EFFECT OF COOKING AND PROCESSING METHODS ON NUTRIENT RETENTION OF VARIOUS FOODS

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

Certified that this project report "**EFFECT OF COOKING AND PROCESSING METHODS ON NUTRIENT RETENTION OF VARIOUS FOODS**", is the bonafide work of SUCHITRA.R and SHRUTIPRIYANKA.J who carried out the project work under my supervision.

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Dedicated to our Beloved Parents

&

Respected Guide

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Abstract

ABSTRACT

Reducing malnutrition is a major and emerging problem as a consequence of variety social and environmental factors. Combating it requires availability of nutritious food and improved health services. Poverty seriously constrains accessibility to nutritious diets. Diets are nutritious if they contain high protein quality, adequate micronutrient, macro-minerals and essential fatty acids, low anti-nutrient content (bioavailability), high nutrient density. Largely plant-source-based diets do not meet these requirements and need to be improved by certain processing methods and most plant based foods have to be cooked to be palatable. The objective of the study was to formulate a highly nutritious supplement and to determine the nutrient loss occurring due to cooking and to determine the best cooking and processing method for the food Samples of soy, chick pea, spinach, pomegranate seeds, almonds, Indian gooseberry2 were analyzed for total protein, calcium, magnesium, phosphorous, potassium, iron, carbohydrates and vitamin C. The protein content was found to be the highest in almonds followed by chick pea and soy. Spinach contained the highest amount of iron followed by chick pea. Based on the nutritive content of all the samples in their raw form, a supplementary food was formulated containing a mixture of the samples. Sugar was added to the food in order to sweeten it. The formulated food was subjected to nutrition estimation after cooking by boiling and micro waving. The protein and magnesium content increased on boiling and microwaving while calcium, iron and phosphorous content decreased significantly on boiling and microwaving. Cooking by Microwaving retained most nutrients. However, minimal losses of calcium and phosphorus were observed. Microwaving without addition of water appears to be a good method of cooking since it retains almost all nutrients. The moisture content in the food material itself aids in the cooking process.

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LIST OF ABBREVIATIONS & SYMBOLS

DNA	Deoxyribo Nucleic Acid
RNA	Ribo Nucleic Acid
g	Gram
mg	Milligram
ml	Millilitre
EDTA	Ethylene Diamine tetra acetic acid
°C	Degree Celsius
K cal	Kilo calories
ATP	Adenosine Triphosphate
N	Normality
ppm	Parts per Million

Introduction

1. INTRODUCTION

Most foods contain a mix of some or all of the nutrient classes .Some nutrients can be internally stored (e.g.; fat soluble vitamin), while others are required more or less continuously. Poor health can be caused by a lack of nutrients or, in extreme cases, too much of a required nutrient. For example, both salt and water (both absolutely required) will cause illness or even death in too large amounts.

1.1 MAJOR NUTRIENTS IN FOOD:

There are six major classes of nutrients: Carbohydrates, Fats, Minerals, Proteins, Vitamins, and water. These nutrient classes can be categorized as either macronutrients (needed in relatively in large amounts) or micronutrients (needed in smaller quantities). The macronutrients are carbohydrates, fats, fibre, proteins and water. The micronutrients are minerals and vitamins.

The macronutrients (excluding fibre and water) provide structural material for e.g. amino acids from which proteins are built, and lipids from which cell membranes and some signalling molecules are built. Some of the structural material can be used to generate energy internally, and in either case it is measured in Joules or kilocalories. Carbohydrates and proteins provide 17 k J approximately of energy per gram, while fats provide 37k J per gram. (Berg *et al*, 2002).Though the net energy from either depends on such factors as absorption and digestive effort, which vary substantially from instance to instance. Vitamins, minerals, fiber and water do not provide energy, but are required for other reasons. A third class dietary material, fibres (non-digestible material such as cellulose), are also required, for both mechanical and biochemical reasons, though the exact reasons remain unclear.

1.1.1 Carbohydrates:

Carbohydrates may be classified as monosaccharides, disaccharides and polysaccharides depending on the number of monomer units they contain. They constitute a large part of foods such as rice, noodles, bread, and other grain-based products. Monosaccharides contain one sugar unit, disaccharides contain two and polysaccharides three or more. Polysaccharides are often referred to as complex carbohydrates because they are typically long multiple branched chains of sugar units.

Polysaccharides serve for the storage of energy (e.g., starch and glycogen) and as structural components (e.g., cellulose in plants and chitin in arthropods). Cellulose and chitin are examples of structural polysaccharides. Cellulose is used in the cell walls of plants and other organisms, and is considered to be the most abundant organic molecule on earth. It has many uses such as a significant role in the paper and textile industries, and is used as a feedstock for the production of rayon, cellulose acetate, celluloid, and nitrocellulose. Chitin has a similar structure, but has nitrogen-containing side branches, increasing its strength. It is found in arthropod exoskeletons and in the cell walls of some fungi. It also has multiple uses, including surgical threads.

The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g., ATP, FAD, and NAD) and the backbone of the genetic molecule RNA. The related deoxyribose is a component of DNA. Sugars and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, pathogenesis, blood clotting, and development.

1.1.2 Proteins:

Proteins (also known as polypeptides) are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. The amino acids in a polymer are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The sequence of amino acids in a protein is defined by the sequence of a gene, which is encoded in the genetic code. In general, the genetic code specifies 20 standard amino acids; however, in certain organisms the genetic code can include selenocysteine – an in certain archaea – pyrrolysine. Shortly after or even during synthesis, the residues in a protein are often chemically modified by post-translational modification, which alters the physical and chemical properties, folding, stability, activity, and ultimately, the function of the proteins. Proteins can also work together to achieve a particular function, and they often associate to form stable complexes.

1.1.3 Minerals:

Dietary minerals are the chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen and oxygen that are present in nearly all organic molecules. The term "mineral" is archaic, since the intent is to describe simply the less common elements in the diet. Some dieticians recommend that these be supplied from foods in which they occur naturally or at least as complex compounds. Some are absorbed much more readily in the ionic forms found in such sources. On the other hand, minerals are often artificially added to the diet as supplements; the most prominent is the iodized salt which prevents goiter.

TABLE 1.1.3.1: Minerals and their Functions:

MINERALS	FUNCTIONS
Calcium	Builds bones and teeth, muscle contraction, heart action, nerve impulses, blood clotting
Magnesium	Bones, liver muscles, transfer of intercellular water, alkaline balance, neuromuscular activity
Sodium	Electrolyte balance, nerve impulse condition
Potassium	Cell membrane potential, nerve impulse conduction, acid base balance
Copper	Hemoglobin formation, production of RNA, elastic tissue formation, cholesterol utilization.
Zinc	Protein synthesis, carbon dioxide transport, sexual function.
Iron	Hemoglobin formation, electron transport, oxygen transport.
Manganese	Carbohydrate metabolism, vitamin B1 utilization
Chromium	Insulin activity, cholesterol utilization
Phosphorus	Builds bones and teeth, energy production, acid-base balance, calcium absorption
Molybdenum	Enzyme activator, uric acid formation, oxidation enzymes
Selenium	Peroxidase scavenger, glutathione peroxidase, anti-carcinogenic, Vitamin F synergist
Silicon	Bone formation, collagen formation, cartilage formation.
Boron	Reduces calcium loss
Vanadium	Strength of bones and teeth , lowers blood lipids.
Nickel	Activates some liver enzymes

1.1.4 Fats:

Fat is a nutrient that is an important source of calories. One gram of fat supplies 9 calories - more than twice the amount we get from carbohydrates or protein. Fat also is needed to carry and store essential fat-soluble vitamins, like vitamins A and D. There are two basic types of fat. They are grouped by their chemical structure. Each type of fat is used differently in our bodies and has a different effect on our health.

Dietary fats are concentrated source of food energy. They are also the source of linoleum acid, an essential nutrient, and the fat-soluble vitamins A, D, E and K. While we all need some dietary fat each day, a tablespoon is generally sufficient when cutting back on fats; it is helpful to know which the most dietary culprits are.

Saturated Fats: Saturated fats are the only fatty acids that raise blood cholesterol levels. Saturated fats are found in meats and whole dairy products like milk, cheese, cream and ice cream. Some saturated fats are also found in plant foods like tropical oils (coconut or palm kernel oil). When margarine or vegetable shortening is made from corn oil, soybean oil or other vegetable oils, hydrogen atoms are added making some of the fat molecules "saturated". This also makes the fat solid at room temperature. Butter, margarine, and fats in meat and dairy products are all especially high in saturated fat.

We can reduce the saturated fats in our diets by using skim milk and low fat cheeses instead of whole milk and cheese. We can also use less fat, oil, butter, and margarine. At the table, use tub margarine instead of butter. Another way to cut down on fat is to drain and trim meats and take the skin off poultry. Simply reducing the total amount of fat we eat goes a long way toward reducing saturated fats.

Unsaturated fats: Unsaturated fats are usually liquid at room temperature. They are found in most vegetable products and oils. An exception is a group of tropical oils like coconut or palm kernel oil which is highly saturated. Using foods containing "polyunsaturated" and "monounsaturated" fats does not increase our risk of heart disease.

However, like all fats, unsaturated fats give us 9 calories for every gram. So eating too much of these types of fat may also make us gain weight. We can reduce the fat and unsaturated fats in our diets by using less fat, oil, and margarine. We can also eat more low-fat foods like vegetables, fruits, breads, rice, pasta and cereals.

1.1.5 Vitamins:

A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. The term 'vitamin' first became popular in the early 1800's as a contraction of the words 'vital' and 'mineral', though the actual meaning of the word has developed somewhat since that time. A compound is called a vitamin when it cannot be synthesized ion sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and the particular organism. For example, ascorbic acid functions as Vitamin C for some animals but not others, and Vitamins D and K are required in the human diet only in certain circumstances. The term vitamin does not include other essential nutrients such as dietary minerals, essentially fatty acids, or neither essential amino acids, nor does it encompass the large number of nutrients that promote health but are otherwise required less often.Vitamins are essential for the normal growth and development of a foetus.

Dietary supplements, often containing vitamins, are used to ensure that adequate amounts of nutrients are obtained on a daily basis, if optimal amounts of the nutrients cannot be obtained through a varied diet. Scientific evidence supporting the benefits of some vitamin supplements is well established for certain health conditions, but others need further study. In some cases, vitamin supplements may have unwanted effects, especially if taken before surgery, with other dietary supplements or medicines, or if the person taking them has certain health conditions Dietary supplements may also contain levels of vitamins many times higher, and in different forms, than one may ingest through food

TABLE 1.1.5.1: Vitamins and their Functions:

VITAMINS	FUNCTIONS
Vitamin A	Promotes growth and repair of body tissues, bone formation and healthy skin and hair. Essential for night vision
Beta-Carotene	Serves as an antioxidant and my help protect against certain cancers, cataracts and hearth disease. Converted to Vitamin A in the body.
Vitamin D	Aids in the absorption of calcium and helps to build bone mass and prevent one loss. Helps maintain blood levels of calcium and phosphorus.
Vitamin E	Helps protect cells from free radical injury. Serves as an antioxidant and may help protect heart disease, cataracts, and certain cancers. Needed for normal growth and development.
Vitamin K	(phylloquinone) Needed for normal blood clotting and bone health.
Vitamin C	Promotes healthy cell development, wound healing, and resistance to infection. Serves as an antioxidant and may help protect against cancers, cataracts, and heart disease.
Riboflavin (B2)	Helps in red blood cell formation, nervous system functioning, and release of energy from foods. Needed for vision and may help pyridoxine (B6) – Essential for protein metabolism, nervous system and immune function. Involved in synthesis of hormones and recd blood cells.
Vitamin B12	Vital for blood formation and healthy nervous system.

1.1.6 Fiber:

Dietary fiber is a carbohydrate (or a polysaccharide) that is incompletely absorbed in humans and in some animals. Like all carbohydrates, when it is metabolized it can produce similar energy per gram. But in most circumstances it accounts for less than that because of its limited absorption and digestibility. Dietary fiber consists mainly of cellulose, a large carbohydrate polymer that is indigestible because humans do not have the required enzymes to disassemble it. There are two subcategories: soluble and insoluble fiber. Whole grains, fruits (especially plums, prunes, and figs), and vegetables are good sources of dietary fiber. There are many health benefits of a high-fiber diet. Dietary fiber helps reduce the chance of gastrointestinal problems such as constipation and diarrhea by increasing the weight and size of stool and softening it. Insoluble fiber, found in whole-wheat flour, nuts and vegetables, especially stimulates peristalsis - the rhythmic muscular contractions of the intestines which move digests along the digestive tract. Soluble fiber, found in oats, peas, beans, and many fruits, dissolves in water in the intestinal tract to produce a gel which slows the movement of food through the intestines. This may help lower blood glucose levels because it can slow the absorption of sugar. Additionally, fiber, perhaps especially that from whole grains, is thought to possibly help lessen insulin spikes, and therefore reduce the risk of type 2 diabetes

1.1.7 Water:

Pure water is calorie-free. The body does not store water so consuming it in plenty is a must in order to replenish losses and maintain healthy function of all body cells. Water contains minerals as well. Fluoride is present naturally in many water resources. Hard water contains calcium and magnesium. These minerals can be neutralized with salty crystals in order to produce softer water that leaves less scaling.

Cooking often involves water which is frequently present as other liquids, both added in order to immerse the substances being cooked (typically water, stock or wine), and released from the foods themselves. Liquids are so important to cooking that the name of the cooking method used may be based on how the liquid combined with the food, as in steaming, simmering, boiling, braising and blanching.

1.2 OTHER NUTRIENTS:

1.2.1 Antioxidants:

As cellular metabolism/energy production requires oxygen, potentially damaging compounds known as free radicals can form. For normal cellular maintenance, growth, and division, these free radicals must be sufficiently neutralized by antioxidant compounds. Some are produced by the human body with adequate precursors (glutathione, Vitamin C) and those the body cannot produce may only be obtained in the diet via direct sources) or produced by the body from other compounds (Beta-carotene converted to Vitamin A by the body, Vitamin D synthesized from cholesterol by sunlight). Phytochemicals and their subgroup polyphenols are the majority of antioxidants; about 4,000 are known. Different antioxidants are now known to function in a cooperative network, e.g. vitamin C can reactivate free radical-containing glutathione or vitamin E by accepting the free radical itself, and so on. Some antioxidants are more effective than others at neutralizing different free radicals. Some cannot neutralize

1.2.2 Phytochemicals:

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. It is well-known that plants produce these chemicals to protect itself but recent research demonstrate that they can protect humans against diseases. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. They are not essential nutrients and are not required by the human body for sustaining life.

There are many phytochemicals and each works differently. These are some possible actions:

- Antioxidant - Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes).

- Hormonal action - Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.

- Activation of enzymes - Indoles, which are found in cabbages, stimulates enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

- Interference with DNA replication - Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.

- Anti-bacterial effect - The phytochemical allicin from garlic has anti-bacterial properties.

- Physical action - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.

1.3 NUTRIENT LOSSES DURING PROCESSES:

Nutritional importance of vegetables cannot be neglected in our daily meals. Vegetables are the major source of vitamins and minerals, but in vegetable, protein is in poor quality. Vitamins and minerals are the chief regulators in metabolism in humans. All the foods we eat, with exception of fruit and vegetables are subjected to some sort of cooking and processing. Minerals and other nutrients are affected by both peeling and cooking. Method such as temperature and duration of cooking may also effect significantly on the nutritive vales of vegetables. Some of the important nutrients such as ascorbic acid and folic acid which are susceptible to oxidation are readily oxidized by brisk cooking. Excessive cooking may also cause an adverse effect on the digestibility of the vegetables.

The common cooking methods are steaming, pressure cooking, boiling, roasting and frying.The effect of food processing on nutrient content will depend on the sensitivity of the nutrient to the various conditions prevailing during the process such as heat, oxygen, pH and light. The nutrient retention may also vary with a combination of conditions such as characteristics of the food being processed, and the concentration of the nutrient in the food.

TABLE1.3.1: Nutrient losses during processing:

NUTRIENT	EFFECT OF PROCESSING
Fat	Oxidation accelerated by light.
Vitamin C(Ascorbic acid)	Decreases during drying processing, heating, oxidation ,cell damage. Losses due to oxidation catalyzed by copper, iron. Stable to heat under acidic conditions.
Vitamin B3(Niacin)	The most stable vitamin. It is stable to heat and light. Leaches into cooking water,
Vitamin B6(Pyridoxine)	Heat stable in alkaline and acidic conditions.
Vitamin A	Very heat labile-easily destroyed by heat. Easily oxidized.
Protein	Denatured by heat(Improves digestion)

Nutrients are lost during cooking by the following mechanisms:

1. If vegetables are cooked in water containing salt and the cooking water is discarded, minerals like sodium, potassium and calcium are lost.

2. Cutting vegetables into small pieces and exposing them to air may result in loss of Vitamin C.

3. Water soluble vitamins are lost during excessive washing as in repeated washing of legumes, rice, etc. Washing may remove as much as 40% thiamine and nicotinic acid.

4. Cooking for long periods results in vitamin loss. If fat is repeatedly used in frying. It may contain toxins due to peroxidation and rancidity.

5. Excessive heating of milk with lactose and other foods with jiggery may affect protein quality as it may lead to browning.

1.4 METHODS OF FOOD PROCESSING:

1.4.1 Lyophilization:

Freeze-drying (also known as lyophilization or cryodesiccation) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to the gas phase.

Freeze-drying also causes less damage to the substance than other dehydration methods using higher temperatures. Freeze-drying does not usually cause shrinkage or toughening of the material being dried. In addition, flavors, smells and nutritional content generally remain unchanged, making the process popular for preserving food.

Freeze-dried products can be rehydrated (reconstituted) much more quickly and easily because the process leaves microscopic pores. The pores are created by the ice crystals that sublimate, leaving gaps or pores in their place. This is especially important when it comes to pharmaceutical uses. Freeze-drying can also be used to increase the shelf life of some pharmaceuticals for many years.

1.4.2 Microwaving:

Microwaves cause water molecules in food to vibrate, producing heat that cooks the food. That's why foods high in water content, like fresh vegetables, can be cooked more quickly than other foods. The microwave energy is changed to heat as it is absorbed by food, and does not make food "radioactive" or "contaminated.

Any form of cooking will destroy some nutrients in food, but the key variables are how much water is used in the cooking, how long the food is cooked, and at what temperature. Microwave ovens do convert vitamin B12 from the active to inactive form, making approximately 30-40% of the B12 contained in foods unusable by mammals.

Spinach retains nearly all its folate when cooked in a microwave; in comparison, it loses about 77 percent when cooked on a stove, because food on a stove is typically boiled, leaching out nutrients. Steamed vegetables tend to maintain more nutrients when cooked on a stovetop than in a microwave. Bacon cooked by microwave has significantly lower levels of carcinogenic nitrosamines than conventionally cooked bacon

Microwave cooking can be more energy efficient than conventional cooking because foods cook faster and the energy heats only the food, not the whole oven compartment. Microwave cooking does not reduce the nutritional value of foods any more than conventional cooking. In fact, foods cooked in a microwave oven may keep more of their vitamins and minerals, because microwave ovens can cook more quickly and without adding water

Unlike frying and baking, microwaving does not produce acrylamide in potatoes, however unlike deep-frying, it is of only limited effectiveness in reducing glycoalkaloid (i.e. Solanine) levels Acrylamide has been found in other microwaved products like popcorn.

1.4.3 Boiling:

Boiling is a method of cooking foods by just immersing them in water at 100 degree celcius and maintaining the water at that temperature till the food is tender. Loss of heat labile nutrients occurs such as C and B vitamins if the water is discarded Decrease in fat, total ash, carbohydrate fractions, antinutritional factors, minerals and vitamin B is also associated with boiling.

1.4.4 Frying:

Frying is the cooking of food in oil or fat. Fats can reach much higher temperatures than water at normal atmospheric pressure (Tannahill, Reay, 1995) . Through frying, one can sear or even carbonize the surface of foods while caramelizing sugars. The food is cooked much more quickly and has a characteristic crispness and texture. Depending on the food, the fat will penetrate it to varying degrees, contributing richness, lubricity, and its own flavour. Frying techniques vary in the amount of fat required, the cooking time, the type of cooking vessel required, and the manipulation of the food

1.4.5 Roasting:

Roasting is a cooking method that uses dry heat, whether an open flame, oven, or other heat source. Roasting usually causes caramelization or Maillard browning of the surface of the food, which is considered a flavor enhancement[1] Meats and most root and bulb vegetables can be roasted. Any piece of meat, especially red meat that has been cooked in this fashion is called a roast. Additionally, large uncooked cuts of meat are referred to as roasts.

It uses high heat just at either the beginning or the end of the cooking process, with most of the cooking at a low temperature. This method produces the golden brown texture and crust people desire but maintains more of the moisture than simply cooking at a high temperature, although the product will not be as moist as low temperature cooking the whole time.

1.4.6 Steaming:

Steaming works by boiling water continuously, causing it to evaporate into steam; the steam then carries heat to the nearby food, thus cooking the food. The food is separated from the boiling water but has direct contact with the steam, resulting in a moist texture to the dishes. This differs from double boiling, in which contact with steam is undesired. Steaming also results in a more nutritious food than boiling because fewer nutrients are leached away into the water, this is usually discarded. Comparison between steaming and boiling vegetables shows the most affected nutrients are folic acid and vitamin C. The only drawback of steaming is that it requires more energy to cook than other techniques because the heat energy does not carry to the food directly—most energy is lost to the low-efficient medium, water.

1.4.7 Pressure cooking:

Pressure cooking is a method of cooking in a sealed vessel that does not permit air or liquids to escape below a preset pressure. Because the boiling point of water increases as the pressure increases, the pressure built up inside the cooker allows the liquid in the pot to rise to a higher temperature before boiling

The food to be cooked is placed in the pressure cooker, with a small amount of water. The lid is closed, the pressure setting selected and the pressure cooker is placed on a heat source at the highest heat until the cooker reaches full pressure. The higher temperature causes the food to cook faster; cooking times can typically be reduced by about 70 percent. Pressure cooking is often used to simulate the effects of long braising or simmering in shorter periods of time. Since pressure cooking depends on the production of steam, the process cannot easily be used for methods of cooking that produce little steam, such as roasting, pan frying or deep frying.

1.5 FORMULATING SUPPLEMENTARY FOODS:

A dietary supplement, also known as food supplement or nutritional supplement, is a preparation intended to provide nutrients, such as vitamins, minerals, fiber, fatty acids, or amino acids, which are missing or are not consumed in sufficient quantity in a person's diet. Some countries define dietary supplements as foods, while in others they are defined as drugs.

Supplements containing vitamins or dietary minerals are included as a category of food in the Codex Alimentarius, a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety. These texts are drawn up by the Codex Alimentarius Commission, an organization that is sponsored by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

1.5.1 Nutritional Aspects:

The selection of ingredients for the formulation of Formulated Supplementary Foods should be made and taking into account the following aspects:

- Nutrient content of staple food;
- Dietary habits;
- Other socio-economic aspects as determined by the national authorities dealing with nutrition;
- Availability and costs of raw materials and other ingredients

1.5.2 Energy:

The energy density of a mixture of milled cereals and pulses and defatted oilseed meals and flours is relatively low.

The energy density of the food can be increased by:

(a) the addition of fats(to reduce bulkiness) and oils, and/or digestible carbohydrates including, in moderation, sugars; and/or,
(b) processing the basic ingredients.

One hundred grammes of the food should provide at least 400 kcal.

1.5.3 Proteins

Cereals, legumes and/or oilseed flours, alone or preferably mixed, can constitute an appropriate source of proteins, provided they are prepared in such a way that in the finished product the proteins in the mixture satisfy the criteria below.

The amino-acid score (previously called the chemical score) corrected in accordance with the true digestibility of the crude proteins, should not be less than 70 per cent of that of casein. Higher values should be required if calculation of the score was based not, as is usually the case, on the most limiting amino acid, but on two or more key amino acids such as lysine, methionine, cystine, threonine and tryptophan.

If, for technical reasons, the amino acid score and the digestibility of a protein cannot be determined, the protein quality should be measured by biological assays. Alternatively, the protein quality may be computed from published data on essential amino acid patterns of dietary proteins and their digestibility.

The addition of methionine, lysine, tryptophan or other limiting amino acids, solely in the L-form (except for DL-methionine, which may be used in foods for children over 12 months of age) should be contemplated only when, for economic and technological reasons, no mixture of vegetable and/or animal proteins makes it possible to obtain an adequate protein quality.

Taking into account the preceding considerations, the protein content should be in the order of 15 g per100 g of the food on a dry matter basis.

1.5.4 Fat:

Incorporation of adequate quantities of fats and/or oils, as technologically feasible, is recommended in order to increase the energy density of the product. A level of between 20% and 40% of energy derived from fat would be desirable. This corresponds to between about 10 g and 25 g of fats and/or oils in 100 g of the food.

The level of linoleic acid (in the form of glycerides) should not be less than 300 mg per 100 kcal or 1.4 g per 100 g of product. Where it is not feasible to include all of the desired fats and/or oils in the formulation of the food, the instructions for use on the label should recommend the addition of a specified quantity of fats and oils during the preparation of the feed.

1.5.5 Carbohydrates:

Starch is likely to be a major constituent of many supplementary foods for older infants and young children. To ensure that its energy value is realized, this starch should be provided in a readily digestible form.

Dietary fibers and other non-absorbable carbohydrates are partially fermented by the intestinal flora to produce short-chain fatty acids, lactate and ethanol which may subsequently be absorbed and metabolized. Increasing the intake of dietary fibers enhances stool bulk. They also may affect the efficiency of absorption of various nutrients of significance in diets with a marginal nutrient content, so the dietary fiber content of the food should be reduced to a level not exceeding 5 g per 100 g.

1.5.6 Vitamins and Minerals:

The addition of vitamins and minerals should take into account local nutrition and health conditions as well as the requirements stipulated by national legislation. When establishing the specifications for the premix of vitamin compounds and mineral salts, the vitamin and mineral content of the other ingredients used in the formulation of the food should be taken into account.

Objectives

2. OBJECTIVES:

1. To determine nutrient loss during process and cooking of chosen plant based foods.

2. To formulate processing and cooking method to reduce nutrient loss.

3. To formulate easily cookable high nutrient containing food for adults.

Literature Review

3. LITERATURE REVIEW:

Domestic cooking practices such as boiling, microwaving and frying provoke a partial loss of the flavanols (Tudela *et al*, 2002) .There is considerable cooking loss of vitamins , and information on vitamin contents of cooked foods is essential for assessing the adequacy of vitamin intakes. The cooking losses were very high in the case of ascorbic acid, thiamine, riboflavin, beta carotene and folic acid (Agte *et al*, 2002). In one study cooking of zucchini beans and carrots with smaller amounts of water resulted in significantly higher content of phenolic phytochemicals in the vegetables compared to cooking with larger water volumes (Andlauer *et al*, 2003). Lycopene is a bioactive carotenoid present in many fruits and vegetables. Tomatoes constitute the major dietary source of lycopene. In addition to lycopoene, tomatoes are a rich source of beta carotene, folate, potassium, vitamin C, flavanoids and vitamin E.

Microwaved cooking without water better retains flavanoids and ascorbic acid. Results show that all antioxidant enzyme activities decrease in the vegetables/ fruits, except tomato, when they are thermally treated (boiling, microwaving and baking). However, it has been observed that GSH-Px and CAT activities increase in the thermally treated tomato and SOD activity increases in the boiled tomato whereas it decreases in the microwaved and baked tomato.

3.1 NUTRITIONAL LOSSES DURING DRYING:

There are many techniques of drying but the cheapest most commonly used is sun-drying. This method however results into combination with dust and microorganisms and is sometimes too slow to accomplish the desired purpose .Improved drying methods include oven, solar, osmotic and freeze drying. (Adom *et al*, 1997) Drying vegetables results into both nutrient and culinary losses. The losses however vary with the drying technique employed (Negi and Roy, 2001).

Indian spinach is one of the most cultivated of all spinaches. The typical storage life of the leaf is less than seven days at ambient conditions. Attempt to process and preserve the leaves by blanching and freezing results into sliminess of the leaf. Drying for preservation offers a good processing method. All the leaves were dried to approximately 4% moisture content before storage at 28^0 C. The samples gained some moisture during storage but the moisture gained was negligible. Both bacteria and fungi were found on the dried leaves but there was no rottenness in the dried samples. All the organisms detected are common contaminants in the environment. The *Aspergillus* and *Penicillium* species are commonly reported in association with various dried food materials.

All the drying reduced the ascorbic acid content by 43-48%, the drying method did not matter much.. Shade dried leaves retained 52%, but sun and oven dried retained 57% and 55%.Vitamins and minerals loss have been reported with various processing methods including shedding and drying (Oboh *et al.*, 2001).Drying and storage caused a reduction in calcium, magnesium, sodium, potassium, iron, manganese and zinc .Shade dried samples was lowest in calcium, magnesium, sodium, potassium, iron, manganese and zinc immediately after drying. There was drastic reduction in sun and oved dried samples by the twelfth week of storage, but the contents of calcium. Magnesium, sodium and iron increased in shade dried leaves during storage. Sun drying technique retained calcium more than the other two techniques.

Similarly drying reduces the Vitamin C content of certain plant based foods. Indian gooseberries are very rich in anti-oxidants and abundant in vitamin C. (Dharmananda, 2003) reported a vitamin C content of 625 mg per 100 g of fruit. They also contain chromium which is believed to give it the ability to temper diabetes.

Vitamin C content of the Indian gooseberry powder dried at different temperatures is shown to have a significant loss in vitamin C content at the level of 0.05 was found in the samples dried at 140°C. The vitamin C content of the powder dried at 120°C was 0.513 mg/ml while in the powder dried at 140°C was 0.321 mg/ml. When compared with the fresh juice (0.846 mg/ml), the loss of vitamin C at120°C and 140°C was 39.4% and 62.1% respectively. The loss in vitamin C content during drying involves oxidation and hydrolysis. The ascorbic acid is oxidised to dehydroascorbic acid, followed by hydrolysis to 2, 3-diketogulonic acid and further oxidation and polymerisation to form a wide range of other nutritionally inactive products (Gregory III, 2008). Because of the high solubility of vitamin C in aqueous solution, there was potential for significant losses by leaching from freshly cut fruit. The loss can also occur during storage and handling (Gregory III, 2008).

3.2 NUTRITIONAL LOSSES DUE TO FOOD PROCESSING:

Removal of the inedible fractions of plant foods is an essential stage of processing to improve palatability. However, these processes can incur losses of nutrients; with careful control of the processes, nutrient losses can be minimised.

Cereals: Cereals provide about three-quarters of both the energy and protein intake of the world's population. All cereals require some form of processing prior to consumption, thereby introducing potential sources of nutrient loss. Milling, involving the mechanical separation of the endosperm from the germ, seed coat and pericarp, results in changes to the micro-nutrient composition. Most of the B vitamins, iron and calcium are concentrated in the outer layers of grain, which are removed by milling .After milling, flour is largely composed of the endosperm, although the composition can be varied and regulated by altering the processing conditions.

Flours can be produced to a range of different extraction rates1, depending upon the amount of bran, germ and pericarp that are removed. Flours of high extraction rate retain many more of the micro-nutrients than those of a lower extraction rate .When the nutritional quality of a single cereal is responsible for the health and nourishment of an entire community, particular importance needs to be paid to its milling and subsequent processing to minimise nutrient losses. Because of the non-uniform distribution of nutrients throughout the grain, the nutrient loss due to processing are non-linear and is characteristic for each nutrient. Thiamine is most concentrated in the scutellum and the aleurone layer while riboflavin is more evenly spread throughout the grain, although it is predominantly concentrated in the germ. Commercial milling removes about 68% of thiamine, 58-65% of riboflavin and 85% of pyridoxine from whole wheat. Iron and zinc, which are located at the periphery of the kernel, are also considerably reduced by commercial extraction rates.

Generally, it is thought that the mineral composition of refined flour is reduced to about 30% of that of the whole grain. Despite the nutritional losses incurred by milling at low extraction rates, there is also a beneficial loss of phytic acid which is concentrated in the aleurone layer (Kent, 1974). Phytic acid forms insoluble complexes with calcium and iron, thereby reducing the bioavailability of these minerals. The reduction in phytic acid through milling may improve the bioavailability of the remaining nutrients. In populations with low calcium intake the use of 100% wholemeal flour may be a nutritional disadvantage. Three years after the introduction of 100% wholemeal flour in Dublin in 1943, the incidence of rickets rose from a negligible level to 50%. Supplementation can be achieved using calcium carbonate (chalk) in flour.

Rice: Rice cannot be consumed raw and therefore has to be processed to make it edible. The most common forms of processing are milling and polishing, and the extent of nutrient loss varies according to the severity and type of process and the distribution of nutrients within the grain.

Legumes: Legumes provide a valuable source of protein in the diet of many of the world's poor. Most legume seeds contain between twenty to forty percent protein on a dry matter basis. This is in comparison with cereals, which contain between seven and fifteen percent protein. Despite their high nutritional value, legumes contain several anti-nutritional compounds. Processing to remove these anti-nutritional compounds is an essential stage in the preparation of most legumes.

All legumes are milled to separate the seed coat from the endosperm prior to consumption. Decortication is known to improve the absorption of water, thereby decreasing the cooking time, and removes the polyphenols and phytate present in the seed coat. Due to the reduction in polyphenols and phytates, the mineral availability from decorticated legumes is higher than from whole seeds. Soaking and cooking also reduce the levels of polyphenols (tannins) in legume seeds through leaching of the water soluble components. See the section on 'bioavailability of micro-nutrients' for further information on the roles of phytate and phenolic compounds.

Fruit and vegetables: Fruit and vegetables are a valuable source of micro-nutrients in the diets of the poor in the tropics. In regions where the consumption of animal-based foods is limited, fruit and vegetables are the predominant source of provitamin A (ß-carotene). Washing and peeling result in the loss of much of the water soluble vitamins B and C since these nutrients are more concentrated in the peel and outer layers. Peeling is an essential stage of processing in most roots and tubers. To minimise losses of vitamins and minerals peeling must be minimised without affecting palatability.

Brocolli: The effects of five different cooking methods (steaming, microwaving, boiling, stir-frying, and stir-frying/boiling) on the contents of chlorophyll, vitamin C, total carotenoids, total soluble sugars, total soluble proteins and glucosinolates were investigated in the present study. The degree of greenness, due to chlorophyll content, is important in determining the final quality of green vegetables (Nisha *et al.*, 2004). It was also reported that chlorophyll and its derivatives exerted beneficial effects such as anticarcinogenic and antimutagenic activities (Turkmen *et al.*, 2006). Green vegetables exhibit poor color quality and decreased chlorophyll content as compared with the raw material after thermally processed (Adebooye *et al.*, 2008; Turkmen et al., 2006). In the present study, boiling, stir-frying/boiling, stir-frying, and microwaving led a great loss of chlorophyll in broccoli. In contrast, steaming did not cause any significant loss of chlorophyll content.

Vitamin C is one of the most important nutrients in broccoli as well as in many other horticultural crops and has many biological functions in the human body (Lee and Kader, 2000). The concentration of ascorbic acid (the predominant form of vitamin C) in broccoli generally was decreased after cooking (Serrano et al., 2006). Previous studies have shown that boiling and microwaving caused a great loss of vitamin C (López-Berenguer *et al.*, 2007; Sikora *et al.*, 2008; Vallejo *et al.*, 2002). Our study also shows that boiling and stir-frying/boiling caused a dramatic loss of vitamin C. Hudson *et* *al.*(1985) and Vallejo *et al.* (2002) reported that steaming caused less loss of vitamin C than boiling and microwaving, which is consistent with our study. Stir-frying caused a considerable loss of vitamin C (16%), however, much less than stir-frying/boiling and boiling (38% and 33%, respectively). Vitamin C is water soluble, stir-frying/boiling and boiling might cause great losses of vitamin C by leaching into surrounding water other than thermal degradation. Microwaving, as well as stir-frying and stir-frying/boiling, caused a high loss of glucosinolates.

3.2.1 Blanching

Blanching is used to inactivate enzymes as a pre-requisite to the preservation of vegetables. Blanching can be carried out by immersion in boiling water or by treatment with hot air or steam. Boiling water is the most common form of blanching used at the small scale. Nutrient retention during blanching differs according to the method used and the type and size of vegetable.

Inevitably there is some loss of water soluble nutrients such as mineral salts, protein, sugar and vitamins during the blanching and subsequent water cooling, but these can be kept to a minimum by attention to detail. Losses are greater from food with a large surface to volume ratio. The loss of nutrients is not uniform throughout the blanching period, with most losses occurring in the early stages.

3.2.2 Parboiling as a technique to reduce nutrient loss in rice

Parboiling is a technique used to process rice and which is claimed to improve the retention of proteins, vitamins and minerals during subsequent cooking. Raw rice is soaked in water and partially steamed prior to drying and milling. The effects of parboiling on the retention of nutrients within the grain are two-fold. Some B vitamins migrate further into the grain, thus reducing their loss during milling. It is reported that parboiled rice may contain up to 2μg/g of thiamine compared to only 0.7 μg/g for the untreated grain (Bender, 1978). Secondly, as the grain becomes partially gelatinised during processing, it is less susceptible to damage during milling and has improved keeping qualities. The protein quality of parboiled rice is also slightly improved.

A modified technique, known as converted rice has been used to reduce vitamin losses in rice and to provide an alternative to parboiling. Converted rice undergoes a vacuum treatment while soaking, followed by steaming under pressure. This technique has the advantage of avoiding the characteristic taint of parboiled rice, but is probably unsuitable for use at the household level by the poorest communities due to the unavailability of pressure cooking facilities. The vitamin content of converted rice is similar to that of parboiled rice, with approximately five times more thiamine and niacin than untreated rice, three times more B6, riboflavin and pantothenate and twice the amount of biotin (Bender, 1978).

In addition to milling and polishing, washing of rice prior to cooking is another potential source of nutrient loss. In most Asiatic countries, rice is washed or soaked in water for lengthy periods prior to cooking. During this time, considerable amounts of water soluble vitamins (B group) are leached out into the wash water, which is then discarded (Juliano, 1993). Washing for shorter periods of time will help to conserve some of these water soluble vitamins.

3.3.3 Inevitable losses due to processing and cooking.

Cooking and food processing have both positive and negative effects on the nutritional value of foods. The digestibility of proteins and starches is improved by cooking and many anti-nutritional components are destroyed. However, there is some inevitable leaching of nutrients into the cooking water during processing and cooking.

Dark green leafy vegetables are an important source of ß-carotene, the precursor of vitamin A. However, processing and cooking can result in significant losses of this compound. Boiling leafy vegetables results in higher losses than both steaming and frying

3.3.4 Losses through improper processing and storage:

Fluctuations in temperature and humidity, undue access to air and insect or rodent infestation during storage all result in considerable nutrient losses. These are all unnecessary losses that could be reduced or avoided with efficient storage methods.

At optimum storage conditions (ambient temperature of 15 to 18°C and low moisture content) cereals can be stored for long periods with no significant effect on nutrient composition. Milling and polishing of rice is known to increase the susceptibility to nutrient damage during storage.

Some vegetables are used in raw form as salad but most of them require cooking for the improvement of digestibility and palatability. Some other vegetables require peeling to decrease their useless fiber(Thane and Reddy,1997). Minerals and other nutrients are affected by both peeling and cooking. Peeling is considered an inevitable treatment for rendering them more digestible and may result in fairly heavy loss of nutrient especially of vitamins. Peeling before boiling increases the loss of ascorbic acid, folic acid, or other vitamins of group B(Bennion,1980).

Methods, temperature and duration of cooking may also effect on the nutritive values of vegetables. Some nutrients such as ascorbic acid and folic acid are susceptible to oxidation are readily oxidized by brisk cooking. Minerals are also affected by high temperature, flavour may be lost by brisk cooking Excessive cooking may also cause an adverse affect on the digestibility of the vegetables.

Materials And Methods

4. MATERIALS AND METHODS

4.1 SAMPLE PREPARATION:

The formulated food prepared was a composition of several food items and in order to make it to a powdered form several steps were taken for each of the samples based on their characteristics. The samples were powdered in order to increase their solubility in water .Required amounts of every sample was taken for every estimation.

4.1.1 Chickpea:

Chickpea is the major constituent in the formulated food. The sample was ground directly.

4.1.2 Soy:

Soy was also another major constituent which was ground into a fine powder.

4.1.3 Almond:

Almond was ground into powder.

4.1.4 Pomegranate seeds:

Pomegranate was peeled, it was crushed and the seeds were filtered and dried at room temperature for 2 days and ground.

4.1.5 Gooseberry:

Gooseberry was grated and stored in the freezer at -30^{0}C .It was the lyophilized for 8 hours at -70^{0}C.

4.1.6 Spinach:

Spinach was cut to minute pieces and stored in the freezer at -30^{0}C and lyophilized for 10-12 hours at -70^{0}C.

4.2 METHODS:

4.2.1 Protein Estimation (Folin Lowry's method,Rosebourgh,Farr & Randall,1951):

Reagents:

Reagent A: 2% sodium Carbonate in 0.1 N Sodium Hydroxide.

Reagent B: 0.5% Copper Sulphate in 1% potassium sodium tartrate

Alkaline Copper Reagent: Mix 50ml of A and 1ml of B prior to use.

Folin-Ciocalteau Reagent: Dilute with equal amount of water.

Protein Solution (Stock Standard); Weigh accurately 50mg of Bovine Serum Albumin and dissolve in distilled water and make upto 50 ml in a standard flask.

Working Standard: Dilute 10ml of the stock solution to 50ml with distilled water in a standard flask. One ml of this solution contains 200 mcg proteins.

Procedure:

Extraction of Protein from Sample:

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

Estimation of Protein:

Pipette out 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard into a series of test tubes. Pipette out 0.1 ml and 0.2ml of sample extract in two test tubes. Make up the volume to 1ml in all the test tubes. A tube with 1ml of water serves as the blank. Add 5ml of reagent C to each tube including the blank. Mix well and allow to stand for 10 minutes. Then add 0.5ml of reagent D, Mix well and incubate in room temperature in the dark for 30 minutes. Blue color is developed. Take the readings at 660 nm. Draw the standard graph and calculate the amount of protein in the sample.

4.2.2 Phosphorus estimation (Vanado- Molybdate method, hanson, 1950):

Reagents:

Vanadate-Molybdate composite reagents:

Dissolve20g ammonium molybdate in 400ml warm water (50^0C) and cool. Dissolve 1.0g of ammonium vanadate in 300ml boiling distilled water, cool and add 140ml conc. Nitric acid gradually with stirring . Then add the molybdate solution gradually to the acid vanadate solution with stirring and dilute to 1 litre with water.

Standard phosphate solution:

Prepare a stock solution containing 3.834g potassium dihydrogen phosphate per litre. Dilute 25 ml to 250ml.

Preparation of standard graph:

To a series of 100 ml volumetric flasks add 0, 2.5,5,10,20,30,40,50 ml of standard phosphate solution and dilute each to 50-60ml with water. Add a few drops of Ammonia solution (0.88) and make just acid with nitric acid (1:2). Add 25ml of vanadate – molybdate reagent, dilute to the mark and mix. Allow to stand for 10 minutes and measure the optical density in a 2.5 Or 10mm cell at 470 nm.

Procedure:

Transfer a suitable volume of the sample to a 100ml volumetric flask. If the determination is carried out on the ash, boil the ash with 10ml of 5M hydrochloric acid and wash the solution into the 100ml flask with water, filtering if necessary. Neutralize with dropwise addition of 0.88 ammonia , and proceed as for the standard graph i.e. make just acid with dilute nitric acid , add 25ml of vanadate-molybdate solution dilute to the mark and measure the optical density after allowing to stand for 10 minutes .

4.2.3 Calcium and Magnesium Estimation:

Reagents:

Buffer Solution:

Dissolve 16.9g NH_4Cl in 143ml NH_4OH. Add 1.25g magnesium salt of EDTA to obtain sharp change in color of indicator and dilute to 250ml. If magnesium salt of EDTA is unavailable, dissolve 1.179g disodium salt of EDTA (AR grade) and 780mg $MgSO_4.7H_2O$ or 644mg $MgCl_2.6H_2O$ in 50ml Distilled water. Add to above solution of NH_4CL. in NH_4OH and dilute to 250ml.

Inhibitor:

Dissolve 4.5g hydroxylamine hydrochloride in 100ml of 95% ethyl alcohol or isopropyl alcohol.

Erichrome black T indicator:

Mix 0.5g dye with 100g NaCl to prepare dry powder.

Murexide Indicator:

Prepare a ground mixture of 200mg of murexide with 100g of solid NaCl.

Sodium hydroxide 2N:

Dissolve 80g NaOH and dilute to 1000ml.

Standard EDTA solution 0.01M:

Dissolve 3.723g EDTA sodium salt and dilute to 1000ml. standardize against standard Ca solution, 1ml= 1mg calcium carbonate.

Standard Calcium solution:

Weigh accurately 1g AR grade Calcium carbonate and transfer to 250ml conical flask. Place a funnel and add HCl till $CaCO_3$ dissolves completely. Add 200ml distilled water and boil for 20-30 min to expel CO_2.Cool and add few drops of methyl red indicator. Add NH_4OH 3N dropwise till intermediate orange color develops. Dilute to 1000ml to obtain 1 ml = 1 mg $CaCO_3$

Procedure:

A)Total Hardness:

Take 25 or 50 ml well mixed sample in porcelain dish or conical flask. Add 1-2ml buffer solution followed by 1ml inhibitor. Add a pinch of Eriochrome black T and titrate with standard EDTA (0.01M) till wine red colour changes to blue. Note down the volume of EDTA required.(A).Run a reagent blank. Note the volume of EDTA (B).

Calculate volume of EDTA required by sample,
C = (A-B) from volume, of EDTA required in steps 3 & 4.

B) Calcium Hardness:

Take 25 or 50 ml sample in a porcelain dish. Add I ml of NaOH to raise pH to 12.0 and add a pinch of murexide indicator. Titrate immediately with EDTA till pink color changes to purple. Note the volume of EDTA required (A'). Run a reagent Blank. Note the ml of EDTA required and keep it aside to compare end points of sample titrations. Standardize the EDTA (0.1M) solution following the procedure of calcium hardness from 1 to 4 using standard calcium solution

Calculation:

Calcium Hardness as $CaCO_3$

(mg/l) $= \frac{A' * D * 1000}{\text{ml sample}}$

where

A'= Volume of EDTA used by sample

D= mg $CaCO_3$equivalent to 1 ml EDTA titrate.

Magnesium hardness as $CaCO_3$ = Total Hardness as $CaCO_3$–Ca hardness as $CaCO_3$

4.2.4 Iron estimation (phenanthroline method):

Reagents:

Sodium citrate solution:

25g/l in distilled water; Hydroquinone solution – 1% solution in distilled water; Prepare fresh and store in amber coloured bottle ; O- Phenanthroline solution – 0.25% in distilled water containing 10% ethanol ; Prepare fresh and store in amber coloured bottle;

Standard iron solution:

Dissolve 0.28 gm of ferrous ammonium sulphate in 50ml of distilled water containing 1ml of sulphuric acid. Transfer this solution to a 1litre volumetric flask and make up the volume upto the mark by adding distilled water

Experimental procedure:

Calibration curve:

Pipette 10ml aliquot of standard iron solution into a beaker and test the pH with the help of an indicator paper. Add sodium citrate solution drop wise until a pH of about 3.5 is obtained. About 1.5ml of citrate solution will be needed which should be dispensed with the help of a burette or pipette. Now pipette a second aliquot of 10ml of iron solution in a 100ml volumetric flask. Add the same amount of sodium citrate solution drop wise, followed by 2ml hydroquinone solution and 3ml O-phenanthroline solution. This solution now contains 4ppm (4mg/ml) of iron. Prepare three other standard solutions (2.0, 0.8, 0.4 ppm/ml) by pipetting 5,2 and 1ml respectively of the standard iron solution into 100ml volumetric flasks. Add the appropriate amount of sodium citrate solution to each flask as described above. Allow the standard solution to stand for 10 minutes and measure the absorbance of each solution at 508 nm against a blank containing all the above mentioned reagents except the iron solutions. Construct a calibration curve by plotting absorbance versus ppm of iron.

Sample Determination:

The sample could be water from any source or a biological fluid. Take 75ml of the sample in a 250ml beaker, Add 25ml 6 N HCl and slowly boil for 15 minutes.Filter the boiled sample directly into a 100ml volumetric flask and make up the volume with distilled water. A 10ml aliquot of this filtrate is pipetted into a 100ml beaker and the pH is adjusted to 3.5 if needed by adding sodium citrate solution dropwise. A second aliquot of 10ml is pipetted into a 100ml volumetric flask and about 3.5ml of sodium citrates solution, 2ml hydroquinone and 3ml O-phenanthroline solution are added. The solution is diluted upto the mark with distilled water. After 10 minutes, the absorbance is read at 508nm. The concentration of iron is determined by interpolation of the sample absorbance value on the standard calibration curve. The calibration curve is linear in the absorbance range used.

4.2.5 Vitamin C Estimation (DCPIP method, Harris &Ray, 1935)

Reagents:

Oxalic acid 4%

Dye solution:

Weigh 42mg of sodium bicarbonate into a small volume of distilled water. Dissolve 52mg of 2, 6- dichlorophenol indophenol in it and make upto 200ml with distilled water.

Stock standard solution:

Dissolve 100mg ascorbic acid in 100ml of 4% oxalic acid solution in a standard flask (1mg/ml)

Working Standard:

Dilute 10ml of stock solution to 100ml with 4% oxalic acid. The concentration of the working standard is 100mg/ml.

Procedure:

Pipette out 5ml of the working standard solution into a 100ml conical flask. Add 10ml of 4% oxalic acid and titrate against the dye. (V_1ml) End point is the appearance of pink colour which persist for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.5 – 5gm depending on the sample) in 4% oxalic acid and titrate against the dye (V_2ml)

Calculation

$$\text{Amount of ascorbic acid (mg/100gsample)} = \frac{0.5\text{mg} * V2 * 100\text{ml} * 100}{(V_1\text{ml} * 5\text{ml} * \text{weight of the sample})}$$

4.2.6 Carbohydrate Estimation (Phenol- Sulphuric Method)

Materials required:

1. Phenol 5%- Redistilled phenol (50g) dissolved in water and diluted to 1 litre.
2. Sulphuric acid 96% reagent grade.
3. Standard glucose: Stock 100mg in 100ml of distilled water..
4. Working standard: 10ml of stock diluted to 100ml with distilled water.

Procedure:

Weigh 100mg of the sample into boiling tube. Hydrolyse by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N Hcl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100ml and centrifuge. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes.Pipette out 0.2 and 0.2ml of the sample solution in two separate test tubes .Make up the volume in each test tube 1ml with water.. Set a blank with 1ml of water.Add 1ml of phenol solution to each tube. .Add 5ml of 96% sulphuric acid to each tube and shake well. After 10 minutes shake the contents in the tubes and place in a water bath at 25-30 C for 20 minutes. Read the colour at490nm.Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

4.2.7 Moisture content:

The moisture content of the various materials used in this study were estimated following the method described by Johnson and Ulrich.

About 5g of freshly collected samples were placed in bottles and dried in a hot air oven for 12-16 hours. The weight of the samples were noted after 16 hours. The difference in weight was expressed as percentage of moisture on oven dry basis.

Calculation:

Weight of samples before drying in a hot air oven	=	A
Weight of samples after drying in a hot air oven	=	B
Loss in weight of samples:	=	A-B
Moisture content	=	(A-B) x 100/A

4.2.8 Determination of crude fibre (IS, 1990)

Reagents

Sulphuric acid – 0.255 N (1.25 % v/v), accurately prepared

Sodium hydroxide solution 0.313 N (1.25 % m/v), accurately prepared

Procedure

The dried material (2 g) was extracted for fat content with petroleum ether or hexane, using Soxhlet extracter (alternatively, the residue from the crude fat determination can be used). The fat free dry residue was transferred to 1 L conical flask. Boiling dilute sulphuric acid (200 ml) was added to the flask with the fat free material and immediately the flask was connected with a reflux condenser and heated for 30 min. The contents were filtered through fine linen held in a funnel. The residue was washed with boiling water.

The residue on the linen was washed into 200 ml of boiling sodium hydroxide solution.

Immediately the flask was connected with the reflux condenser and boiled for 30 min. The solution was filtered, washed with boiling water and transferred to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. The residue was washed first with hot water and then with 15 ml of 95% (by volume) ethyl alcohol. The Gooch crucible and the contents were dried at 105^0 C in the hot air oven to constant mass. It was cooled and weighed. The contents of the Gooch crucible were incinerated at 600^0

C in a muffle furnace until all the carbonaceous matter was burnt. The crucible containing the ash was cooled in a desiccator and weighed.

Calculation

Percentage of crude fat = 100 * (M1- M2) / M
(on moisture free basis)

Where,

M1=Mass in g of Gooch crucible and contents before ashing.

M2=Mass in g of Gooch crucible containing asbestos and ash.

M=Mass in g of the dried sample taken for the test.

Results and Discussion

5. RESULTS AND DISCUSSION:

5.1 CHOICE OF FOOD MATERIAL:

The following foods were chosen based on their high nutrient content and easy availability

S.No	SAMPLE	SCIENTIFIC NAME OF PLANT	PREDOMINANT NUTRIENT	NUTRIENT/100g (USDA values)
1	Soy	*Glycine max*	Protein	34.54g
2	Chickpea	*Cicer arientinum*	Phosphorus	168mg
3	Almond	*Prunus dulcis*	Calcium	264mg
4	Gooseberry	*Phylanthus emblica*	Vitamin C	600mg
5	Spinach	*Spinacea oleracea*	Iron	3.57mg
6	Pomegranate Seeds	*Punica granatum*	Potassium	259mg

5.1.1 Soy (*Glycine max*):

For human consumption, soybeans must be cooked with "wet" heat in order to destroy the trypsin inhibitors (serine protease inhibitors). Raw soybeans, including Edamame should not be eaten by humans, swine, chickens, in fact, all monogastric animals. Soybeans are considered by many agencies to be a source of complete protein. A complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body's inability to synthesize them. For this reason, soy is a good source of protein, amongst many others, for vegetarians and vegans or for people who want to reduce the amount of meat they eat.

Soy protein is essentially identical to that of other legume seeds. Moreover, soybeans can produce at least twice as much protein per acre than any other major vegetable or grain crop, 5 to 10 times more protein per acre than land set aside for grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production.

Consumption of soy may also reduce the risk of colon cancer, possibly due to the presence of sphingolipids.

5.1.2 Pomegranate seeds (*Punica granatum*):

Pomegranate seeds are loaded with numerous antioxidant properties that are supposedly three times higher than the green tea. As we know, antioxidants are very important to control the free radicals from the body that can cause several troubles to the body. Antioxidants also maintain and repair the damaged body cells. Pomegranate seeds are high in vitamin C, vitamin A, folic acid, vitamin E and have a lot of fibers along with potassium, iron and calcium.

Due to all these pomegranate seeds nutrition content, this 'magic fruit' is a bliss for the heart health, as regular intake of pomegranate seeds or pomegranate juice can evade heart diseases and reduce risk of strokes and heart attacks. Pomegranate seeds help in thinning the blood, reduce blood pressure levels, increase blood flow towards heart, maintain good cholesterol levels and reduce arterial plaques. Apart from these many benefits for heart, pomegranate seeds are also a blessing for cure of arthritis, osteoporosis, skin allergies, skin disorders, urinary tract infections, sore throats, tapeworms, digestive disorders, blood impurities, osteoarthritis and diabetes. Read on for health benefits of pomegranate juice.

5.1.3 Chick pea (*Cicer arientinum*):

Chickpeas are a helpful source of zinc folate and protein. They are also very high in dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes. Chickpeas are low in fat and most of this is polyunsaturated. Nutrient profile of desi chana (the smaller variety)is different , especialy the fibre content which is much higher than the light coloured variety. One hundred grams of mature boiled chickpeas contains 164 calories, 2.6 grams of fat (of which only 0.27 grams is saturated), 7.6 grams of dietary fiber and 8.9 grams of protein. Chickpeas also provide dietary calcium (49–53 mg/100 g), with some sources citing the garbanzo's calcium content as about the same as yogurt and close to milk.

5.1.4 Indian Gooseberry (*Phylanthus emblica*):

Gooseberry) has been found to be the most abundant source of Vitamin C in the plant kingdom, containing as much as 20 times that of an orange. What makes this even more extraordinary is that unlike many other natural sources, the vitamin C content in Amla (Gooseberry) or gooseberry does not diminish with cooking. The fresh fruit contains more than 80% of water besides protein, carbohydrates, fibre, minerals and vitamins Minerals and vitamins mainly include calcium, phosphorus, iron, carotene, Vitamin C and B complex. It also contains gallic acid, which is a potent polyphenol.

5.1.5 Spinach (*Spinacea oleracea*):

Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (and especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron, vitamin B2, calcium, potassium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids. Recently, opioid peptides called rubiscolinshave also been found in spinach. It is a source of folic acid (Vitamin B_9), and this vitamin was first purified from spinach. To benefit from the folate in spinach, it is better to steam it than to boil it. Boiling spinach for four minutes can halve the level of folate.

5.1.6 Almonds (*Prunus dulcis*):

Almonds are a unique package of nutrients – a good source of protein (6 grams per one ounce) along with dietary fibre, phosphorus, calcium, potassium, magnesium, manganese, copper, zinc, iron and vitamin E. In fact, one ounce of almonds provides about 7.4 grams of alpha-tocopherol vitamin E, 50 percent of the RDA. Almonds are the only good source of protein that is also an excellent source of vitamin E.

As a protein, almonds are rich in arginine and low in lysine. Research indicates that diets rich in arginine, low in lysine are thought to reduce the risk of coronary disease. Almonds are an ideal source of arginine in the absence of lysine, hence reducing the likelihood of competing amino acids. Also, eating a mixed diet that includes almonds and other protein sources can provide lysine in adequate and balanced quantities. Almonds are also unique in that they provide various minerals that are essential for bone health. Calcium, magnesium, manganese, and phosphorus have been implicated in maintaining bone mineral density

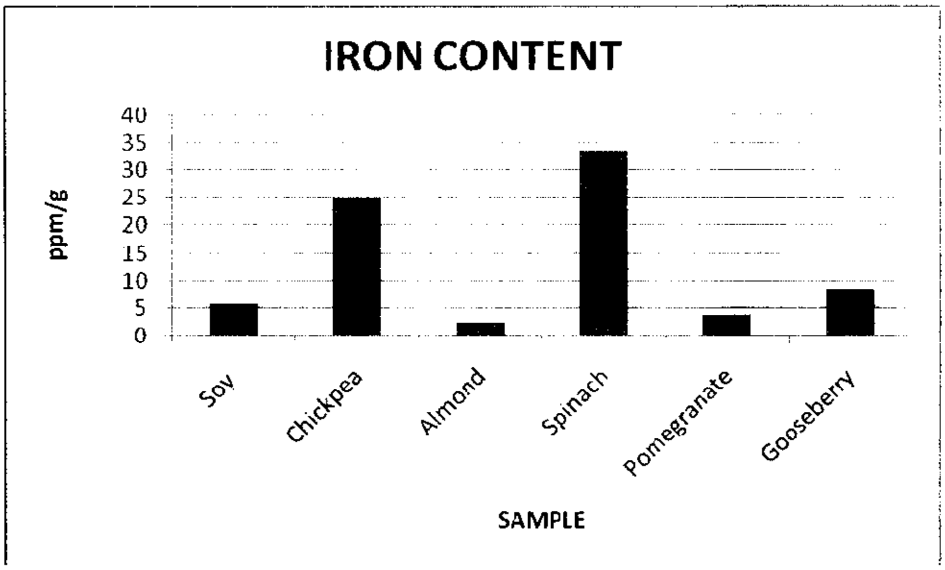
5.2 DETERMINATION OF NUTRIENT CONTENT OF RAW FOODS:

Table 5.2.1 Protein Content of the raw foods

S.No	SAMPLE	PROTEIN CONTENT (mg/g)
1	Soy	35.4
2	Chickpea	62.38
3	Almond	64.38
4	Gooseberry	46.2
5	Spinach	58.752
6	Pomegranate Seeds	25.08

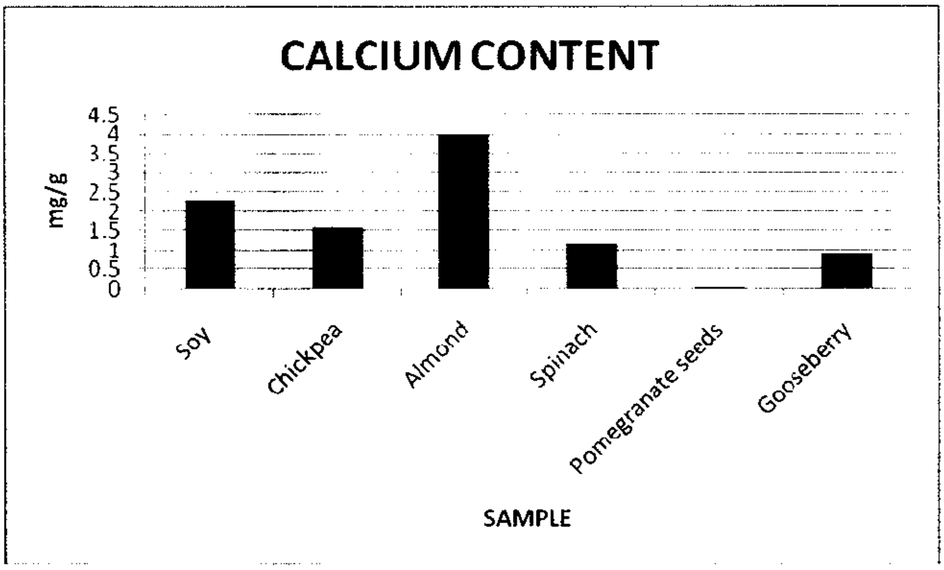
The Protein levels were estimated by the Folin lowry method and ranged between 25-65 g/100g. Maximal levels were found in soybean, Chickpea, almonds and spinach. Almonds are relatively costly and the cheapest source of protein for the formulated food is in the form of soybean and chickpea flour. Spinach on account of its light density should be added in large amounts for providing similar levels of protein.

Fig 5.2.1 Iron Content of raw foods:



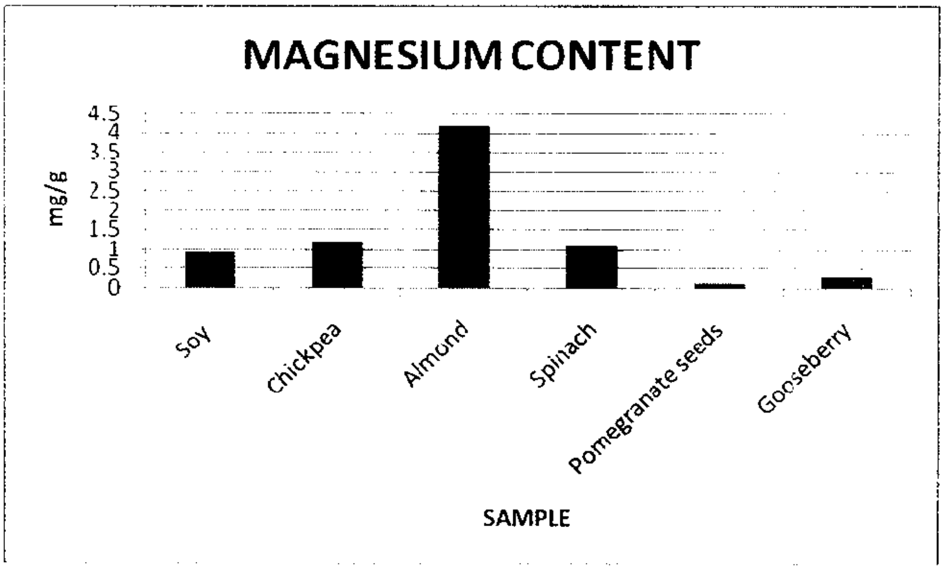
Rich source of total iron was spinach and Chickpea. It is well known that green leafy vegetables are a good source of dietary iron. Among the foods taken in the present study chick pea which has high iron and also high protein is a good candidate as an ingredient for formulated food. Least Iron content was in Almond and Pomegranate seeds.

Fig 5.2.2 Calcium Content of raw foods:



Calcium Content was found to be high in Almond while a very low value was noted in Pomegranate Seeds. Moderate amounts of calcium was also found in soy and chickpea flour. Calcium controls muscle contraction and relaxation, is responsible for nerve impulse transmission and the transfer of information between our brain cells. Calcium controls osmosis and diffusion through the cell membranes, and also the passing of information within the cell. Calcium controls the rhythm of the heart, the formation of enzymes and hormones, and also the DNA formation in chromosomes. Calcium is involved in blood clotting, urine filtration, and in the formation and maintenance of bones and teeth. And perhaps most importantly, Calcium is the main buffer used in the body to neutralize acids and maintain the proper pH.

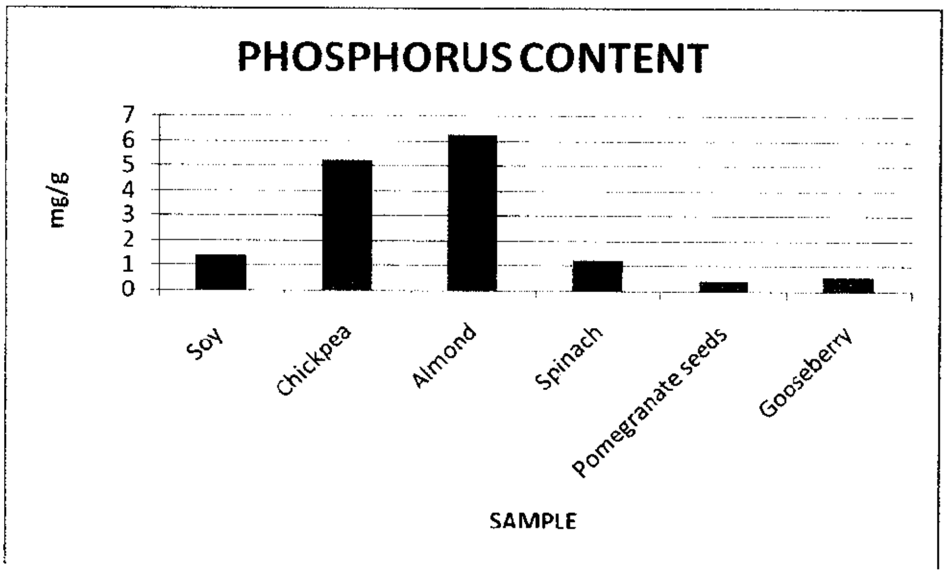
Fig 5.2.3 Magnesium Content:



Magnesium is an important cofactor for several enzymes, therefore availability of which is important. Magnesium levels were maximal in the case of almond while minute quantities were noted in Gooseberry and Pomegranate seeds. The magnesium levels closely mirrored the calcium levels.

Magnesium occurs typically as the Mg2+ ion. For example, ATP (adenosine triphosphate), the main source of energy in cells, must be bound to a magnesium ion in order to be biologically active (Mg-ATP). Similarly, magnesium plays a role in the stability of all polyphosphate compounds in the cells, including those associated with DNA and RNA synthesis.

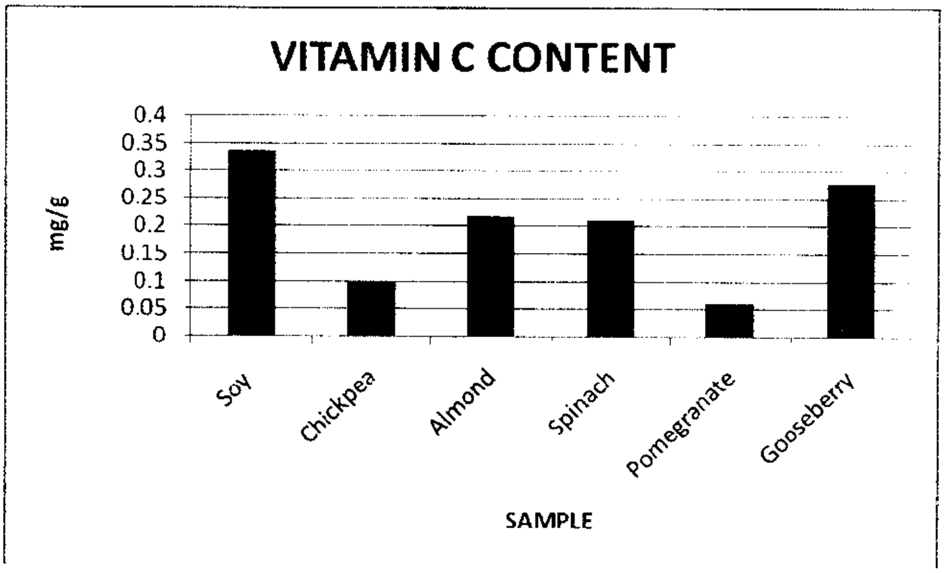
Fig 5.2.4 Phosphorus Coentent:



Highest Phosphorus content was noted in Almond and Chickpea .Least Phosphorus content was noted in Gooseberry and Pomegranate Seeds.

Phosphorus is a key element in all known forms of life. Inorganic phosphorus in the form of the phosphate PO_4^{3-} plays a major role in biological molecules such as DNA and RNA where it forms part of the structural framework of these molecules. Living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy obtains it in the form of ATP. ATP is also important for phosphorylation, a key regulatory event in cells. Phospholipids are the main structural components of all cellular membranes. Calcium phosphate salts assist in stiffening bones.

Fig 5.2.5 Vitamin C Content:



As expected Vitamin C content was found to be high in gooseberry and soy snd lower levels were noted in the case of chickpea and pomegranate seeds.Vitamin C is an important antioxidant and upto 500mg/ day is recommended for humans. Vitamin C has radical scavenging activity .

Table 5.2.2 Moisture content:

SAMPLE	MOISTURE CONTENT (g/gm)
Soy	0.39
Chickpea	0.48
Almond	0.17
Pomegranate seeds	0.59
Gooseberry	0.5
Spinach	0.63

Gooseberry, Spinach and Pomegranate Seeds intrinsically contained moisture predominantly . However, Presence of moisture leads to microbial contamination and therefore, the above samples were dried for use in formulation. The dried samples had similar moisture content in the range of 0.3- 0.6 g/g. Almond powder had the least moisture content.

5.3 FORMULATION OF HIGH NUTRIENT CONTENT SUPPLEMENTARY FOOD:

Table 5.3.1 Formulation of supplementary food

S.No	SAMPLE	FORMULATED CONTENT(%)
1	Soy	25
2	Chickpea	30
3	Almond	20
4	Gooseberry	4
5	Spinach	8
6	Pomegranate Seeds	1
7	Sugar	12

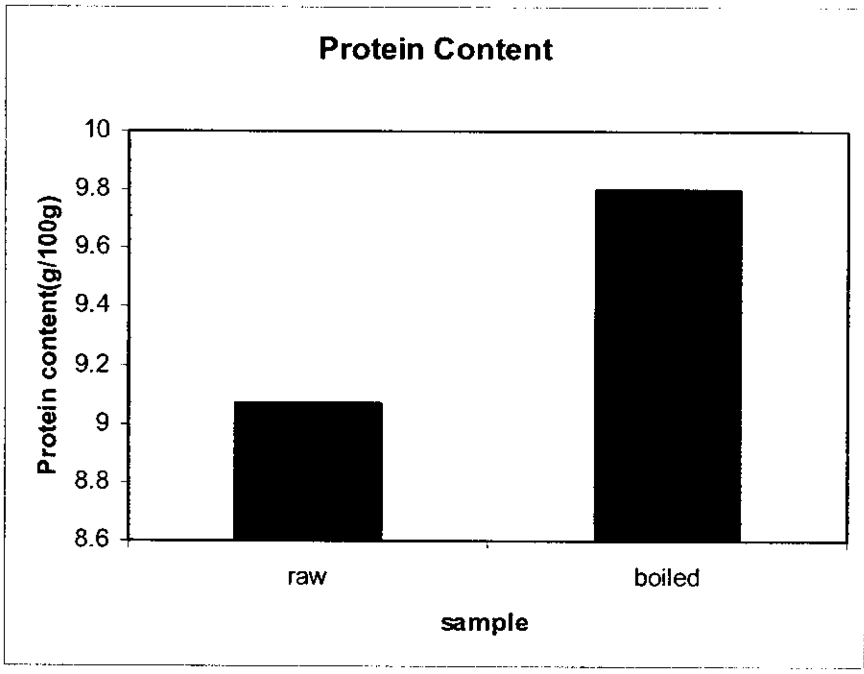
Based on the nutrient content estimated for the raw foods a nutritious food mixture was formulated. Considerations for formulations in order of priority are as follows:

1. High Nutrient content
2. Availability in dry/low moisture form
3. Relative ease of availability
4. Ease in processing and/or processing cost
5. Low cost

Based on these considerations maximal quantity of food in the mixture was prepared from chick pea (30%), soy flour (25%) and almond powder (20%). Goosberry and spinach are moisture rich foods and processing them to remove moisture is time and labour intensive. Therefore goosberry and spinach were added to the mixture at 4% and 8% respectively. Pomeogreanate seeds were both time and cost intensive to prepare and were added to the mixture a very low extent.

5.4 EFFECTS OF BOILING ON NUTRIENT CONTENT OF FORMULATED SUPPLEMEMNTARY FOOD:

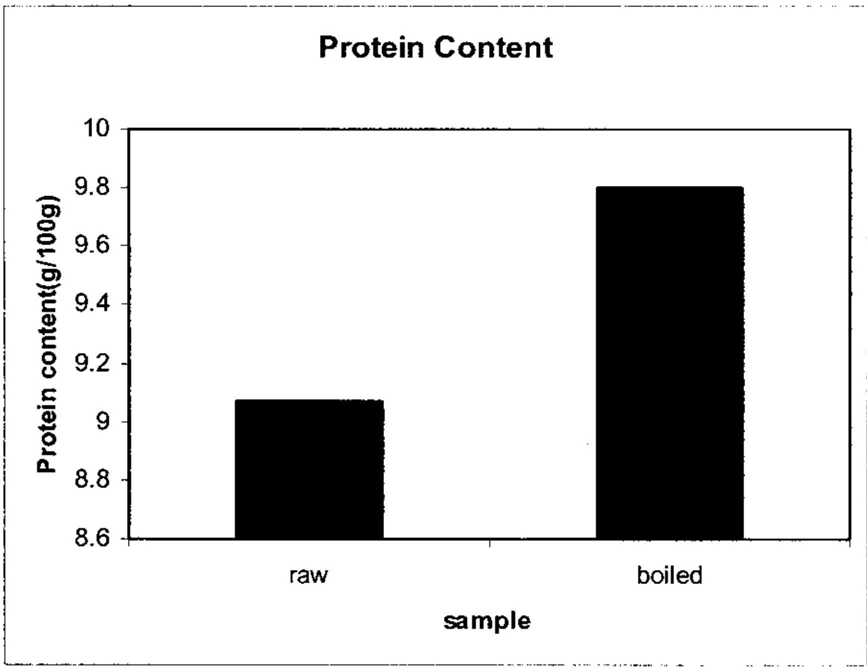
Fig 5.4.1 Effects of boiling on carbohydrate content of formulated food.



Cooking improves digestibility. However nutrient losses are inevitable consequences to study the loss of various nutrient on cooking, the formulated raw and boiled form.

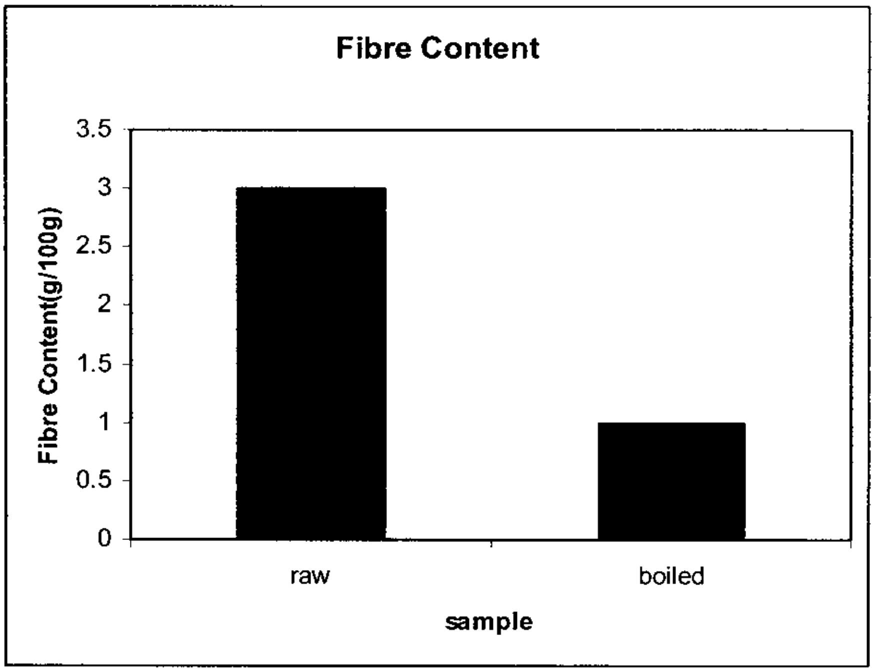
Phenol-sulphuric method was used to determine the carbohydrate content of the formulated supplementary food in its raw and boiled form.

Fig 5.4.2 Effects of boiling on protein content of formulated food



Protein estimation was carried out by Folin Lowry's method for raw and boiled sample of the formulated supplementary food. It was found that the boiled sample had more protein content when compared to the raw sample. It is possible that protein is inaccessible in the raw form and is made accessible during boiling process.

Fig 5.4.3 Effects of boiling on fibre content of formulated food



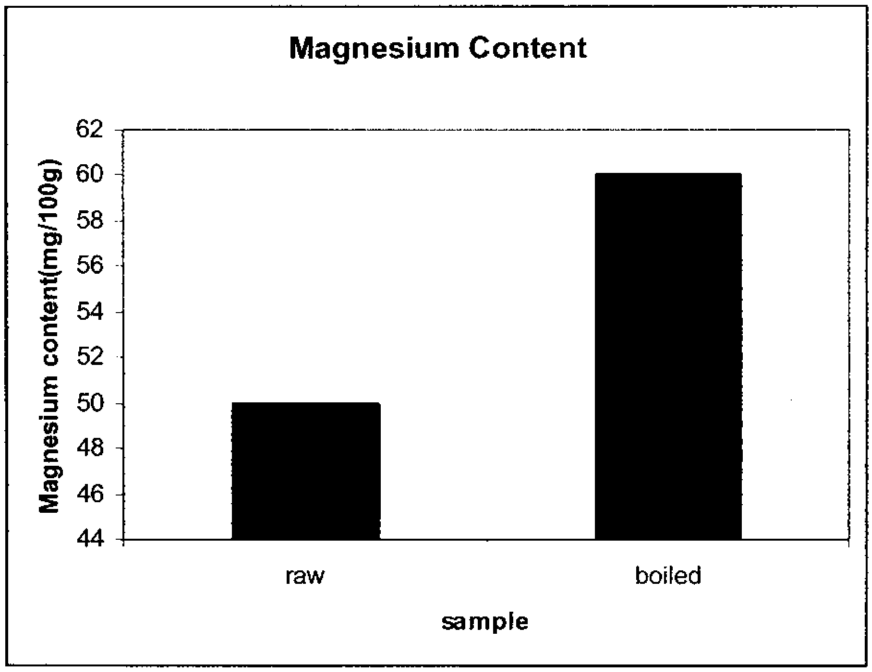
Fibre is recognised as an important dietary constituent . Fibre improves digestive function .Fibre is of two types Soluble and Insoluble . It is found that the fibre content of the boiled samples reduces by upto 65%. Possibly due to loss of the soluble fibre during boiling

Table5.4.1 Effects of boiling on calcium content of formulated food

	Sample	Calcium Content(mg/100g)
1.	Raw Sample	140
2.	Boiled Sample	123

Calcium content for the raw and boiled samples of the formulated supplementary food was measured using the EDTA Titration method. The results showed that the boiled sample lost about 10% of the calcium content when compared to the raw sample and compared to other nutrients this loss is minimal.

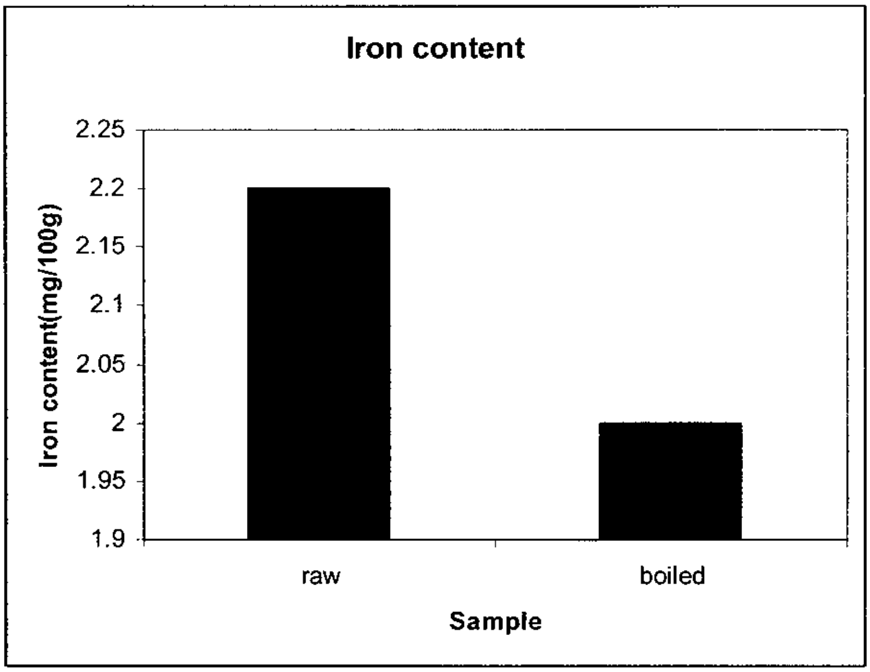
Fig 5.4.4 Effects of boiling on Magnesium content of formulated food



Magnesium content of the raw and boiled sample of the formulated supplementary food was carried out using the EDTA Titration method.

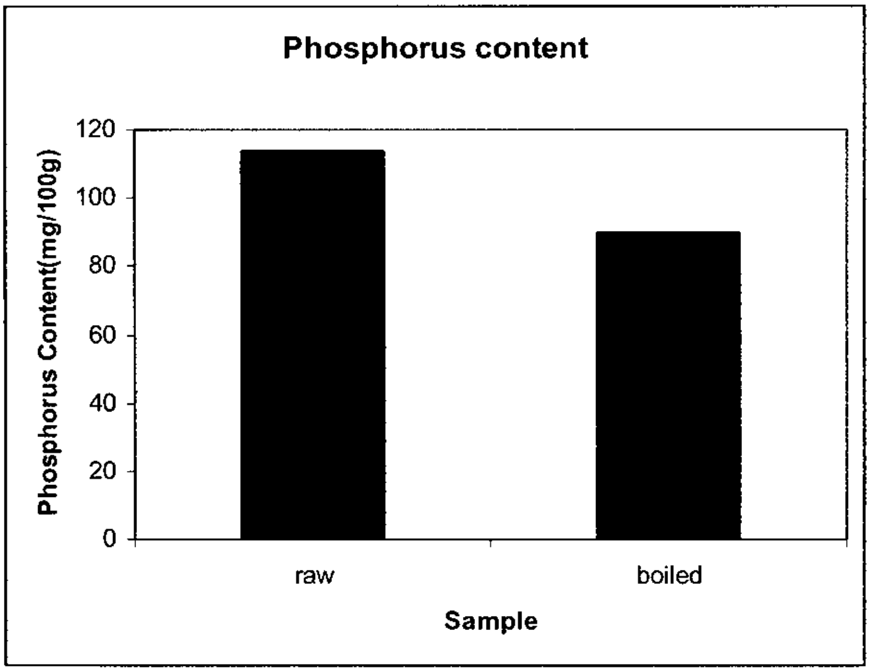
It was determined from the results that the boiled sample contained the maximum amount of magnesium followed by raw sample. Similar to the trend observed for protein the boiled supplemented food contains a higher magnesium content compared to the raw form. This could be due to the release of magnesium upon boiling.

Fig 5.4.5 Effects of boiling on Iron content of formulated food



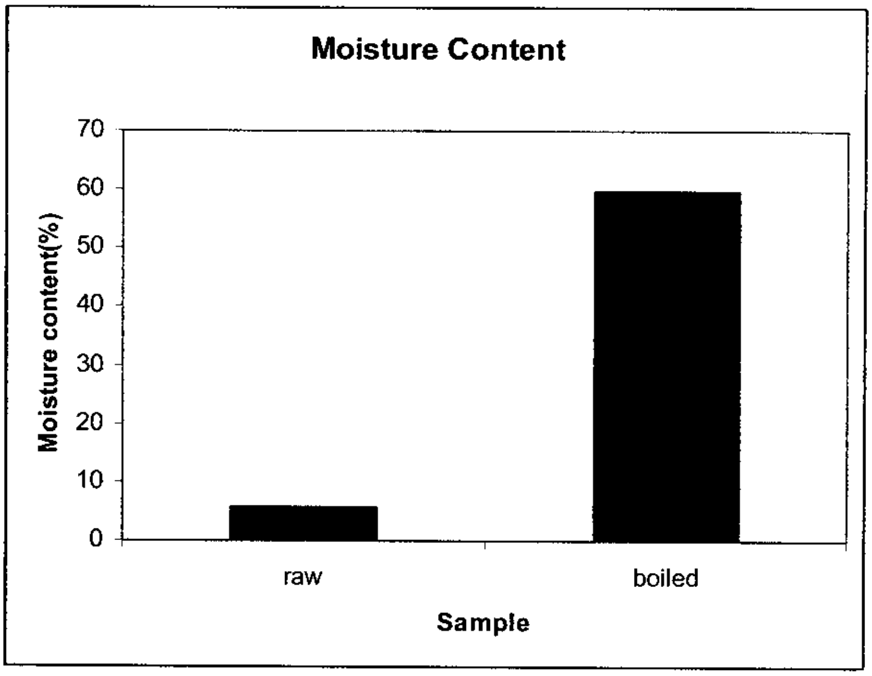
Iron estimation was carried out by Phenanthroline method for raw, boiled samples of the formulated supplementary food. Boiling resulted in iron losses by more than 50%. Iron exists as ionic and protein bound forms. The lost iron maybe the ionic iron. Further studies are necessary to establish the nature of the lost and retained iron.

Fig 5.4.6 Effects of boiling on Phosphorus content of formulated food



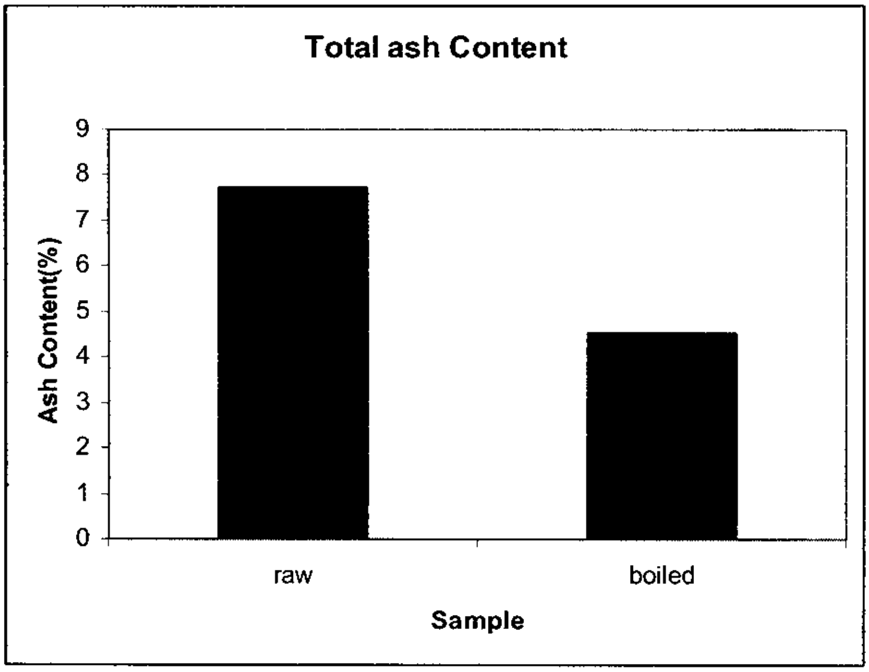
Phosphorous content was determined by the Vanado-molybdate method for raw and boiled samples and the following results were obtained.Phosphorus was more or less retained upon boiling the supplemented food. So loss of phosphorus is not a major concern in formulation of supplemented food.

Fig 5.4.7 Effects of boiling on Moisture content of formulated food



As expected the boiled sample retained 10 times the moisture content compared to the raw.

Fig 5.4.8 Effects of boiling on Total Ash content of formulated food



Total Ash reduced by about 40% upon boiling , the reasons for which are not clear. It is probable that some sizeable amount of nutrients and non- nutrients is lost and this is responsible for the lowered ash content.

5.5 EFFECT OF MICROWAVE HEATING ON NUTRIENT RETENTION:

Table 5.5.1 Effect of Microwave heating on Nutrient Retention

NUTRIENTS	RAW (/100g)	MICROWAVED (/100g)	RESULTANT CHANGE wrt RAW
Protein	9.07g	9.66g	Negligible
Carbohydrate	67.15g	68.88g	Negligible
Calcium	140mg	111mg	Decreased
Iron	2.20mg	1.80mg	Negligible
Magnesium	50mg	59.4mg	Negligible
Phosphorus	114mg	90.14mg	Decreased
Fibre	3g	1.20g	Decreased
Ash	7.72%	8.39%	Negligible
Moisture	5.68%	2.40%	Decreased

Cooking by Microwaving retained most nutrients. However, minimal losses of calcium and phosphorus were observed . Microwaving without addition of water appears to be a good method of cooking since it retains almost all nutrients. The moisture content in the food material itself aids in the cooking process.

Conclusion

6. CONCLUSION

- Maximal levels were found in soybean, Chickpea, almonds and spinach. Almonds are relatively costly and the cheapest source of protein for the formulated food is in the form of soybean and chickpea flour.

- Rich source of total iron was spinach and Chickpea. It is well known that green leafy vegetables are a good source of dietary iron

- Calcium Content was found to be high in Almond while a very low value was noted in Pomegranate Seeds. . Calcium controls the rhythm of the heart, the formation of enzymes and hormones, and also the DNA formation in chromosomes.

- Magnesium levels were maximal in the case of almond while minute quantities were noted in Gooseberry and Pomegranate seeds. The magnesium levels closely mirrored the calcium levels

- Highest phosphorous content was noted in Chick Pea and almond. Least phosphorous content was noted in pomegranate seeds and gooseberry.

- As expected Vitamin C content was found to be high in gooseberry and soy snd lower levels were noted in the case of chickpea and pomegranate seeds

- Gooseberry, Spinach and Pomegranate Seeds intrinsically contained moisture predominantly . However, Presence of moisture leads to microbial contamination and therefore, the above samples were dried for use in formulation.

Formulated supplementary food.

- It was found that the boiled sample had more protein content when compared to the raw sample.It is possible that protein is inaccessible in the raw form and made
- accessible during boiling process.

- Fibre is recognised as an important dietary constituent It was found that the fibre content of the boiled samples reduces by upto 65%.

- It was determined from the results that the boiled sample contained the maximum amount of magnesium followed by raw sample. Similar to the trend observed for protein the boiled supplemented food contains a higher magnesium content compared to the raw form. This could be due to the release of magnesium upon boiling.

- The results showed that the boiled sample lost about 10% of the calcium content when compared to the raw sample and compared to other nutrients this loss is minimal.

- From the results that the boiled sample contained the maximum amount of magnesium followed by raw sample. Similar to the trend observed for protein the boiled supplemented food contains a higher magnesium content compared to the raw form.this could be due to the release of magnesium upon boiling.]

- Boiling resulted in iron losses by more than 50%. Iron exists as ionic and protein bound forms. The lost iron maybe the ionic iron.

- Phosphorus was more or less retained upon boiling the supplemented food.

- Total Ash reduced by about 40% upon boiling , the reasons for which are not clear. It is probable that some sizeable amount of nutrients and non- nutrients is lost and this is responsible for the lowered ash content

- Cooking by Microwaving retained most nutrients. However, minimal losses of calcium and phosphorus were observed . Microwaving without addition of water appears to be a good method of cooking since it retains almost all nutrients. The moisture content in the food material itself aids in the cooking process

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