

Project report
on
**STUDIES TO ASSESS THE QUALITY OF FOOD
SAMPLES**

Submitted in Partial Fulfilment of the Requirement for the Degree of
M.Sc. Biochemistry

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May 2020



SCHOOL OF BASIC AND APPLIED SCIENCES

CERTIFICATE

This is to certify that Ms. **Shahalam Khursheed** has carried out her project work entitled **“Studies to Assess the Quality of Food Samples”** under my supervision. This work is fit for submission for the award of Master Degree in Biochemistry

A handwritten signature in blue ink, appearing to read 'S. Mythily S.', is written over a light yellow horizontal line.

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Dean, SBAS

DECLARATION

I hereby declare that the dissertation entitled “**Studies to Assess The Quality of Food Samples**” submitted by me in partial fulfillment for the degree of M.Sc. in Biochemistry to the Division of Biochemistry, School of Basic and Applied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India is my original work. It has not been submitted in part or full to this University or any other Universities for the award of diploma or degree.



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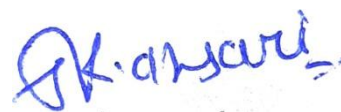
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Signature

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ABSTRACT

The diet is selection of food eaten by an individual. A balanced diet is essential for health. It provides the appropriate amount of all nutrients in the correct proportions to meet body requirements. A nutrient in any substances that is digested, absorbed and used to promote body function. And these substance such as (carbohydrates, protein, fats, vitamins, minerals, water).

Quantitative assessment of nutritive value in food consumed by an individual is measured by fatty acid, metals, minerals, proteins, nitrogen and vitamins, moisture contents. And this food quality parameter measures by the various manual and instrumental techniques and my work is demonstrated on the instrumental techniques such as Soxhlet Apparatus for estimation of Fat percentage, High Performance Liquid Chromatography (HPLC) for Vitamin concentration detection, ICP-MS (Inductively Coupled Plasma Mass Spectrometry) and AAS (Atomic Absorption Spectroscopy) for Multi-elemental or minerals analysis, Kjeldahl method for Protein and Nitrogen analysis, Bomb Calorimeter for estimation of Energy Expenditure and Gas chromatography Mass Spectroscopy for Fatty acid analysis.

For estimation of balanced diet not only food but plasma and urine like biological samples also tested for vitamins, metals and minerals present in it which will help to analyse an individual body requirements. Body mass index is another factor that also effect the calorific need of an individual diet.

INTRODUCTION

A balanced diet is one that gives your body the nutrients it needs to function correctly. A diet is all that we consume in a day. And a balanced diet is a diet that contains an adequate quantity of the nutrients that we require in a day. (Lupton et al., 2002)

Nutrients that are broadly classified as macronutrients, micronutrients, and essential nutrients. Macronutrients include carbohydrates, proteins, and lipids; whereas micronutrients include minerals (e.g., sodium and chloride), trace minerals (e.g., iodine and copper), vitamins (e.g., carotenoids and sterols), and organic acids (e.g., citric acid). Essential nutrients include some amino acids (e.g., leucine and valine) and fatty acids (e.g., polyunsaturated fatty acids [PUFAs]) and are defined as those that cannot be synthesized, or are inadequately synthesized *de novo* by animals to meet their physiological demands. (Taşğın E. 2017)

1) Macronutrients- There are three macronutrients: protein, fats and carbohydrates. Macronutrients provide calories, or energy. The body requires large amounts of macronutrients to sustain life.

- a) **Carbohydrates** - Carbohydrates are subdivided into several categories on the basis of the number of sugar units and how the sugar units are chemically bonded to each other. Including sugars, starches, and fibers. Sugars are intrinsic in fruits and milk products. Sugars are also used in food preservation and for functional properties such as viscosity, texture, body, and browning capacity. Vegetables, fruits, whole grains, milk, and milk products are the major food sources of carbohydrates. Grains and certain vegetables including corn and potatoes are rich in starch. Starches, the major plant-energy-reserve polysaccharides used by humans, are stored in plants in the form of nearly spherical granules.
- b) **Protein-** Proteins is essential for optimal growth, development, and health of children, as well as for optimal maintenance, function and health of tissues (including skeletal muscle, brain, and heart, kidneys, liver and gut) in adults.it is also essential to building muscle mass. A protein usually contains various amounts of 20 different amino acids (AA) linked *via* peptide bonds. Each gram of protein contains 4 calories. Protein makes up about 15 percent of a person's body weight.
- c) **Fat** - Fat is essential for several bodily functions. It is an energy source, and it protects the skeleton and nerves. Fats are substances that help the body use some vitamins and keep

the skin healthy; they are also the main way the body stores energy. In food, there are many types of fats -- saturated, unsaturated, polyunsaturated, monounsaturated, and Trans fats. Saturated and Trans fats can raise cholesterol levels and increase disease risk. Unsaturated fats support health and may be monounsaturated or polyunsaturated. Meats, dairy products, snack foods, and baked goods contain saturated and Trans fats.

2) **Micronutrients-** The term micronutrients is used to describe vitamins and minerals in general. Our body needs smaller amounts of micronutrients. Depending on their function, certain micronutrients also play a role in preventing and fighting disease.

a) **Vitamins and Minerals-** Vitamins and minerals are essential nutrients because they perform hundreds of roles in the body. Vitamins and minerals can be divided into four categories: water-soluble vitamins, fat-soluble vitamins, macro minerals and trace minerals. Vitamins and minerals are absorbed in similar ways in your body and interact in many processes. Vitamins are not stored in our body

b) **Trace elements-** Trace elements are minerals present in living tissues in small amounts. Trace elements function primarily as catalysts in enzyme systems; some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen. (underwood E. 2012)

What nutrients does to the brave soldier of our Nation?

In addition to exercise, proper nutrition plays a major role in maintaining total fitness of our brave soldiers. Eating a variety of foods and maintaining adequate energy balance are basic guidelines for a healthy diet. For Soldiers to get enough energy from the food they eat and to obtain the variety of foods needed for nutritional balance, they eat three meals a day. Without a regular supply of food and water, no army can hope, or expect to successfully prevail in its principal role: war fighting. (Hill N et. al, 2019). Nutrition is a major contributor to the wound healing process in those who are injured, as well as influencing their subsequent recovery and rehabilitation. Inadequate nutrition can result in poor physical and cognitive performance (e.g. inability to carry out physical tasks, poor concentration and decreased vigilance). The long-term effects of both macro- and micro-nutrient imbalances include increased risk of vitamin and mineral deficiencies (an increased risk of stress fractures and rickets), obesity, hypertension, coronary heart disease, diabetes, osteoporosis and kidney failure. The implications of having a

poorly nourished force may result in an increased risk of ill health reduced manning levels owing to absenteeism and ultimately a reduced state of operational readiness. (Ahmed M et. al, 2019)

Table.1 Types of macro and micro nutrients and their function and major source

NUTRIENT	FUNCTION	MAJOR SOURCES
Proteins (4 calories/gram)	Form important parts of muscles, bone, blood, enzymes, some hormones, and cell membranes; repair tissue; regulate water and acid-base balance; help in growth; supply energy	Meat, fish, poultry, eggs, milk, legumes, nuts
Carbohydrates (4 calories/gram)	Supply energy to cells in brain, nervous system, and blood; supply energy to muscles during exercise	Grains (breads and cereals), fruits, vegetables, milk
Fats (9 calories/gram)	Supply energy; insulate, support, and cushion organs: provide medium for absorption of fat-soluble vitamins	Animal foods, grains, nuts, seeds, fish, vegetables
Vitamins	Promote (initiate or speed up) specific chemical reactions within cells	Abundant in fruits, vegetables, and grains; also found in meat and dairy products
Minerals	Help regulate body functions; aid in growth and maintenance of body tissues; act as catalysts for release of energy	Found in most food groups
Water	Makes up 50-60% of body weight; provides medium for chemical reactions; transports chemicals; regulates temperature; removes waste products	Fruits, vegetables, liquids

AIM AND OBJECTIVES

Aim-

Macro and micro nutrient analysis by various techniques

Objective-

- To estimate the calorific value of the food sample.
- To estimate Vitamins by High Performance Liquid chromatography
- To quantitative fat content by Soxhlet Apparatus.
- To estimate minerals by AAS/ICP-MS.

REVIEW OF LITERATURE

A balanced and varied diet is the best source of essential vitamins and minerals; however, nutrient deficiencies occur, including in populations with bountiful food supplies and the means to procure nutrient-rich foods. Deficiencies in one or more micronutrients may lead to serious health issues. A common reason people take multivitamin and mineral (MVM) supplements is to maintain or improve health. Micronutrients are required for nearly all metabolic and developmental bodily processes. Some people are at risk of micronutrient deficiencies due to excessive losses (e.g., through hemodialysis), abnormal metabolism (e.g., genetic polymorphisms, alcoholism, conditions that impair fat absorption), and/or inadequate synthesis (e.g., insufficient sunlight exposure to allow vitamin D synthesis). (Coates PM et. al, 2010)

Vitamins (e.g., vitamin A, B, C, D, and E) are organic compounds that are essential to maintaining health. Minerals, on the other hand, are inorganic substances that humans need to maintain their health (e.g., calcium, iron, zinc). Vitamins and minerals are considered essential nutrients—because acting in concert, they perform hundreds of roles in the body. They help shore up bones, heal wounds, and bolster your immune system. They also convert food into energy, and repair cellular damage. Vitamins and minerals are often called micronutrients because your body needs only tiny amounts of them. (Steenbock H, Black A. 1924)

Mineral salts are responsible for structural functions involving the skeleton and soft tissues and for regulatory functions including neuromuscular transmission, blood clotting, oxygen transport, and enzymatic activity. Calcium, phosphorus, and magnesium are required in relatively large amounts and are designated as macro minerals. (Bailey RL et. al, 2011)

Vitamins and minerals can be divided into four categories: water-soluble vitamins, fat-soluble vitamins, macro minerals and trace minerals.

- 1) **Water soluble vitamins-** These vitamins are soluble in water and are not easily stored in our body and get flushed out with urine when consumed in excess.

Table.2 Water soluble vitamins and their source and functions

S.NO.	Water soluble vitamins	Source	Function	RDA or AI (adults > 19 years)
1.	Vitamin B1(thiamine)	Whole grain, meat, fish	Helps convert nutrients into energy	1.1-1.2mg
2.	Vitamin B2 (riboflavin) (Lippincott Williams & Wilkins; 2014)	Organ meat, eggs, milk	Necessary for energy production, cell function and fat metabolism	1.1-1.3mg
3.	Vitamin B3(niacin)	Meats, salmon, leafy greens, beans	Drives the production of energy from food	14-16mg
4.	Vitamin B5(pantothenic acid)	Organ meats, mushrooms, tuna, avocado	Necessary for fatty acid synthesis	5mg
5.	Vitamin B6(pyridoxine)	Fish, milk, carrot, potatoes	Helps your body release sugar from stored carbohydrates for energy and create red blood cells	1.3mg
6.	Vitamin B7(biotin)	Eggs, almonds, spinach, sweet potatoes	Plays a role in the metabolism of fatty acids, amino acids and glucose	30mcg
7.	Vitamin B9(folate)	Beef, liver, black eyes peas, spinach, asparagus	Important for proper cell division	400mg
8.	Vitamin B12(cobalamin)	Clams, fish, meat	Necessary for red blood cell formation and proper nervous system and brain function	2.4mcg
9.	Vitamin C(ascorbic acid)	Citrus fruits, ball peppers, Brussels sprouts	Required for the creation of neurotransmitters and collagen, the main protein in your skin	75-90mg

- 2) **Fat soluble vitamins**-These vitamins are dissolve in fat. Fat-soluble vitamins are stored in liver and fatty tissues for future use.

Table.3 Fat soluble vitamins and their source and function.

S.NO.	Fat soluble vitamins	Source	Function	RDA or AI(adults > 19 years)	References
1.	Vitamin A	Retinol (liver, dairy, fish), carotenoids (sweet potatoes, carrots, spinach)	proper vision and organ function	700-900 mcg	Nutr.J 2008
2.	Vitamin D	Sunlight, fish oil, milk	Promotes immune function and assists in calcium absorption and bone growth	600-800 IU	Food data central department of agricultural 2019
3.	Vitamin E	Sunflower seeds, wheat germ, almond	Assists immune function and acts as an antioxidant that protects cells from damage	15mg	Food data central department of agricultural 2019
4.	Vitamin K	Leafy green, soybeans, pumpkin	blood clotting and proper bone development	90-120mcg	Food data central department of agricultural 2019

3) **Macro minerals**- Macro minerals are needed in larger amounts to body.

Table.4 Macronutrients and their source and functions.

S.NO.	Macro minerals	Source	Functions	RDA or AI (adults > 19 years)	References
1.	Calcium	Milk product, leafy green, broccoli	Proper structure and function of bones and teeth. Assists in muscle function and blood vessel contraction	2000-2500mg	Knochel JP.et, al 2016
2.	Phosphorus	Salmon, yoghurt,	Part of bone and cell membrane structure	700mg	Knochel JP.et, al 2016
3.	Magnesium	Almond, cashew, black beans	Assists enzyme reactions, and regulation of blood pressure	310-420mg	Food data central department of agriculture 2019
4.	Sodium	Canned soup, salt	Electrolyte that aids fluid balance and maintenance of blood pressure	2300mg	Food data central department of agriculture 2019
5.	Chloride	Seaweed, salt,	Combination with sodium. Helps maintain fluid balance and is used to make digestive juices	1800-2300mg	Food data central department of agriculture 2019
6.	Potassium	Lentils, squash, bananas	Electrolyte that maintains fluid status in and helps with nerve transmission and muscle function	4700mg	Food data central department of agriculture 2019
7.	Sulfur	Garlic, onion, mineral water, eggs	Found in living tissue and contained in the amino acids methionine and cysteine	None established	Food data central department of agriculture 2019

Trace elements- Trace elements are minerals present in living tissues in small amounts. All trace elements are toxic if consumed at sufficiently high levels for long enough periods. It act as a catalyst in enzyme system.

Table.5 various trace elements and their source and functions.

S.NO.	Trace element	Sources	Function	RDA or AI (adults > 19 years)
1.	Iron	Oysters, white beans, spinach	provide oxygen to muscles and assists in the creation of certain hormones	8-18mg
2.	Manganese	Pineapple, pecans, peanuts	Assists in carbohydrate, amino acid and cholesterol metabolism	1.8-2.3mg
3.	Copper	Liver, crabs, cashew	Connective tissue formation, brain and nervous system function	900mcg
4.	Zink	Oysters, chickpeas, crabs	Growth, immune function and wound healing	8-11mg
5.	Iodine	Seaweed, cod, yogurt	Assists in thyroid regulation	150mcg
6.	Fluoride	Fruit juice, water, crab	Development of bones and teeth	3-4mg
7.	Selenium	Brazil nuts, sardines, ham	Thyroid health, reproduction and defense against oxidative damage	55mcg

(Reference - Institute of Medicine. Food and Nutrition Board 2001)

METHODOLOGY

The following methods and equipment were used to carry out the analysis.

1. Halogen Moisture Analyzer for determination of moisture content in food using
2. Soxhlet Extraction Methods for fat extraction from food sample
3. Bomb calorimeter for quantification of calorific value of food samples
4. Muffle Furnace for estimation of Ash Content in food samples
5. High Performance Liquid Chromatography (HPLC) for the estimation of fat soluble vitamins in blood plasma samples
6. Inductively Couple Plasma - Mass Spectroscopy (ICPMS) for Metals and Minerals Analysis

1) Halogen Moisture Analyzer for determination of moisture content in food using

- Moisture analyzer work on the principle of thermo gravimetric and used moisture test for food in laboratories.
- In this a sample is weight first and then heated to a point where it releases its moisture completely.



Fig.1 Moisture Analyzer unit with food sample.

2) Soxhlet Extraction Methods for fat extraction from food sample

- Prepare thimbles and weight them.
- Rinse all the glass apparatus by petroleum ether and dry it in the oven at 102°C and after removing it keep in the desiccator.
- Weigh 3 gram of grounded and dried sample and place it in the thimble.
- Place the thimble in the soxhlet extractor.
- Take a 150ml round bottom flask and clean it and fill the flask with 90 ml petroleum ether.
- Place the whole setting on a heating mantle and allow the petroleum ether to boil.
- Leave for 24 hrs for estimation fat content.

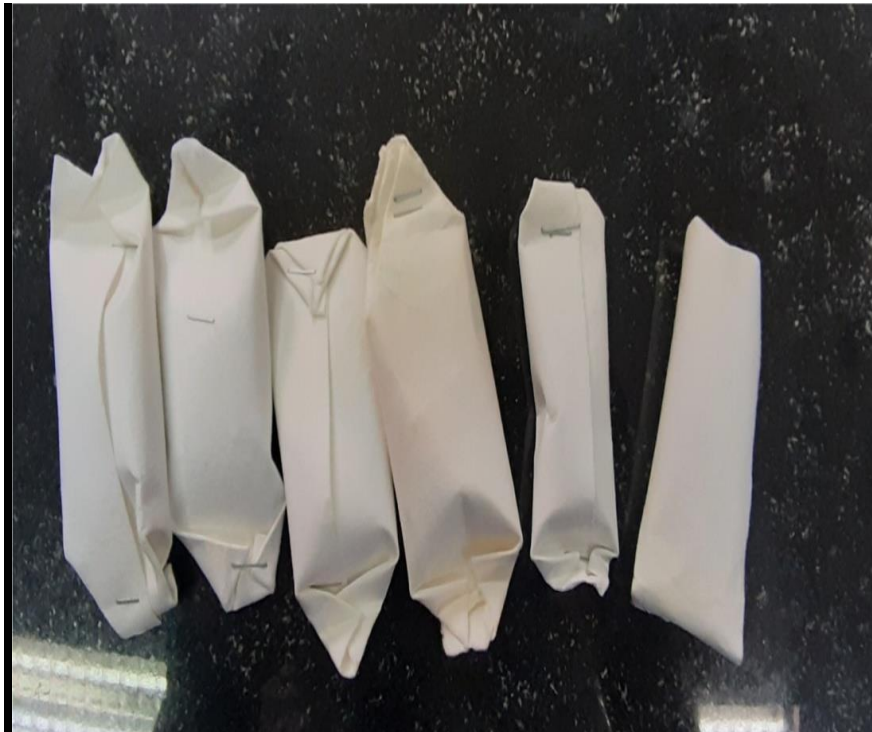


Fig.2 Extraction thimbles for soxhlet



Fig.3 soxhlet extraction apparatus.

3) Bomb calorimeter for quantification of calorific value of food samples

- Weight 1g of standard sample in crucible
- Put 5ml of milli Q water in bomb
- Put the crucible on cap
- Place crucible into bomb and fix it
- Fill the oxygen in bomb after capping the bomb



Fig.4 Bomb calorimeter unit.

4) Muffle Furnace for estimation of Ash Content in food samples

Principle- A muffle furnace is a furnace with an extremely heated chamber, the wall of which radiantly heat the contents of the chamber, so that the material being heated has no contact the flame.

Furnace consists of-

- A vented heating chamber
- A temperature controller
- A door safety switch for operator safety



Fig.5 Muffle furnace unit.



Fig. 6 crucibles containing various types of food samples

5) High Performance Liquid Chromatography (HPLC) for the estimation of fat soluble vitamins in blood plasma samples

Method- sample preparation for HPLC

- Take 400 μ l plasma from plasma sample. Then centrifuge in 15ml centrifuge tube.
- Add 400 μ l cold ethanol and vortex for 8 min for proper dissolution.
- Add 1ml n-Hexane than vortex for 5 min each sample for proper separation.
- And centrifuge for 10 min at 1000rpm
- And collect supernatant in amber color eppendrof.
- Again add 1ml Hexane and vortex for 5 min.
- Again centrifuge for 10 min at 1000rpm.
- And 2nd time collect supernatant in amber color eppendrof.
- Than speed vac for 2 hrs. For dry the sample.
- Dried sample reconstructed to add the 200 μ l ethanol.
- Transfer it in the insertion tube and place in the HPLC chamber for vitamins examine.

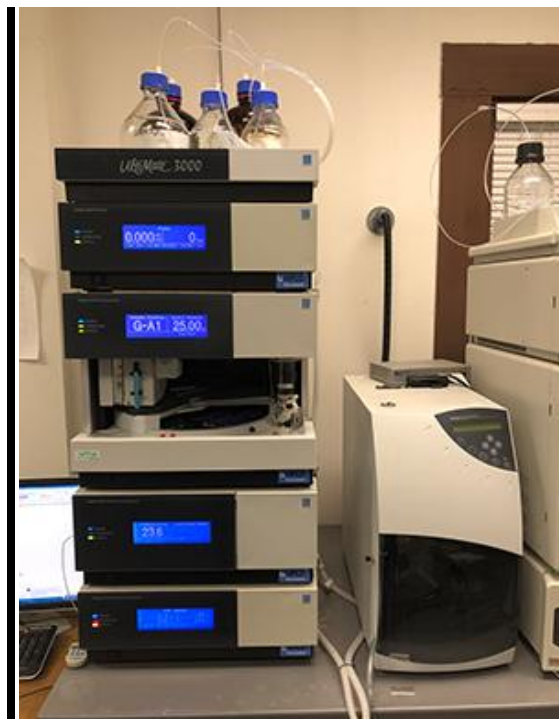


Fig. 7 High performance liquid chromatography (HPLC).

RESULTS

Table 6. Results and data interpretation for moisture analyzer of food sample

Altitude	High Altitude			Sea level Altitude		
	Sample No.	Sample Type		Sample No.	Sample Type	
		Vegetarian Food Moisture %	Non Vegetarian Food Moisture %		Vegetarian Food Moisture %	Non Vegetarian Food Moisture %
	1.	58.88	56.69	1.	84.62	58.62
	2.	78.69	73.21	2.	68.42	68.63
	3.	62.81	75.35	3.	79.01	86.36
	4.	82.73	69.65	4.	58.99	85.26
	5.	72.87	67.01	5.	77.08	60.09
Mean value		71.196	68.478		73.624	71.792

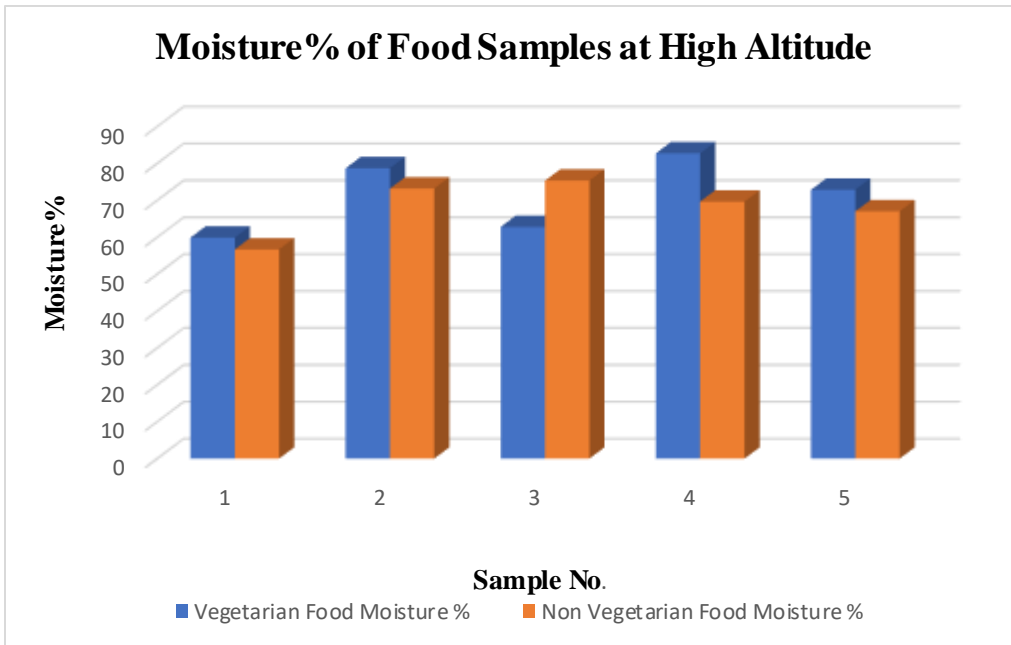


Fig.8 Graph for estimated moisture of food sample consumed at high altitude.

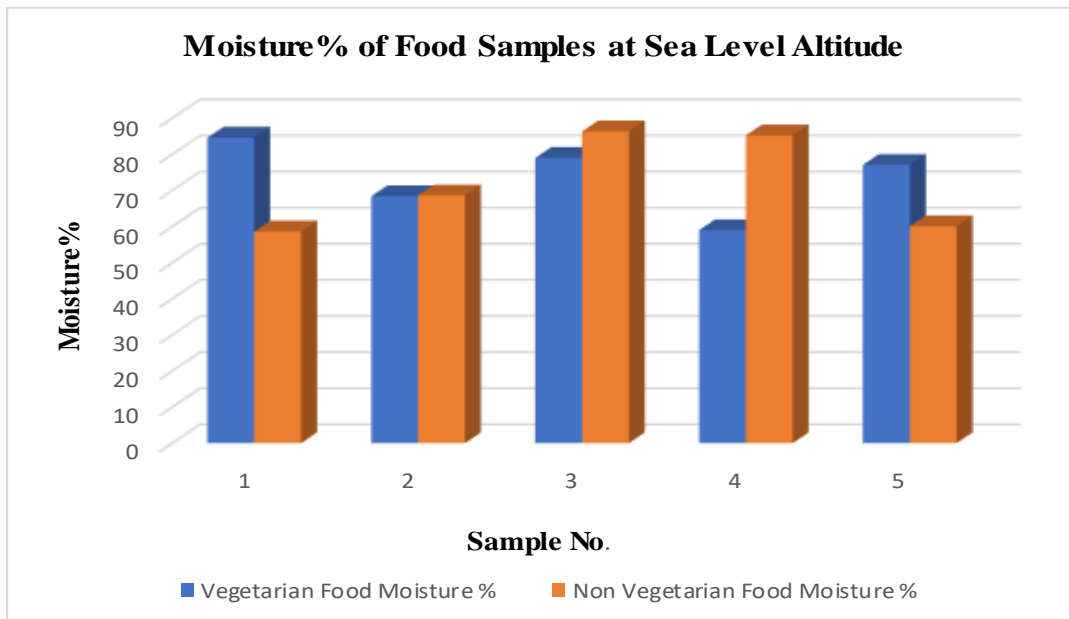


Fig. 9 Graph for estimated moisture of food consumed at sea level.

Table.7 Result and data interpretation for fat % of food sample.

Altitude	High Altitude			Sea level Altitude		
	Sample No.	Sample Type		Sample No.	Sample Type	
		Vegetarian Food fat %	Non Vegetarian Food fat %		Vegetarian Food fat %	Non Vegetarian Food fat %
	1.	5.62	7.03	1.	6.09	5.06
	2.	11.12	11.82	2.	6.94	9.93
	3.	7.89	13.97	3.	4.74	8.87
	4.	11.03	8.28	4.	8.96	4.44
	5.	17.73	10.29	5.	10.81	6.02
Mean value		10.678	10.278		7.508	6.864

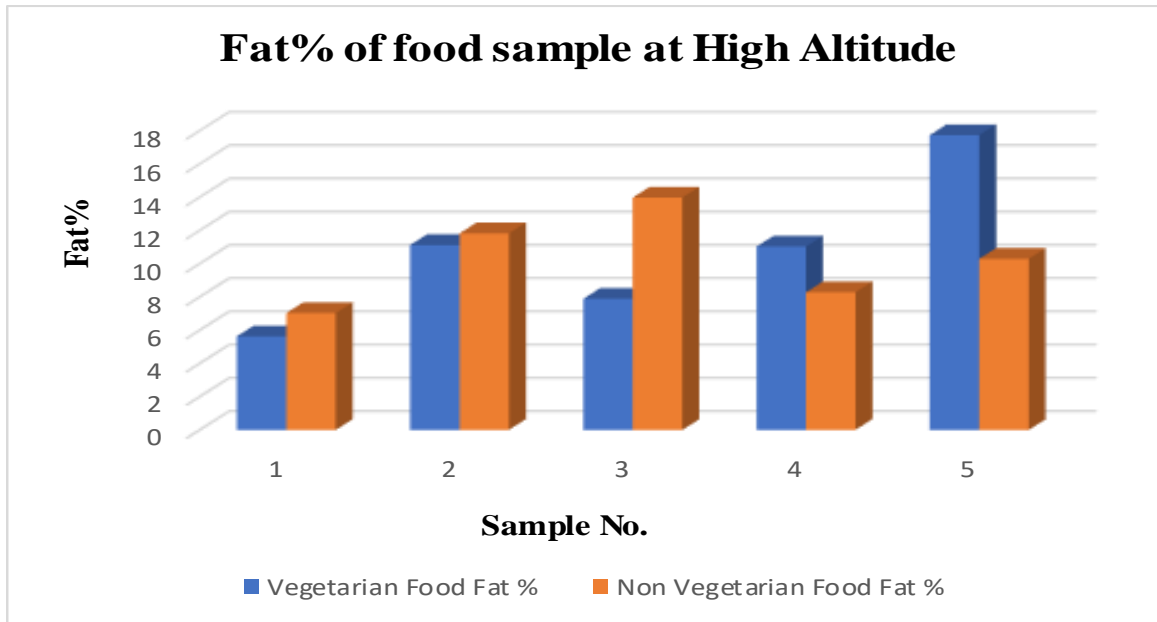


Fig.10 Graph for estimated fat % of food samples consumed at High Altitude.

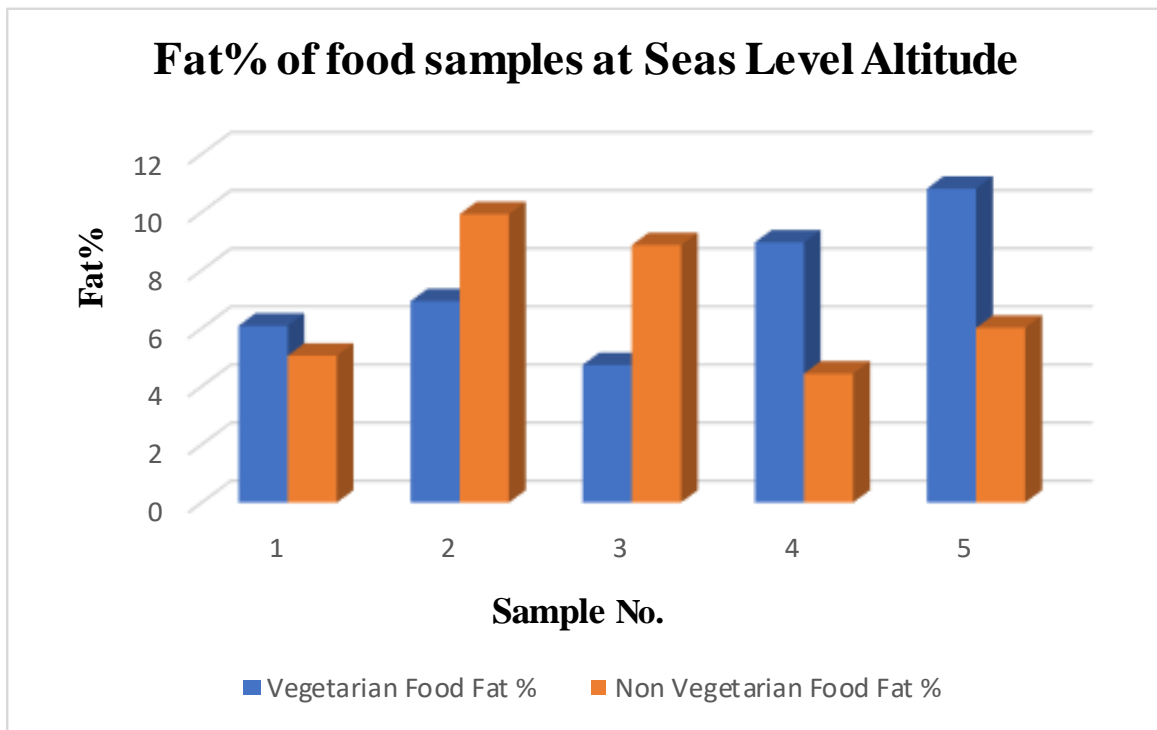


Fig.

Fig. 11 Graph for estimated Fat % of food samples consumed at sea level.

Table. 8 Result and data interpretation for calorific value of food samples.

Altitude	High Altitude			Sea level Altitude		
	Sample No.	Sample Type		Sample No.	Sample Type	
		Calories/g in Vegetarian Food	Calories/g in Non-Vegetarian Food		Calories/g in Vegetarian Food	Calories/g in Non-Vegetarian Food
	1.	4833.61	4833.9	1.	4436.94	4574.31
	2.	4397	4764.92	2.	5088.94	4359.52
	3.	5367.9	5011.27	3.	4341.09	4617.1
	4.	4698.67	5464.33	4.	4917.56	4204.42
	5.	4681.38	5388.01	5.	5229.48	4623.65
Mean value		96795.712	5092.486		4802.802	4475.8

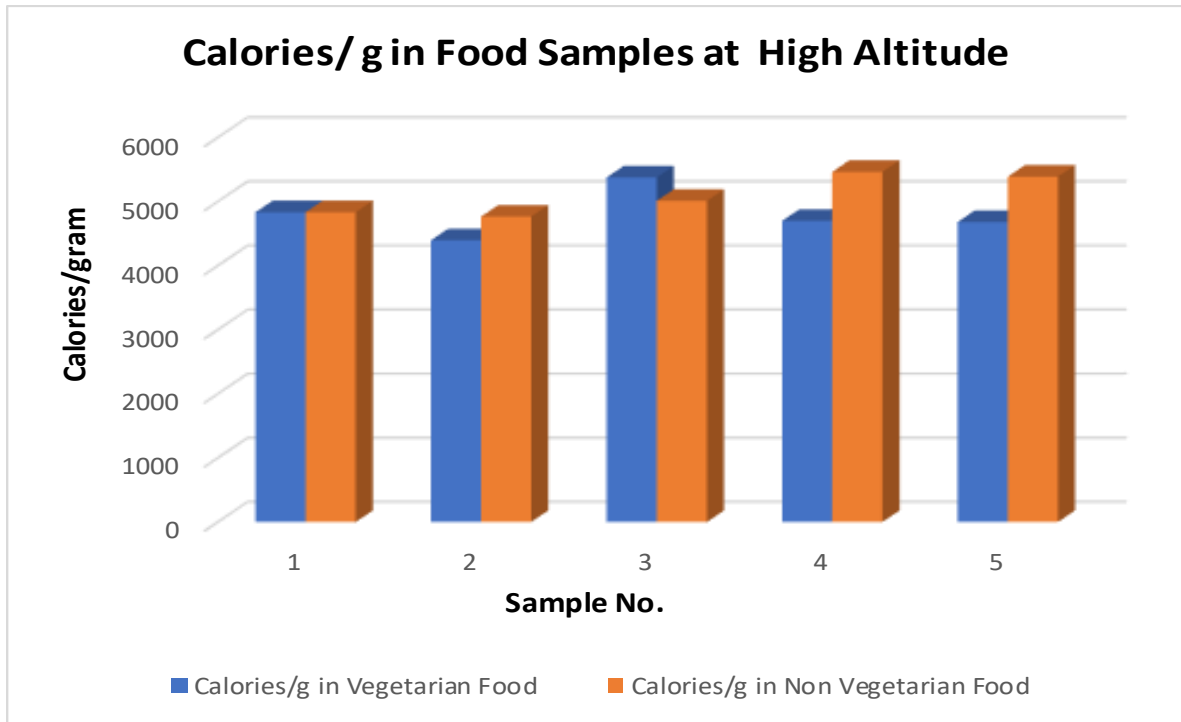


Fig. 12 Graph for estimated calorific value of food samples consumed at High Altitude.

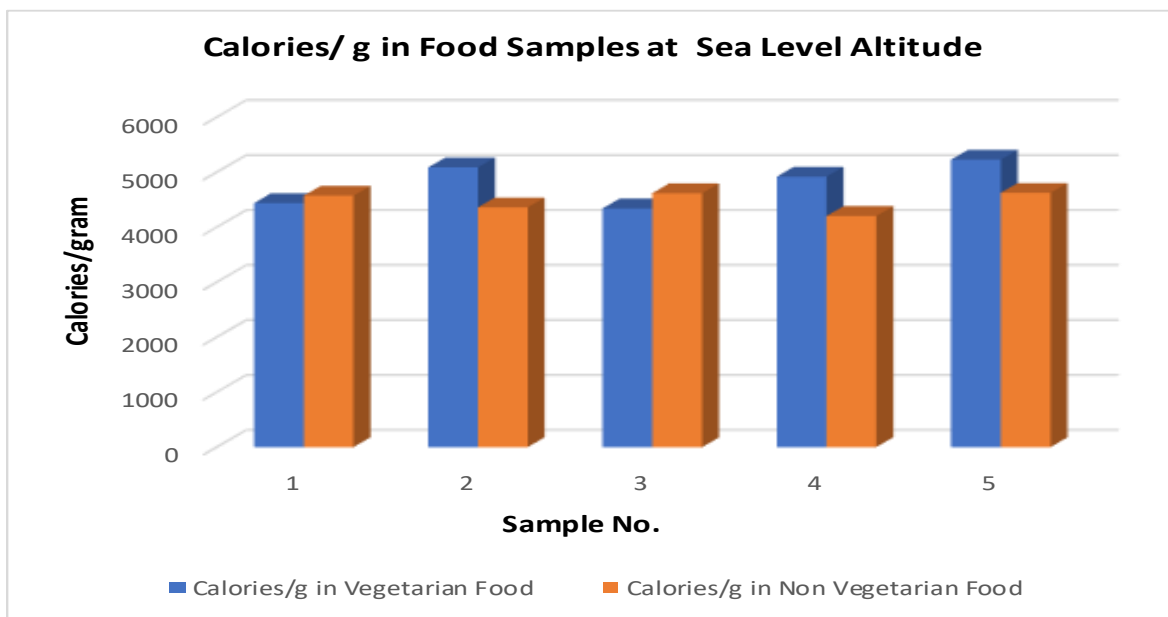


Fig.13 Graph for estimated calorific value of food samples consumed at sea level

Table. 9 Result and data interpretation for Ash content in food samples

Altitude	High Altitude			Sea level Altitude		
	Sample No.	Sample Type		Sample No.	Sample Type	
		Ash % in vegetarian food	Ash % in Non-Vegetarian		Ash % in Vegetarian Food	Ash % in Non-Vegetarian Food
	1.	0.81	0.84	1.	0.74	0.64
	2.	0.78	0.76	2.	0.8	0.65
	3.	0.92	0.79	3.	0.71	0.73
	4.	0.96	0.72	4.	0.72	0.53
	5.	0.86	0.69	5.	0.81	0.86
Mean value		0.866	0.76		0.756	0.682

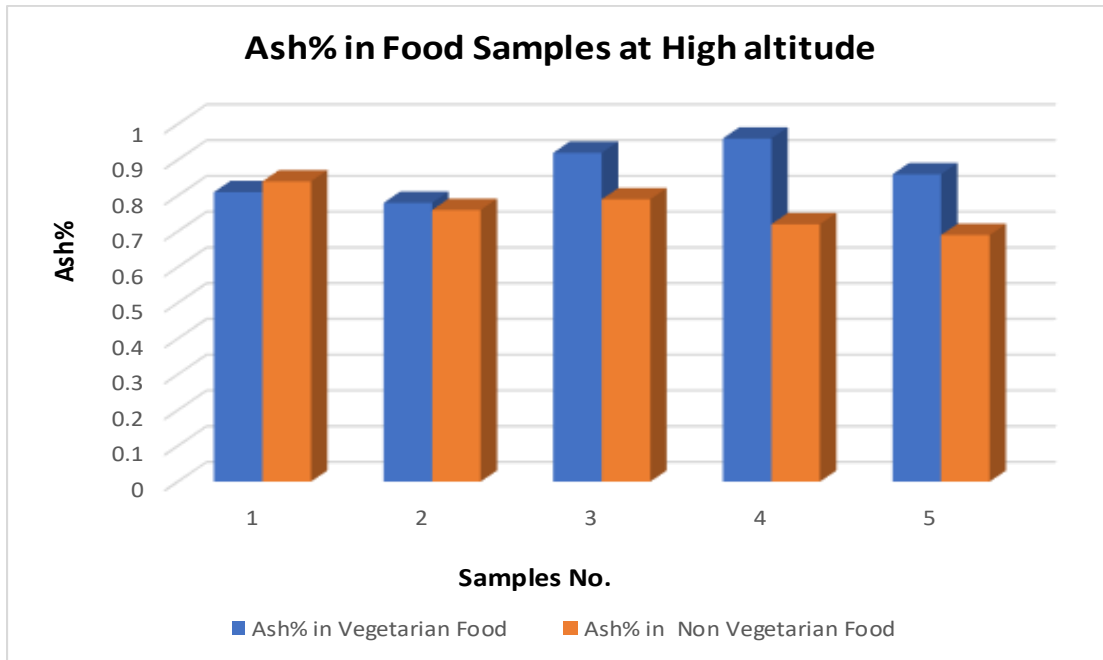


Fig.14 Graph for estimated Ash content in food samples consumed at High Altitude.

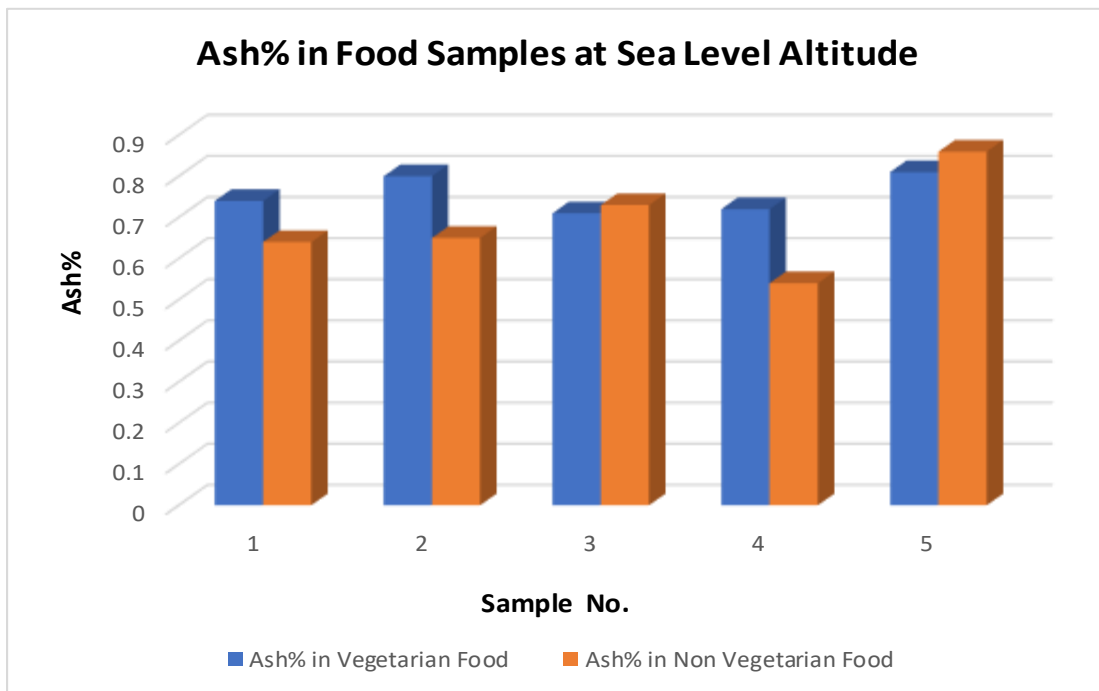


Fig. 15 Graph for estimated Ash content in food samples consumed at sea level

CONCLUSION

This report describes how the nutrition and food sciences have advanced enormously in this century and contributed to the development of an abundant, health promoting food supply and better health for people around the world. In this report Soxhlet extractor is the tool to extract the fat from food sample. HPLC methods as standard techniques for the quantitative and qualitative determination of both water and fat soluble vitamins.

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