohydrate, fats and dietary fibre were estimated using the standard procedure. Micro nutrients like calcium, phosphorous, iron and vitamin C were also estimated to note the nutrient content of the value added biscuits. Physiochemical properties were also studied in terms of bulk density, spread ratio, diameter, thickness, and volume and water absorption capacity.

Microbial content and cost effectiveness of the value added biscuits were carefully assessed to study the shelf life of the products affordability of the consumers to promote their health status especially bone health. Packaging and labelling were considered as a part of the study for maintaining the quality and quantity of value added biscuits and labelling helps to communicate the nutritional information between the consumers and manufactures and helps us to make healthier and safer food choices by understanding the nutrient content.

The salient findings of the present research are discussed in the following pages.

1. Acceptability of Value Added Biscuits

The obtained scores of the Standard biscuits in terms of colour, texture, flavour, taste, appearance and overall acceptability were 8.4, 7.75, 8.15, 8.2, 8.5, and 8.2 respectively. Variation A biscuits secured scores of 8.25, 8.5, 8.05, 7.75, 8.15, and 8.4 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively. The Variation B secured scores of 7.8, 8.4, 8, 7.55, 8.15, and 7.9 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively. The Variation C secured scores of 7.5, 8.35, 8.05, 7.75, 7.75, and 8.05 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively while the Variation D secured scores of 7.9, 8.1, 8.05, 7.975, 7.7, and 7.95 in terms of colour, texture, flavour, taste, appearance and overall acceptability respectively. Among these variations, Variation A secured the maximum scores for colour, texture, taste, flavour, appearance and overall acceptability were distinctly high and considered for further analysis. The standard deviation of overall acceptability score of the control biscuit was $8.2 \pm$

0.61. Variation A obtained the overall acceptability score of 8.4 ± 0.59 while the Variation B obtained the score as 7.9 ± 0.55 and Variation C obtained the score as 8.05 ± 1.09 and the last product's was 7.95 ± 1.05 . ANOVA was used for statistical analysis of sensory evaluation and was expressed in terms of colour, texture, taste, flavour, and overall acceptability remained substantial at 1 per cent level. It indicated that there was a substantial variance in terms of texture, flavour and overall acceptability amongst the different variations. The criteria for the taste and flavour is different and is less significant as compared to all other variations.

2. The Physical Properties of Value Added Biscuits.

The physical properties of control biscuits were expressed in terms of Control biscuits Bulk Density (1.25 g/cm^3), spread ratio (the 10.75 per cent), diameter (the 4.3 cm), thickness (0.6 cm) and weight (5g), height control biscuits (0.4 cm) and volume (2 ml) and the water absorption capacity is the 22.6 per cent. Whereas in Variation A biscuits. Bulk Density was 3.75 g/cm^3 , spread ratio was 9 per cent, diameter was 4.5 cm, thickness was 0.7 cm and weight was 7.5g, height. The biscuits was 0.4 cm volume 2 ml and the water absorption capacity was 22.6 per cent. By conclusion the control and variation A is the similar in volume and water absorption capacity whereas other physical properties were different in nature.

3. Proximate Composition of Value Added Flour Used for The Preparation of Biscuits

Standard Biscuit used for analysis of the proximate composition are expressed in terms of energy, carbohydrates, protein, fat, calcium, phosphorus, iron and dietary fibre. Control biscuit had the energy of 341kcal, protein 12.1g, carbohydrates 69.4 g, fat 1.7 g, phosphorus 355mg, calcium 48mg, iron 4.9mg and dietary fibre 12.5g.

Proximate composition of value added flour used in Variation A were expressed in terms of total energy, carbohydrates, protein, fat, calcium, phosphorus, iron and dietary fibre. The control biscuit had the energy (425.5 kcal), protein (18.5 g), carbohydrates (41.8 g,) fat (23.2 g), phosphorus (242.8mg), calcium (396.7mg), iron (9.2mg) and dietary fibre (13.98g). Variation B depicted the all ingredients proximate composition of value added flour of total energy,

carbohydrates, protein, fat, calcium, phosphorus, iron and dietary fibre. The control biscuit had the energy as 409.1 kcals, protein 17 g, carbohydrates 75 g, fat 6.35 g, phosphorus 421.2 mg, calcium 318 mg, iron 6.8 mg and dietary fibre 14.42g. Variation C depicted the all ingredients proximate composition of value added flour of total energy, carbohydrates, protein, fat, calcium, phosphorus, iron and dietary fibre. The control biscuit had the energy as 419 kcals, protein 16.1 g, carbohydrates 79 g, fat 23g, phosphorus 437 mg, calcium 314 mg, iron 9.2 mg and dietary fibre 13.38 g. Variation D depicted the all ingredients proximate composition of value added flour of total energy, carbohydrates, protein, fat, calcium, phosphorus, iron and dietary fibre. The control biscuit had the energy as 420 kcals, protein 15.9 g, carbohydrates 79.4 g, fat 23g, phosphorus 433.3 mg, calcium 329.3 mg, iron 9.2 mg and dietary fibre 13.38 g. Among the four variations.

4. Nutrients Analysis of Value Added Biscuits

Variations A contributed more amount of nutrients especially calcium, phosphorous and considered for further nutrient analysis. Nutrient content of energy, protein, fat, carbohydrates, dietary fibre, calcium, phosphorus, and iron of the value added biscuits. The values obtained for the control biscuits is given as follows – energy content was as 420 kcal, protein was 8.0g, fat was 15.2g, carbohydrates was 63g, dietary fibre was 12.5g , calcium was 98mg, phosphorus was 110mg and iron was 1.0mg. Variation A had its energy content of 436 kcal, protein as 9.4g, fat as 17.2g, carbohydrates as 61g, dietary fibre as 8.0g, calcium as 115mg, phosphorus as 206mg and iron 5.2mg.

5. Chemical Components of Value Added Biscuits

Chemical components of value added biscuits the ash and moisture content. The ash of the flour was 0.44 per cent and the moisture was 3.6, whereas control biscuits ash content was 0.12 per cent and the moisture content was 3.3 percent. Ash content of Variation A was 0.16 and moisture content was 2 percent.

6. Microbial Load and Peroxide Analysis of Value Added Biscuits

Microbial load and peroxide analysis represented that the total bacterial count of control and Variation A were within the desirable limit. At the time of

preparation of biscuits, there was no or nil bacterial count. But after seven days, the colony forming units in the control biscuits were 18×10^2 cfu/g and the Variation A had the total bacterial count as 15×10^2 cfu/g. Peroxide value is an indicator of rancidity development which is observed during storage time. No rancidity development was observed in formulated value added. The studied conducted by Aruna (2000) also indicated that no rancidity development was observed during storage of biscuits.

7. Cost Effectiveness of Value Added Biscuits

The ingredients cost of the 100 g of control was Rs.35.66 and Variation A biscuits founded prize is Rs 41.16. Increased cost was mainly due to value addition in terms of functional foods which help to promote and preserve health and also prevent diseases.

Conclusion

The value added biscuits mostly of used for the bone health. The health benefits of the value added biscuits are helpful for bone health. People consumed the value added biscuits for their healthy life especially for bone health. The general properties of chia and flax seeds are helpful in lowering blood cholesterol level, good for diabetes, heart disease, postmenopausal women, heart beneficial, and increase immunity and also good for bone health. The study concluded that the value added biscuits are healthy and helpful for bone health and prevent health problems especially related to bones.

Recommendations

- Further studies consisting of snack will be included systematically in the daily dietaries of the vulnerable group of population.
- To formulate a healthy varieties value added products like biscuits, bars, breads extruded products help to meet the nutritional demands of the population.
- Incorporation of high quality protein in the health mix also enhance the nutritional quality and quantity of products.

“Any investments towards improving the nutritional status of the population is an investment for the health and wealth of the nation”.

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ANNEXURE – I

HEDONIC SCALE USED FOR SENSORY EVALUATION

Name -----

Date -----

Product -----

Test these samples & check how much you like or dislike each one. Hedonic scale use appropriate to show your point of view by give point that like yours devotion about the sample. The truthful expression of your feelings will help us.

S. No	Colour	Texture	Flavour	Taste	Appearance	Overall acceptability
Control						
Variation A						
Variation B						
Variation C						
Variation D						

Rate Organoleptic Score

Like Extremely	9
Like very much	8
Like Moderately	7
Like Slightly	6
Neither Like Nor Dislike	5
Dislike slightly	4
Dislike Moderately	3
Dislike very much	2
Dislike Extremely	1

ANNEXURE – I (A)

Determination of Total Protein by Lowry's Method

Reagents

- 2% Sodium carbonate in 0.1 N Sodium hydroxide (Reagent A)
- 0.5% Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 1% Potassium sodium tartrate in 100 ml distilled water (Reagent B)
- Alkaline copper solution: Mix 50 ml of A and 1 ml of B prior to use (Reagent C)
- Folin-Ciocalteu Reagent
- Protein Solution (Stock Standard) - Weigh accurately 50 mg of bovine serum albumin and dissolve in distilled water and make up to 50 ml in a standard flask
- Working Standard- Dilute 10 ml of the stock solution to 50 ml with distilled water in a standard flask. 1 ml of this solution contains 200 μg protein

Procedure

- Extraction of Protein from sample: Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500 mg of the sample and grinded well with a pestle and mortar in 5-10 ml of the buffer. Centrifuged and used the supernatant for protein estimation
- Pipette 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes
- Pipette 0.1 ml and 0.2 ml of the sample extract in two other test tubes
- Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of water serves as the blank
- Add 5 ml of reagent C to each tube including the blank. Mix well and allow standing for 10 minutes.
- Then add 0.5 ml of reagent D, mix well and incubated at room temperature in the dark for 30 minutes. Blue colour is developed.
- Take the readings at 660 nm.
- Draw a standard graph and calculate the amount of protein in the sample and express the amount of protein in mg/g or 100 g sample.

Calculation

$$\text{Concentration of the Protein (\%)} = \frac{\text{OD (test)}}{\text{OD (STD)}} \times \frac{\text{Conc (STD)}}{\text{Aliquot (test)}} \times 100$$

ANNEXURE – I (B)

Determination of Total Carbohydrates by Anthrone Reagents

Reagents

- 2.5N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% Sulphuric acid. Prepare fresh before use.
- Standard Glucose (stock): Dissolve 100 mg in 100 ml distilled water.
- Working standard: 10 ml of stock diluted to 100 ml with distilled water. Store refrigerated after adding a few drops of Toluene.

Procedure

- Weigh 100 mg of the sample in to a boiling tube
- Hydrolyze by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cool to room temperature
- Neutralize with solid Sodium carbonate until the effervescence ceases
- Make up the volume to 100 ml and centrifuge
- Collect the supernatant and take 0.5 and 1 ml aliquots for analysis
- Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, '0' serves as blank
- Make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water
- Then add 4 ml of Anthrone reagent
- Heat for 8 minutes in a boiling water bath
- Cool rapidly and read the green to dark green colour at 630 nm
- Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis
- From the graph calculate the amount of carbohydrate present in the sample tube

Calculation

$$\text{Concentration of the Carbohydrate (\%)} = \frac{\text{OD (test)} \times \text{Conc (STD)}}{\text{OD (STD)} \times \text{Aliquot (test)}} \times 100$$

Estimation of Energy, (Protein value X 4)
+ (Carbohydrate x 4) + (Fat x

ANNEXURE - I (C)

STANDARD OPERATING PROCEDURE FOR TESTING OF IRON IN SAMPLE

Apparatus:

- Atomic absorption spectrophotometer with air acetylene flame
- Cathode Lamp-Fe – 248.3 nm.

Reagent:

- Fe (*NIST traceable*)
- Nitric acid (1:499).
- CaCl₂ solution
- Dissolved 630 mg CaCO₃, 50 ml of 20% v/v HCL, if required boil gently to obtained complete solution. Cool and dilute to 1000 ml with distilled water.

Procedure

- Take 100 ml standard flask.
- Prepare Iron standards (*Nist traceable*) to 0.05, 0.1, 0.125, 0.15, 0.20&0.25 mg/l in nitric acid (1:499) from 1000 ppm solution.
- Prepare a blank solution in 100ml distilled water.
- Pipette out 100 ml of sample in a beaker and digest with 0.5 ml. of conc. Nitric acid and add 25 ml CaCl₂ till the volume reduced to three fourth.
- Make up to 100 ml. with distilled water.
- Process the blank also in the above manner.
- Set the AAS as per the specific work instruction.
- Aspirate the blank, standards and DIGESTED FOOD SAMPLE solutions.
- Measure the absorbance of the iron at 248.3nm.

Calculation:

Draw the standard calibration graph by plotting the absorbance Vs standard conc. for each standard.

ANNEXURE – I (D)

Procedure for Testing Of Calcium in Sample

Procedure

- The fruit sample was dried in an air oven at 105°C for 3 hours. The dried sample was next charred. The charred sample was ashed in a muffle furnace at 550°C until the whitish or greyish ash was obtained. The ash was treated with concentrated hydrochloric acid, transferred to a volumetric flask and made up of 100 ml.
- Take above 100ml in conical flask.
- Add 2-3 drops of sodium hydroxide 1N solution (4.1) and to raise the pH 12 -13.
- Add a pinch of Patton &Reeder (4.3) indicator and stir well.
- Titrated against the solution with 0.01M EDTA (4.4).
- The end point is appearance of blue colour.

Calculation:

Calcium (Ca), mg/100g = $\frac{\text{Volume of EDTA} \times \text{Eq.wt of Ca} \times 100}{\text{Weight of sample}}$

ANNEXURE – I (E)

Standard Operating Procedure for Testing Of Phosphorous

Procedure

Calibration

- Dilute the standardized solution in such a manner as to obtain solutions containing exactly 0.4, 0.2, 0.1, 0.05, and 0.01 mg of phosphorous per ml. Utilize these solutions in order to plot the calibration curve of the instrument in such a way that this curve expresses the result in mg of phosphorous per ml of the final solution.

Determination

- Weigh 1 gm exactly of sample into the crucible or porcelain dish, and ignite. Allow to cool, then weigh, to within 1 mg about 0.1 to 10g of fat, according to its presumed phosphorous content. Burn off the fat. Ignite to a white ash in the furnace at 800 to 900°C.
- Dissolve the magnesium containing ash in exactly 5 ml of the aqueous nitric acid solution with the aid of a 5 ml pipette. Add exactly 20 ml of a mixture of 10 ml of the aqueous ammonium molybdate solution and 10 ml of the acid aqueous ammonium vanadate solution.
- Mix and allow to stand for 20 min. Prepare a blank test, not containing fat, under exactly the same conditions. Transfer the test solution into the cell of the apparatus, Measure the extinction at 460 nm against the blank solution. Read the absorbance.

Calculation:

$$\text{Phosphorous content (\%)} = \frac{2.5 m_1}{m}$$

Where

m_1 = Concentration of phosphorous read from the curve, in mg per ml

m = Mass of the test portion, in g.

ANNEXURE – I (F)

Standard Operating Procedure for Testing Of Vitamin C

Procedure

- Pipette a 20 mL aliquot of the sample solution into a 250 mL conical flask and add about 150 mL of distilled water and 1 mL of starch indicator solution.
- Titrate the sample with 0.005 mol L⁻¹ iodine solution. The endpoint of the titration is identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex.
- Repeat the titration with further aliquots of sample solution until you obtain concordant results (titres agreeing within 0.1 mL)

Calculation

- Calculate the average volume of iodine solution used from your concordant titers.
- Calculate the moles of iodine reacting.
- Using the equation of the titration (below) determine the number of moles of ascorbic acid reacting.
- ascorbic acid + I₂ → 2 I⁻ + dehydro ascorbic acid
- Calculate the concentration in mol L⁻¹ of ascorbic acid in the solution obtained from the Sample.

ANNEXURE – I (G)

Determination of Total Dietary Fiber

- Following enzymatic breakdown heat 220 ml ethanol 95 % to 60 °C and add to the contents of each beaker.
- Allow the precipitate to settle for at least 1 h at room temperature and then, decanting under slight vacuum, filter through the prepared glass filter crucibles (wash residues remaining in the beaker into the crucible with small amounts of ethanol 78 %).
- Prepare the glass filter crucibles used as follows: Heat the thoroughly cleaned glass crucibles for 1 h at 525 °C, add approximately 1 g Celite® 545 (ignited overnight at 525 °C, cooled, and stored in a stoppered container), dry overnight at 105 °C, cool and weigh to the nearest 0.1 mg.
- In order to avoid negative blank values, new crucibles will need to be prepared repeatedly as described. Crucibles should be cooled and stored in a desiccator until required.
- Wash the residues with 3 × 15 ml ethanol 78 %, then rewash with 2 × 10 ml ethanol 95 % and 3 × 10 ml acetone.
- Dry overnight at 105 °C. Cool and weigh to the nearest 0.1 mg. When determining protein and ash, use separate filter crucibles for sample and blank.
- Calculation Dietary fiber content w in % $w = \frac{mR - mP - mA - mB}{m} \times 100$

ANNEXURE – I (H)

Total Bacterial Count

Scope

- This Method Gives general guideline for the Detection of *Total Microbial Count* organism Present in Food Sample.

Apparatus and Glass Wares

- Weighing Balance , Measuring Cylinder, Conical Flask, Auto clave, Bio-safety cabinet, PH Meter, Bunsen Burner , BOD Incubator, Sterile Petri plates, Micropipettes, Water bath, Incubator, Test tube, Colony counter

Culture Media and Reagents

- Plate Count Agar, Peptone water (PH-7.0)

Preparation of Test Sample:

- Blended the sample in a sterile blender jar for 2 minutes or macerate with sterile mortar , using 10 ml of diluting fluid per gram of sample(In Pour plate technique diluting fluid , with a 1g inoculum of a 1/10 Suspension). The diluting fluid for preparing the homogenate should be 0.1 percent peptone.

Procedure

- Take two sterile petri dishes using a sterile pipette, transfer to each dish 1 ml of the test sample.
- Take two other sterile petri dishes, using a fresh sterile pipette, transfer to each dish 1 ml of the First decimal dilution (10^{-2}) of the test sample and repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.
- Aseptically pipette 1 ml each of the sample and 1 ml each of suitable dilutions into the duplicate petriplate and pour 10-15 ml of agar medium (cooled to 45° -50° c) and rotate the Petri dishes clockwise and anti-clockwise for uniform distribution of the inoculums and allow to Solidify.
- Pour the media and diluents control plates. (The elapsing time between the dilution and media.

- Plating is not to exceed 15 minutes).
- Carefully mix the inoculums and allow to solidified, after solidified invert and incubate the Plates for 72 hours at 30°C.

Observations

- After the specified the period of incubation count the colony using colony counting equipment.
- The colonies in each dish containing not more than 300 colonies.

Method of Calculation (counting of total colonies or typical colonies)

- For a result to be valid, it is generally considered necessary to count the colonies on at least one dish containing at least 10 colonies [total colonies, typical colonies or colonies complying with identification criteria, Calculate the number N of microorganisms present in the test sample as a weighted mean from two successive dilutions using Equation (1):

$$N = \frac{\sum C}{V \times 1.1 \times d}$$

- Where, $\sum C$ = The sum of the colonies counted on the two dishes retained from two successive dilutions, at least one of which contains a minimum of 10 colonies; V = The volume of inoculum placed in each dish, in millilitres;
- d = the dilution corresponding to the first dilution retained [d 1 when the undiluted liquid product (test sample) is retained].

Results

TPC 1 g of sample at 30°C is expressed in CFU.

TPC: Total Microbial Count

CFU: Colony Forming Unit