

**FORMULATION, PREPARATION AND NUTRITIONAL EVALUATION
OF SUPPLEMENTARY PRODUCT FOR HOSPITALIZED
CARDIOVASCULAR DISEASE PATIENTS**

by

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**Formulation, Preparation and Nutritional Evaluation of Supplementary
Product for Hospitalized Cardiovascular Disease Patients**

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Approval letter

This dissertation entitled Formulation, Preparation and Nutritional Evaluation of Supplementary Product for Hospitalized Cardiovascular Disease Patients submitted by Bipin Karki has been accepted as the partial fulfillment of the requirements for the Bachelor degree in Nutrition and Dietetics.

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Abstract

The objective of this study was to formulate, prepare and evaluate the nutritional content of supplementary product with wholesome nutritional approach and ease of administration for cardiovascular disease patients to overcome mortality and morbidity. Few food ingredients from the basic food groups were selected. For this purpose three formulae were developed based on daily energy and nutrients requirement of heart patients. According to the formulae, three products A, B and C were prepared using cereals (wheat and maize), legumes (soybean and Bengal gram), fruits and vegetables (apple, banana and cauliflower), spices (cinnamon and fenugreek), honey, sugar, soybean oil and nuts (walnut). The product A, B and C was formulated with the variation of fenugreek and honey. Product A, B and C has same ingredient in which product A was formulated with 28g fenugreek and 30g honey, product B with 10g fenugreek and 50g honey and product C with 56.7g fenugreek and 0% honey. The best prepared products among three was analyzed on the basis of sensory evaluation and then analyzed for proximate composition, minerals, microbiological quality, and cost.

On the basis of sensory evaluation, the product B was found to be the best among the three products. The protein, fat, carbohydrate, crude fiber, Vitamin C, sodium, potassium and calcium content of the product B were found as 14.63 gm, 9.077 gm, 60.73 gm, 3.11 gm, 26 mg, 139.53 mg, 800 mg and 279.06 mg per 100 g dry matter respectively. The diet can supply 384.68 Kcal per 100g on dry basis. The moisture content and ash content of the product was found to be 8.9% and 2.32% respectively. The energy contributed by the protein, fat and carbohydrate were found to be 15.21%, 21.34% and 63.52% on the basis of 2000 Kcal respectively. The cost of the diet was calculated Rs 15 per 100g based on the current market price. The TPC and yeast and mold count in the product were 1565 cfu/g and 46 cfu/g respectively. The product was found to be microbiologically safe. This diet can be used by the heart patients and is beneficial to malnourished children and post-operative patients as well.

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List of Abbreviation

Abbreviation	Full Form
ADMA	Asymmetric dimethyl arginine
AMI	Acute myocardial infection
ANOVA	Analysis of variance
AOAC	Association of analytical communities
BMI	Body mass index
CCT	Central campus of technology
CHD	Coronary heart disease
CHF	Coronary heart failure
CVDS	Cardiovascular diseases
DHA	Docosapentanoic acid
DNA	Deoxy ribonucleic acid
EPA	Ecosapentanoic acid
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
GFSP	Germinated fenugreek seed powder

GSHP	Glutathione peroxidase
HDL	High density lipoprotein
HTN	Hypertension
LDL	Low density lipoprotein
LDPE	Low density polythene
LSD	Least square difference
MC	Moisture content
MI	Myocardial infarction
MUFA	Monounsaturated fatty acid
NA	Nutrient agar
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydrogen
NCD	Non-communicable disease
NCEP	National cholesterol education program
NHANES	National health and nutrition examination survey
NPU	Nitrogen protein utilization
PDA	Potato dextrose agar

RMR	Resting metabolic rate
RNA	Riboxynucleic acid
SEARO	South east asia region office
SFA	Saturated fatty acid
TFA	Trans fatty acid
TPC	Total plate count
U.S	United states
UNICEF	United nation international child emergency fund
VLDL	Very low density lipoprotein
WFP	World food programme
WHO	World health organization
YLL	Years of lost life

Part I

Introduction

1.1 General introduction

Atherosclerotic cardiovascular diseases are ischemic heart disease or coronary artery disease (heart attack), cerebrovascular disease (stroke) and diseases of aorta and arteries, including hypertension and peripheral vascular disease (Mendis *et al.*, 2011). Cardiovascular diseases (CVDs) are common in general population and becoming the number one cause of morbidity and mortality. It is estimated that 17.3 million died globally due to cardiovascular diseases in 2008. Ten percent death is attributable to CVDs alone. Cardiovascular diseases are common in general population (Alwan, 2011). Cardiovascular diseases claimed 3.7 million lives. Trend of disease is changed. Premature death before age of 60 were 34 % in the region, compare to 23% on rest of the world. In Nepal, death due to CVDs also increased from 22% in 2004 to 25% in 2008 (Bhandari *et al.*, 2014).

Cardiovascular diseases are diseases of the heart, vascular diseases of brain and diseases of blood vessels. CVDs due to atherosclerosis are coronary heart disease or ischemic heart disease, cerebrovascular disease or stroke, diseases of aorta and arteries including hypertensive and peripheral vascular disease. Other CVDs are congenital heart disease, rheumatic heart disease, cardiomyopathies, and cardiac arrhythmia. Coronary artery disease, ischemic stroke, which until recently were a common only in high income countries, are now becoming the dominant sources of morbidity and mortality worldwide. CVDs are a group of large number of conditions relating to the heart and blood vessel. The major CVDs include hypertensive heart disease. In South East Asia region 7.9 million (55%) deaths were caused by non-communicable diseases in 2008. Cardiovascular diseases alone accounted for 25% deaths in the same year (WHO/SEARO, 2011).

Cardiovascular disease affect the heart and blood vessel and a type of cardiovascular disease that builds the fatty materials in the artery walls, causing them to become narrow and the walls to become thick and hard called atherosclerosis. Once this happen, the heart has to work harder to provide the necessary circulation of the blood. The process of hardening or thickening of the arteries, causing break down in the blood supply to the heart. Both cardiovascular and atherosclerosis are the causes of heart disease (Dewan, 1994).

There are many dietary and lifestyle factors associated with the cardiovascular disease including intake of fat, saturated fat, fruits and vegetables, fiber as well as smoking and activity level (Grosvenor and Smolin, 2000). Many different factors are associated with cardiovascular disease and diet is one of the most important factor for causing a heart disease by providing bad cholesterol to the body. Heart attack remains relatively unknown in Japan because their traditional diet keeps the Low Density Lipoprotein (LDL) cholesterol level very low. Role of nutrition in cardiac disease is very essential. The heart muscles like any other body tissue, is dependent upon the adequate supply of all essential nutrients. Markedly malnourished people of the world frequently manifest cardiac impairment such as dyspnea and palpitation on exertion, enlargement of heart and systolic murmurs (Robinson, 1972). Food has very significant role to play in illness. Diet may have to be modified depending upon the disease, the severity of the problem, the nutritional status of the patient as well as the metabolic change involved (Khanna *et al.*, 2005).

The normal diet is modified according to the disease, symptoms, condition of patient and metabolic changes (Swaminathan, 2000). Nutrient modification which are commonly done are classified as :

- a. Modification in consistency- To provide a normal, soft or fluid diet. In condition like diarrhoea, fever etc.
- b. Modification in energy intake- For example high and low energy diets such as an obese patient required low energy diet, likewise heart patients also need low energy diet for low pressure of heart patient (Srilakshmi, 2002). Loss of the weight by the obese leads to considerable reduction in the work of the heart because the imbalance between the body mass and strength of the heart muscles is corrected. There is a slow heart beat rate, a drop in the blood pressure, and thereby improved cardiac efficiency. So loss of weight can be done by the reduction in the calorie intake (Robinson, 1972).
- c. Modification in content of one or more nutrients- for example high or low protein diets, low sodium diets, high carbohydrate diet, or moderate fat diet. For example, restriction of sodium in hypertension (Robinson, 1972).
- d. Modification in fiber content- such as high or low fiber diets.
- e. Bland diets- such as diets are chemically, mechanically and thermally bland.

Consuming an adequate diet that provides the necessary combination of macronutrients and micronutrients is considered essential for normal human growth and physical development, cognitive development and functioning, and maintenance of a healthy immune system. Dietary

requirements vary according to age, gender, physical activity and health status. Supplementary foods (for example, fortified blended foods) is defined “as specially formulated foods, in ready-to-eat or in milled form, which are modified in their energy density, protein, fat or micronutrient composition to help meet the nutritional requirements of specific populations”. Supplementary foods are not intended to be the only source of nutrients in a given population. Supplementary foods are macronutrients (balanced diet or high protein, high carbohydrate, or high fat diets/foods) given as a supplement in addition to the usual diet (not a total dietary replacement). Supplementary foods can contain added micronutrients (vitamins and minerals), but reviews of only micronutrients will be excluded. Food supplements must be taken orally (Visser *et al.*, 2013).

For a 45-50 years old executive leading an active life, the recommended energy is 1800-2000 Kcal (Grover, 2006), an obese patient required 1000-2000 Kcal energy. It is advisable to undernourish the patient for few days after the heart attack. During this period 1000 Kcal energy per day may be recommended and progressed to energy 1200 Kcal per day few after the heart attack. Injection of the food involves increase cardiac output so as to meet the metabolic demands for digestion, absorption and assimilation of food. Therefore by giving the hypocaloric diet or restricting the food intake, the metabolic activity can be decreased to a level that the weakened heart can accommodate without extra strain (Khanna *et al.*, 2005).

1.2 Statement of the problem

Nepal being a developing country and due to its poor socio-economic condition and lack of public awareness, heart disease become a common disease in Nepal. It is believed that dietary and lifestyle factors including intake of fat, saturated fat, fruits and vegetables, fibre as well as smoking and activity level are associated with the cardiovascular disease. Many different factors are associated with cardiovascular disease. Out of which, diet is one of the most important factor for causing the heart disease by providing cholesterol to the body. The dietary management is necessary for maintenance of good nutrition and for the maximum rest of heart (Khanna *et al.*, 2005). It is recommended that the heart patients should take large amount of fruits and vegetable for the fulfillment of vitamins, minerals, crude fiber and other phytochemicals.

During recent years, the diversity of supplementary feeding has been rapidly increased. In advanced countries, they prefer supplementary product in addition to hospital made blenderized feed to fulfill the nutritional requirement of hospitalized CVDs patient and to decrease the

number of hospital stays of the patient. Cardiovascular disease has been very vulnerable in every part of the world. In the developing countries like Nepal, the condition is being much worse. According to the Department of Health Services Report 2072/73, there are 407 hospitals in Nepal. In these hospitals, the use of supplementary products has not been done till this date. Only the uses of horlicks, viva, Bournvita, Chyawanprash by hospitalized patients has been found considering them as nutrient dense products. These types of products do not provide all the essential nutrients and energy fulfillment and protein requirement can not be fulfilled and also influencing the mortality and morbidity of the patients and also number of hospital staying days.

Hospitalized Cardiovascular disease patients require high energy and high protein but such foods do not contain the required amount of nutrients needed for the patients for proper healing and to prevent patient from undernutrition. Hospitalized patients have generally poor appetite and are not intended to take frequent meals. Therefore it is very essential to formulate and prepare easily digestible and palatable supplementary products for CVDs patients as per their nutritional requirements. It can be used by post-operative and malnourished patients as it is a nutrient dense product. Such diet can solve the problems of hospital patients who are using the products like horlicks, Bournvita, Viva for fulfilling their energy requirements. One of the major nutritional problems faced by heart patients is the management of the diet so patients suffering from heart disease could not get the appropriate nutrients. Therefore for the fulfillment of such deficiency, combination of cereals, legumes, fruits and vegetables, spices, honey and vegetable oil can be converted into single powder form as a supplementary diet. It is believed that, this diet is sufficient to fulfill daily nutrient requirements for the heart patient.

1.3 Objectives

1.3.1 General Objectives

The main objective was to develop a nutritionally concentrated, easily digestible and palatable supplementary product especially for heart patients with the inclusion of the specific, unique pharmacologically important nutrient at low cost.

1.3.2 Specific Objectives

The specific objectives of this study were as listed below:

- i) To formulate and prepare nutritionally concentrated supplementary products for the heart patients.

- ii) To evaluate the best product on the basis of sensory quality of diet.
- iii) To perform Chemical and microbiological analysis of the prepared diet.
- iv) To calculate the cost of the best product.

1.4 Significance of the study

Heart disease has been the major health issue for the cause of mortality and morbidity in developing countries nowadays. The heredity, age, diet and exercise are the important risk factors for this disease. This disease can be controlled by consuming plant based foods which includes cereals, legumes, fruits, vegetables, vegetable oil, sugar, spices, nuts. With the heightened awareness of the prevalence of CVDs in hospitalized patients, supplementary food product has gained new attention. The problem appears to be constant and the rate of deaths due to CVDs has increased to 25% in 2008 in Nepal.

Supplementary feeding is the safe and good method of providing nutrients to a hospitalized CVD patients. Supplementary feeding is used in both emergency and non-emergency situations to address short-term hunger, longer term food shortage, and to improve the nutritional status, or prevent the nutritional deterioration, of specific populations. Supplementary feeding can have direct nutrition and health benefits and can also be used in enteral feeding. It may also contribute to increased service utilisation, with secondary effects on improved health related to increased service uptake. In addition, it may contribute to social goals, such as food supplementation given at schools to improve school attendance. This diet though highlighted as a Supplementary food in this work can be used by the normal person of various ages and physiological states. The study initiated by this dissertation work can be further extended in wide aspect (eg. evaluation of nutrition quality, clinical trials etc) in the future and this diet can be prescribed for heart, malnourished patients, and post operative patients and can be produced in the large scale.

1.5 Limitation

- i) Due to the very short time, it could not evaluate the shelf life of product.
- ii) Analysis of the vitamin and some trace element, amino acids and fatty acid composition of the products could not be performed due to the limitation of the required facilities available in Central Campus of Technology, Dharan laboratory.
- iii) Trials of the product on the CVDs patients has not been done.

Part II

Literature Review

2.1 Background information

Nutrition as a science can be said to have been founded by Lavoisier towards the end of the eighteenth century, but dietetics is much older subject. Hippocrates frequently gave his patients advice about what foods they should eat and since the days of ancient Greece, doctors in all countries have used dietetics as an important part of their treatment (Davidson, 1986). A sick person like a healthy person should be maintained homeostasis. A well-planned diet providing all the specific nutrients to the body helps to achieve nutritional homeostasis in a normal, healthy individual, however in disease condition, the body tissue either do not receive the proper nutrients in adequate amount or can not use the available nutrients owing to faulty digestion, absorption or transportation of food elements, thus affecting the nutritional homeostasis of the sick person (Khanna *et al.*, 2005).

Six million years ago, human primate ancestors lived in the woods of Eastern Africa and their diet was comprised of leaves, roots, fruits, and nuts. The main macronutrients consumed were carbohydrates having low glycemic index and individuals exerted high physical activity. The climate became drier and colder and the forests in which our ancestors lived disappeared and were replaced by arid grassland. This change forced the human ancestors to move to the coastlines. The human diet changed to low carbohydrate, high-protein and became rich in micronutrients such as iron, retinol, zinc, vitamin B₁₂, and unsaturated fatty acids from fish (with a balance of fatty acids omega-3 and omega-6, ratio 1 : 1) as hunted large predators became part of the diet. These new components of the diet provided enough fuel and building blocks to facilitate encephalization and the development of intellectual capacity (Rubio-Ruiz *et al.*, 2015).

The history of coronary syndromes and sudden death, and apoplexy or stroke, goes back to antiquity and has been thoroughly treated by historians and experts from many disciplines. By the beginning of the twentieth century, a heart attack with myocardial infarction was well known to cause death, but comprehension of it as a syndrome that one might survive was much delayed. When that awareness finally came and diffused into the practicing community in the 1920s and after, it had a major effect on the recognition of coronary disease as epidemic after World War II, which, in turn, gave preamble and impetus to CVD epidemiology and preventive cardiology. Because coronary disease was newly epidemic, it was reasoned by a few pioneers that its causes

and conceivably its preventives must therefore lie in changed environment (Rubio-Ruiz *et al.*, 2015).

The Omni Heart (Optimal Macronutrient Intake Trial for Heart Health) study compared three heart healthy diets that were known to lower blood pressure and improve blood lipids. These three diets were based on the DASH diet but differed in the amount of carbohydrate (58%, 48% and 48%), protein (15%, 25% and 15%) and unsaturated fat (27%, 27% and 37%), while being equivalent in calories of 2100 Kcal. The higher protein (25% of calories) and higher unsaturated fat (37% of calories) diets showed the most benefit on blood pressure and blood lipids and reduced the estimated 10-year risk of heart disease compared to the higher carbohydrate diet. Of note, the higher-protein diet emphasized plant sources of protein, which have been associated with reduced blood pressure. A DASH diet of about 2000 Kcal must constitute of 55% of calories from carbohydrate, 15% of calories from protein, 27% of calories from fat, <2300 mg sodium, 4700 mg potassium, 121150 mg calcium and 30 gm fiber (Campbell, 2017).

Two type of ready to eat supplementary food formulations were developed by roller drying based on wheat, soy protein concentrate, whey protein concentrate, and green gram flour and were fortified with vitamins and minerals to meet the one third of the recommended daily allowance. The supplementary food formulations contained 20–21% protein, 0.6-5% fat, 67-71% carbohydrate, 370–390 kcal of energy and 2,300 µg of β-carotene per 100 gm serving. The nutrient composition of both the supplementary food formulations provided one –third of the RDA as recommended by ICMR. The food formulations prepared are suitable to use as supplementary food for children above 6 months, as they provide all the required macro and micronutrients as recommended for the age group (Khanam *et al.*, 2013).

2.2 Global Burden of Cardiovascular Diseases

Cardiovascular diseases are the number one cause of morbidity and mortality. It was estimated that among 36 million death due to non communicable diseases, 17.3 million mortality is attributable to cardiovascular diseases in 2008. Ten percent daily were lost due to CVDS alone in 2008. In 2012, Cardiovascular disease mortality was 17.5 million (31.4%), which is almost half of total non-communicable disease mortality (67.8%) among 55.8 million global deaths. Almost two thirds of the total CVDS is accounted by ischemic heart disease and cerebrovascular diseases in both male and female (WHO/SEARO, 2011). Among top ten leading causes of global death, ischemic heart disease is attributable to 13.2 %, Stroke 11.9% and hypertensive heart disease 2 percent Developing countries are experiencing more inequalities of occurrence and

outcome of these diseases. Over 80 % of CVDS death takes place in low and middle income countries . In all income countries ischemic heart disease and stroke are the top two killer except low income country where these are number one killer after lower respiratory infection and diarrhea (WHO, 2012).

According to disease burden estimate for 2012, 1512 million daily lost due to non-communicable disease, which is 55.1 % of total 2743 million daily. Cardiovascular diseases were responsible for 14.4 % (393 million) daily loss. of which, 165 million were due to ischemic heart disease and 141 million were due to stroke. Among total daily ischemic heart disease was responsible for 6% and stroke was responsible for 5.2 percent loss. In 2012, years of life lost due to premature death caused by CVDs was 370 million, 18.5 % of total years lost in 2003. cardiovascular disease mortality will rise even in future. It is projected that 23.3 million people will die by 2030 only due to CVDs according to projection from 2002 (WHO, 2012). Total proportion of death due to Cardiovascular disease will be 31.7 % even in 2030 (WHO and UNAIDS, 2030).

2.3. Burden of Cardiovascular Diseases in South East Asia Region

In South East Asia region 7.9 million (55%) deaths were caused by Non communicable diseases in 2008. Cardiovascular diseases alone accounted for 3.7 million (25%) death in the same year. Trend of disease is changed (Fric, 2010-2011). In 2012 mortality due to cardiovascular diseases was 3.6 million which is one fourth (26.8 %) of total 13.7 million death in SEA region. Among them ischemic heart disease (11.5%) and Stroke (10.5%) were the top two causes of mortality in 2012. Premature death before age of 60 were 34 % in the region, compare to 23% on rest of the world (Fric, 2010-2011).

Age standardized death rate per 100000 by cardiovascular diseases and diabetes was highest in Bhutan in male, where as it was in Bangladesh in female among SEA countries in 2008. In Bhutan 465 males and 381 females while in Bangladesh 447 males and 388 females per 1,00,000 were died in 2008. It was 203.7 by ischemic heart disease and 108.3 by cerebrovascular disease in 2008 (WHO, 2012). daily lost due to cardiovascular diseases in the region is 97.8 million, 13.2 % of total daily of 739 million in 2012. Among 551 million years of life lost due to premature death globally 93.4 million (17.2%) years were lost by cardiovascular diseases (WHO, 2012). In 2030, cardiovascular disease will be responsible for 5.8 million deaths. It is projected that ischemic heart disease and stroke will be top two killer even in 2030, causing 13.8 % and 12.7% of regional death (WHO and UNAIDS, 2030).

2..4 Burden of Cardiovascular Diseases in Nepal

Cardiovascular diseases are attributable to major mortality and morbidity in Nepal. Death due to CVDS also increased from 22% in 2004 to 25% in 2008 (Mathers, 2013). Among all NCD patients, 40% are admitted after diagnosis of cardiovascular diseases in non-specialized hospital in Nepal. Highest percentage of patients are cardiovascular accident (16%) in non-specialized hospital and Myocardial Infarction(18%) in Specialized hospital (Bhandari *et al.*, 2014) Estimated proportionate mortality due to CVDS also increased from 22% in 2004 to 25% in 2008 (Mathers, 2013). Among total death, due to all cause 41,400 died by cardiovascular diseases in 2012, which was 22.25 % of total deaths (WHO, 2012). According to 2008 estimate NCD accounted 50% of all deaths. Cardiovascular diseases mortality was 25% among all disease. Age standardized death rate per 1,00,000 caused by cardiovascular diseases and diabetes was 400.2 in male more than in female 301.3 in 2008 (Alwan *et al.*, 2011). Ischemic heart diseases and Stroke were accounted for 152.6 and 82.6 deaths per 100000 for both sexes in 2008 (Mendis *et al.*, 2011).

2.5 Types of Heart Disease

2.5.1 Atherosclerosis

Atherosclerosis is the most common contributing factor to the development of CVD (Mahan and Escott-Stump, 2008). Atherosclerosis develops when plaque builds up in the artery walls from Low-density lipoproteins (Dewan, 1994). The thickening and narrowing of the arterial wall is caused by the accumulation of cholesterol, smooth muscle cells, and fibroblasts causing plaque formation. With enough plaque accumulation, blood circulation is slowed or blocked altogether. Before plaque formation, endothelial dysfunction occur causing blood vessels to become constricted. Some of the factors that contribute to endothelial dysfunction are dyslipidemia (abnormality in any lipoprotein, especially LDL, hypertension (HTN), smoking, diabetes, obesity, and diets high in saturated fat and cholesterol (Mahan and Escott-Stump, 2008). Occlusion of arteries can have detrimental effects depending on its location. In the coronary arteries, atherosclerosis can cause angina, myocardial infarction (MI) or sudden death. A cerebral arterial occlusion can cause strokes, ischemic attacks, blood clots, and gangrene (Mahan and Escott-Stump, 2008).

2.5.2 Coronary Heart Disease

Impaired blood flow to the coronary artery is classified as Coronary heart disease (CHD). The incidence of CHD is high; 700000 Americans had a new coronary attack and 500000 had a recurrent attack in 2000 (Mahan and Escott-Stump, 2008). CHD is described as a MI or ischemia of at least one coronary artery. With the occurrence of either, heart tissue is damaged often leading to heart disease and potential death. Symptoms of CHD may or may not be detected. If symptoms are present, they include chest pain and discomfort from the heart not receiving enough oxygen, shortness of breath and fatigue with exertion (Kitchen, 2014).

2.5.3 Congestive heart failure

Improved treatment of cardiovascular disorders such as MI, HTN and valvular heart disease, is projected to increase the occurrence of heart failure (Coulston *et al.*, 2001). Congestive heart failure (CHF) is a sign and symptom resulting from impairment of systolic and/or diastolic functioning of the myocardium or can also be described as inefficient heart pumping. Those individuals with CHF often experience shortness of breath, chest discomfort, exercise capacity limitations, peripheral edema, anorexia and can become fatigued easily. Risk factors of CHF include but are not limited to HTN, obesity, diabetes, atherosclerosis, CHD and dyslipidemia along with excess sodium intake (Mahan and Escott-Stump, 2008). Physiologically, CHF occurs similar to atherosclerosis where there is an asymptomatic phase when damage is occurring unknown to the individual. Damage can be caused by an acute MI or by volume overloading on the heart. The occurrence of damage changes the function and shape of the heart left ventricle producing left ventricular hypertrophy to compensate for the lack of blood flow. Due to an enlarged left ventricle and the compensating overuse of the system, further damage is done allowing for CHF to further progress (Mahan and Escott-Stump, 2008).

2.5.4 Stroke

The two most common forms of CVD are heart attack and stroke. A stroke transpires when cerebral arteries to the brain become blocked or burst often resulting in the death of brain. Blockage occurs from the collection of lipoproteins on the arterial walls. High blood pressure, diabetes, smoking, and obesity can predispose an individual for a stroke (AHA, 2002).

2.5.5 Heart attack

Each year, about 1.1 million people in the U.S. have heart attacks and almost half of them die. CHD, which often results in a heart attack, is the leading killer of both men and women in the

U.S.A. heart attack occurs by either a block in the coronary artery which can be triggered by heavy physical exertion due to an increase in heart rate and blood pressure or a decrease in arterial circumference as a result of atherosclerotic plaque. Chest and upper body discomfort in the arms, neck and back, shortness of breath, nausea, vomiting, lightheadedness, fainting, or breaking out in a cold sweat are symptoms of a heart attack and should elicit help to decrease damage to the heart. Heart attack prevention is linked to diet, exercise and stress factors, all of which can be modified (Mahan and Escott-Stump, 2008).

2.5.6. Ischemic heart disease

It is due to the reducing in blood flow to the heart. Symptoms of the ischemic heart disease are chest pain, exertional dyspnea, orthopnea, cardiomegaly, peripheral oedema etc (Grosvenor and Smolin, 2000).

2.5.7 Tachycardia

This type of the disease is due to the excessively rapid of the heartbeat. Symptoms of tachycardia are rapid heartbeat at rest, palpitations (Grover, 2006).

2.5.8 Rheumatic heart disease

It is the chronic heart condition due to heart damage from rheumatic fever (Grover, 2006).

2.5.9 Pulmonary heart disease

Heart disease resulting from a lung disorder is known as pulmonary heart disease. A complication of lung disorders where the blood flow into the lungs is slowed or blocked causing increased lung pressure. The right side of the heart has to pump harder to push against the increased pressure and this can lead to enlargement of the heart muscle and other problems. Ultimately, congestive heart failure of the right side of the heart can result. Breath, syncope, dyspnea, chest pain are the symptoms of the pulmonary heart disease (Grover, 2006).

2.6 Risk factors of Cardiovascular disease

There are many risk factors associated with CVD. Modifiable risk factors are risks that can be changed to decrease the prevalence of CVD, while non-modifiable risks are factors that can not be altered. These risk factors do not cause CVD but have a positive association with acquiring the disease. CVD may not develop if a risk factor is present but it has been shown that the

presence of multiple risk factors does increase the chance of developing CVD (Mahan and Escott-Stump, 2008).

2.6.1 Modifiable risk factor:

Modifiable risk factors are responsible for approximately 80% of CVD and strokes. These factors include smoking, physical inactivity, alcohol, weight, HTN, diabetes and poor diet (Mahan and Escott-Stump, 2008).

i) Smoking and tobacco

Smoking and tobacco has been recognized for more than 40 years as an increased risk for CVD (Mahan and Escott-Stump, 2008). The cardiovascular risk imposed by both, smoking and tobacco, is magnified by the coexistence of several other coronary risk factors. However, when another risk factor is present in a smoker (i.e.; HTN, high cholesterol) the risk of CVD is further increased (Prasad *et al.*, 2009). Use of any tobacco product also promotes atherosclerosis and fibrinogen, a blood clotting agent. Acute coronary events including thrombus formation, plaque instability and arrhythmias are influenced by tobacco use as well (Mahan and Escott-Stump, 2008). Cigarette smoking is associated with increased levels of inflammatory markers. Inflammatory markers, such as C-reactive protein have been shown to be both prognostic and predictive of future cardiovascular events in several populations (Bakhru and Erlinger, 2005).

ii) Physical activity

Low level of fitness (i.e.; physical inactivity) is an independent risk factor for CVD (Mahan and Escott-Stump, 2008). It has been shown to increase the risk of heart disease and stroke by 50%. Without exercise, Atherogenesis can occur rapidly forming plaque in the arterial walls and decrease the vascularity of the myocardium. Physical inactivity also has an impact on other risk factors including HTN, triglycerides, high density lipoproteins (HDL), diabetes and obesity which when combined with lack of exercise can increase the risk of CVD (Hubert *et al.*, 1983).

iii) Alcohol

Alcohol consumption has been associated with a lower risk of CVD in individuals who are light to moderate drinkers (Mukamal *et al.*, 2010). By consuming two drinks a day for men and one drink a day for women, there is a significant decrease in cardiovascular risk due to alcohol's ability to raise HDL and reduce fibrinogen (Mahan and Escott-Stump, 2008). Heavy drinkers

more than two drinks for men and one drink for women, have an increased risk for HTN because alcohol raises blood pressure and total triglycerides (Beulens *et al.*, 2007).

iv) Weight

Obesity has been shown to be an important long term predictor of CVD incidence among individuals. Obesity is a prevalent CVD risk, 65% of adults are overweight (Body Mass Index (BMI) 25- 29.9) and 31% are obese (BMI>30) (Hedley *et al.*, 2004). In a study conducted by Hubert, CHF increase 2.5 to 3 fold from leanest to heaviest subjects, signifying that those who are overweight have a higher risk when compared to leaner individuals. In addition to weight, waist to height ratio is strongly correlated with cardiovascular risk (Gruson *et al.*, 2010). For men, a ratio between 53 and 58 predicts for an increased CVD risk. For women the ratio is 49 to 54 (Android fat distribution), where weight is centered around the midsection, have a greater chance of CVD than those who carry weight around the hips (Gynoid fat distribution). It has been argued that obesity does not convey an increased risk of CVD unless it is accompanied by elevations in such characteristics as blood pressure or blood lipid. Weight loss can affect CVD (Mahan and Escott-Stump, 2008).

In a study conducted by Pascualet, metabolic syndrome participants who lost weight during the trial decreased systolic and diastolic blood pressures as well as LDL cholesterol. Further observations concluded that the impact of weight loss changes the rate of cardiovascular risk factors and reinforces the necessity to be proactive in achieving weight reduction (Pascual *et al.*, 2009).

v) Hypertension

Hypertension (HTN) is a prevalent and powerful contributor to CVD (Kannel, 1996). HTN is classified as having an average blood pressure higher than 140 mmHg systolic or 90 mm/Hg diastolic. Having high blood pressure predisposes to all cardiovascular diseases including cardiac failure, coronary artery disease, and peripheral artery disease due to an increased stress on the heart and arteries (Kannel, 1996). Stress on the arteries causes microscopic tears that when healed create scar tissue that attracts plaque. Plaque formation in the arteries then eventually leads to atherosclerosis. In response to high blood pressure and an increased workload secondary to obesity, the left ventricle of the heart grows in size. Left ventricular growth is classified as left ventricular hypertrophy and is found to be a strong risk factor for CVD, heart failure (HF) and sudden death (Mahan and Escott-Stump, 2008).

HTN is a public health problem in developed countries. HTN is defined as persistently high arterial blood pressure. It is often referred to as the “silent killer” because individuals with HTN can be asymptomatic for years and then have a fatal stroke or heart attack. There is no cure for HTN however, it is easily detected and can be managed through proper diet, exercise and medications. HTN is caused by multiple factors including a combination of environmental and genetic components. Environmental factors including the increase in BMI may contribute to the increase in prevalence of HTN (Mahan and Escott-Stump, 2008).

vi) Diabetes

With improvements in the management of diabetes and CVD, evidence still suggests that CVD is the leading cause of morbidity and mortality in people with diabetes. The Framingham Study demonstrated that diabetes mellitus is associated with a two to five fold increase in CVD and related death (Kannel and McGee, 1979). Individuals with diabetes lack the ability to make insulin or cannot facilitate their own insulin production and glucose becomes abundant in the blood. The abundance of glucose results from defects in insulin secretion from the β - cells or insulin action/resistance. With glucose build up, arteries become damaged perpetuating CVD. Some of the increased risk for CVD seen in diabetic individuals is attributable to the concurrent presence of other risk factors such as dyslipidemia, HTN and obesity (AHA, 2002).

Management of these risk factors has been shown to effectively reduce the incidence of major coronary events in persons with diabetes. Additionally due to dyslipidemia as a risk factor, LDL cholesterol in a diabetic individual needs to be at 70 mg/dl. With regards to drug treatment, lowering of glucose by medications has not produced a reduction in cardiovascular events, suggesting that elevated glucose may indicate proximal pathology related to adipocyte stress and dysfunction (Rifai *et al.*, 2005).

vii) Diet

Dietary intake high in fat and cholesterol is strongly related to an increased risk for CVD, but more specifically to the proliferation of other risk factors such as obesity, HTN and diabetes. Evidence from prospective studies have shown that dietary patterns are associated with risk and specifically dietary patterns high in saturated fatty acids, cholesterol, and animal fat increase LDL cholesterol levels (Van Horn *et al.*, 2008).

a) Total Fat

Total fat, while necessary in the diet, is often consumed in large amounts in the typical American diet. Total fat intake for adults 19 years of age and older is 20-35% of kilocalories. These ranges are associated with a reduced risk of CVD while providing adequate intake of essential nutrients (U.S. Department of Agriculture., 2010). However, according to National Health and Nutrition Examination Survey (NHANES), the average American between the ages of 20-70 years old consumes 35% of their calories from fat. High fat diets increase postprandial lipidemia and chylomicron remnants, both of which are associated with increased risk of CVD (Mahan and Escott-Stump, 2008).

Hu and Rimm determined in the study of dietary patterns and risk of CVD in men that those individuals who consumed high intakes of red and processed meats, refined grains, sweets, desserts, french fries and high fat dairy had an increased risk for CVD when compared to those who consumed a high intake of vegetables, fruit, legumes, whole grains, fish and poultry. As a whole, the study demonstrated strong evidence that a diet high in vegetables, fruit, legumes, whole grains, fish and poultry and low in red and processed meats, refined grains, sweets, desserts, French fries and high fat dairy may reduce the risk of CVD (Hu *et al.*, 2000). Focusing on dairy, diets containing high fat dairy may also be linked to an increase in blood pressure and CVD. Consumption of high fat dairy products such as cheeses and whole milk may increase systolic blood pressure. In the same study, high fat dairy was also shown to increase body weight compared to low fat dairy consumption (Alonso *et al.*, 2009).

Research has also determined that there is very little evidence from prospective epidemiological studies suggesting that total fat intake independently of dietary fat quality increases the risk of CVD (Erkkila *et al.*, 2008). U.S. Dietary Guideline for Americans (2010) has stated that while a recommendation is made for percent of total fat, the type of fatty acids consumed are more important in influencing CVD than the total amount of fat in the diet.

b) Saturated Fat

Saturated fat has a close relationship to CVD and more specifically to its effects with cholesterol and LDL. A strong body of evidence indicates that higher intake of saturated fatty acids (SFA) are associated with higher levels of blood total cholesterol and LDL cholesterol indicating risk factors for CVD (AHA, 2002). SFA are found mostly in animal foods and contain three different types. SFA include myristic acid (butterfat, coconut and palm kernel oil), lauric acid (palm

kernel and coconut oils), and palmitic acid. Palmitic acid is the most abundant SFA in the diet accounting for 60% of the total SFA intake of Americans (Mahan and Escott-Stump, 2008).

According to the Dietary Guidelines for American 2010, consuming less than 10% of calories from SFAs and replacing them with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) is associated with low blood cholesterol levels and therefore a lower risk for CVD (U.S. Department of Agriculture., 2010). Further reduction of CVD risk can be done by decreasing the percentage of calories from saturated fat from 10% to 7%. A significant correlation between SFA intake, total cholesterol and death was found in the Seven Countries Study. Death rates were positively associated with the percentage of dietary energy from SFA. Saturated fat intake (as percent of calories) was also positively correlated with serum cholesterol levels as well as with a five year incidence of CVD (Coulston *et al.*, 2001).

In an epidemiological study by Gordon (1995), the evidence that lowering serum cholesterol levels by decreasing intake of saturated fatty acids reduced the risk for CVD. The analysis included six dietary trials and demonstrated that lowering serum cholesterol levels by reducing the intake of saturated fatty acids significantly decreased the incidence of CVD by 24%, a 21% decrease in coronary mortality and a 6% decrease in total mortality. Additional research performed by the National Cholesterol Education Program (NCEP) states that for every 1% increase in kilocalories from SFA as a percent of total energy, the LDL cholesterol increases approximately 2%. Conversely, a 1% reduction in SFA will reduce cholesterol by about 2% (AHA, 2002).

c) Trans fatty acids

Trans fatty acids (TFA) are fat that contain a double bond in the trans configuration. TFA are produced through oil hydrogenation that allows hardening of the oil, but some TFA are found naturally in animal fats (AHA, 2002). Solid fat including TFA are abundant in the American diet and can lead to excess caloric intake. Solid fats account for on average 19% of total calories in the American diet However, TFA are not classified as saturated fatty acids, and therefore need to be consumed in small or no quantities Similar to SFA, TFA are shown to modify blood cholesterol levels. TFA lower HDL cholesterol resulting in a worsening of total cholesterol to HDL ratio and LDL to HDL ratios, which in turn increases CVD risk (Mensink and Katan, 1990). TFA also raises LDL cholesterol levels by increases plasma levels of lipoprotein and triglycerides while reducing endothelial function by impairing dilation (Hu and Willett, 2002). In addition, TFA adversely affect essential fatty acid metabolism and prostaglandin balance by

inhibiting enzyme function. Due to TFA having a large negative impact on cholesterol and CVD, research has shown that by substituting MUFAs and PUFAs for SFA and TFA, total cholesterol can be lowered.

d) Cholesterol

Cholesterol can either be made in the body or consumed through food intake. Regardless of where cholesterol comes from its purpose is for physiological and structural functions. Therefore, it is not necessary to consume dietary cholesterol. With that said, the average cholesterol consumption is 256 mg per day with men consuming 331mg and one-third of coming from egg intake (AHA, 2002). Even though cholesterol can be made in the body there are recommendations to consume less than 300mg per day due to its large impact on LDL and HDL. Dietary cholesterol has been shown to raise total cholesterol and LDL cholesterol. Diets high in saturated fat and cholesterol elevate LDL by down-regulating the LDL receptors in the liver (Mahan and Escott-Stump, 2008). With down-regulation, receptors are repressed causing less LDL to be cleared from the plasma.

The abundance of LDL in the plasma allows for oxidation in the arterial walls leading to atherosclerosis. This process of LDL oxidation caused by cholesterol can vary from person to person depending on genetic factors. Cholesterol also affects total to HDL cholesterol ratio. In a meta-analysis study by Weggemans and colleagues it was determined that dietary cholesterol raises the ratio of total to HDL cholesterol adversely affecting serum cholesterol (Weggemans *et al.*, 2001)

2.6.2 Non-modifiable Risk Factors

i) Age and gender

Non-modifiable risk factors include age, gender and family history. In both men and women, as age increases, there is an increase in CVD mortality rates leading to not only gender but age as a risk factor for cardiovascular risks. The incidence of premature CVD in men age 35 to 44 is three times as high as the incidence of women of the same age (Mahan and Escott-Stump, 2008). Therefore, the increase in absolute risk with aging becomes clinically significant for men in their mid 40's and women at about the same time as menopause (AHA, 2002). As gender plays a risk factor role, the Framingham Heart Study has shown that the differences in absolute CVD risk between genders cannot be explained by standard risk factors (AHA, 2002).

ii) Family history/ethnicity

Family history of CVD is a non-modifiable risk factor. A family history is considered to be positive when a MI or sudden death occurs before the age of 55 years in a male first degree relative or the age of 65 in a first degree female relative (Mahan and Escott-Stump, 2008). The risk for CVD can greatly increase when heredity is combined with unhealthy lifestyle choices such as smoking, physical inactivity and consuming a poor diet. Ethnicity may play a role as a non-modifiable risk factor. In a study conducted by Thomas *et al.*, focusing on ethnicity, income and CVD, black men had lower cholesterol levels and high blood pressure than white men. Fewer black men were also classified as being at low or intermediate risk for CVD compared to white men but, more black men were classified as high or very high risk for CVD. In summary, the study determined black men have a greater tendency to develop CVD risk factors versus white men over time (Thomas *et al.*, 2005).

2.7 Nutritional Status of Cardiovascular disease patients

Cardiovascular risk factors for ischemic heart disease and acute myocardial infarction are on the rise in Nepalese population. Recent observation has shown the significant rise of patients in younger age. Coronary Artery Disease (CAD) is the major cause of morbidity and mortality burden in the world. Young patients with CAD are specific subset of population requiring attention. Rheumatic Fever (RF), Rheumatic Heart Disease (RHD) and Congenital Heart Disease (CHD) are the most common cardiovascular diseases among school children and young adults. In 2005, 15.6 million people were estimated to have RF or RHD on the basis of traditional clinical measures that may represent substantial underestimation (Prajapati *et al.*, 2013).

Malnutrition and cachexia are serious consequences of numerous chronic diseases. Severe heart failure patients could be related with marked weight loss. Malnutrition is associated with poor prognosis among heart failure patients. A cross-sectional study was done on 284 randomly selected heart failure patients. The nutritional status of the patients was assessed based on their serum albumin level (normal value 4–5mg/dl) and triceps skin fold thickness. Based on serum albumin and triceps skin fold thickness, 77.8% of patients were malnourished. Mean age of the patients was 48.3 ± 15.9 years. The common cause of heart failure was ischemic heart disease (34.9%). Hypertension (36%) was the commonest co-morbid disease. Forty four percent of patients had class II heart failure (Amare *et al.*, 2015).

Serum hemoglobin was found to be significantly associated with nutritional status of heart failure patients. As serum hemoglobin increases by 1gm/dl, the risk of malnutrition decreased by 15 %. Malnutrition in heart failure is associated with loss of muscles, fat and bone mass. The assessment of malnutrition in heart failure might be done by anthropometric and biochemical tests. Numerous studies had used BMI, mid upper arm circumference (MUAC), calf circumference and triceps skin fold thickness. A study from Spain that assessed the usefulness of body mass index to characterize the nutritional status in patients with heart failure concluded that BMI does not indicate true nutritional status in HF (Amare *et al.*, 2015).

A Canadian study identified anemia by itself had a strong impact on clinical outcome of heart failure patients with higher number of anemic patients fitting to a higher functional class of heart failure. In conclusion, heart failure patients in our setup are relatively young. The major cause of heart failure was ischemic heart disease. Hypertension was the commonest co morbid disease associated with heart failure. Majority of heart failure patients were malnourished. Serum hemoglobin was found to be associated with nutritional status of heart failure patients. The current mean weight of the heart failure patients included was 53.9 ± 11.9 kg. Thirty six patients (12.7 %) reported that they have noticed weight loss in the past (Amare *et al.*, 2015).

2.8 Nutritional Requirement for the heart patients

2.8.1 Energy

The human body utilize the potential energy contained in a food for maintaining life and doing work. The energy yielding food factors are Carbohydrate, Fat and Proteins. Within the body, these nutrients are oxidized in the cells with the help of catalysts such as enzyme, co-enzyme and hormones. The process is one of the continuous utilization of oxygen and production of carbondioxide, water and heat. The energy value of the food depends on the quantity of the carbohydrate, fats and protein present in them. This can be determined by oxidizing a known weight of food in an instrument called bomb calorimeter and measuring the heat produced. The unit of energy value can be expressed in terms of Kilocalories (Cals) means one kilogram calorie is the quantity of the heat required to rise the temperature of 1 kg of water through 1 degree celcius. The physiological energy value of carbohydrate, fat and protein are 4,9 and 4 Kcal/gm respectively (Swaninathan, 2004).

Since the energy requirement reflects total energy expenditure which is composed of resting metabolic rate (RMR), activity energy expenditure and the thermal effect of food (Maliklal *et al.*, 1991). The resting metabolic rate is measured under the standard condition in which the

individual is at rest in a thermos neutral environment but not fasted, measurement are generally made a few hours after a light meal. Since indirect calorimeter is not widely available, the RMR is usually estimated by the Harris-Benedict formula which gives values approximately 22-25 Kcal per kg per day.

2.8.2 Total Energy

Those patients whose weight is at desirable level are permitted a maintenance level of calories during convalescence and their return to activity. The total calories should be restricted so as to reduce the weight to the expected normal for the height, age, and sex. Mild degree of weight loss for the cardiac patient of normal weight is recommended. Loss of weight by obese patients leads to considerable reduction in the work of the heart because basal metabolism is at the lowest level. There is slowing of the heart rate, a drop in the blood pressure and there by improved cardiac efficiency (Khanna *et al.*, 2005).

A brief period of under nutrition during the first few days of heart attack is advisable as increased cardiac output is needed with high intake of food. For a 45-50 years old executive leading an active life, the recommended energy is 1800-2000 Kcal (Grover, 2006) and an obese patient require 1000-1200 Kcal energy. It is advisable to undernourish the patient for few days after the heart attack. During this period, 1000 Kcal energy per day may be recommended and progressed to energy 1200 Kcal per day after the heart attack. Ingestion of the food involves increased cardiac output so as to meet the metabolic demands for digestion, absorption and assimilation of food. Therefore by giving the hypocaloric diet or restricting the food intake, the metabolic activity can be decreased to a level that the weakened heart can accommodate without extra strain (Khanna *et al.*, 2005).

2.8.3 Carbohydrate

The diet being low in fat, energy is mainly derived from carbohydrates as it is suggested that $\geq 55\%$ of total Kcal should be provided from the carbohydrate base (Grodner *et al.*, 1996). It is recommended to include complex starch rather than the simple sugar in the diet. Soluble fibers increases the transit time, delay gastric emptying and slow glucose absorption. In the colon is almost completely fermented to short chain fatty acids which may inhibit the liver cholesterol synthesis and help clear LDL cholesterol. Therefore, liberal use of foods containing water soluble fiber such as whole pulses, legumes, beans, oats ,fruits and vegetables should be made as they have a significant cholesterol lowering effect. In soluble fiber like cellulose and lignin do not have this lipid lowering effect (Joshi, 1995).

2.8.4 Protein

The word protein means necessary for life because it is vital parts of nucleus. Protoplasm of every cell is made of protein only. One sixth of body weight contains protein. One third of total body protein is in muscle, one tenth in skin, one fifth in cartilage and bones and the remaining in body fluids (Maliklal *et al.*, 1991). Protein differs from carbohydrate and fat because it contains 16% nitrogen and size of protein molecule is large. For the heart patient, the normal allowance is as for a normal diet means, 1g protein per kilogram body weight. But animal protein is not suggested for an atherosclerotic patient (Srilakshmi, 2002).

It has been suggested that the intake of protein should be $\leq 15\%$ of the total energy. Animal protein in the diet have not been linked specifically to risk of cardiovascular disease in humans, although increased levels induce hypercholesterolemia and atherosclerosis in laboratory animals. In the humans, substitution of soy protein for the animal protein in the diet reduce the level of serum cholesterol particularly in hypercholesterolemia subjects and there is a evidence that groups eating vegetarian diets have lower average blood cholesterol levels than non-vegetarian population. Avoiding the animal protein, using protein of pulses, as they are not only low in fat but also contain soluble fiber which helps in lowering the serum cholesterol (Khanna *et al.*, 2005).

i) Amino acid essential in special cases

a) Glutamine

Glutamine is the most abundant amino acid in the plasma and although considered a non – essential amino acid, it nevertheless regulates several cell specific processes including growth and gene expression. L-glutamine is among the 20 amino acids encoded by the standard genetic code. It is the most abundant free amino acid of the human body. It comprises about 20% of free amino acids in plasma and more than 50% of the amino acid pool in human skeletal muscle. It plays role in the maintenance and function of many organs and tissues such as the kidneys, liver, intestine, heart, muscle, neurons, lymphocytes, macrophages, neutrophils and pancreatic β -cells. L-glutamine improves cardiac function in patients with acute myocardial infarction (Badole *et al.*, 2014).

b) Arginine

Arginine act as a precursor for creatinine synthesis at all age group of people and its guanidine nitrogen acts as a potential precursor of nitric oxide, the potent endothelial releasing factor. L-

Arginine, a conditionally essential amino acid is the main substrate of nitric oxide synthase (NOS) family enzymes and is responsible for the production of the endothelium-derived relaxing factor nitric oxide (NO) which is involved in regulatory mechanisms of the cardiovascular system. L-arginine has a number of direct effects on endothelial functions via antioxidant activity, decrease blood viscosity, inhibition of angiotensin-converting enzyme, stimulation of fibrinolysis, and some hormones such as glucagon, prolactin and growth hormone (Bahadoran *et al.*, 2016).

It has been indicated that any defect in L-arginine metabolism such as increased levels of asymmetric dimethyl arginine (ADMA), a competitive inhibitor of NO synthase, are related to endothelial dysfunction and increased risk of cardiovascular disease. There is a potential protective effect of plant-derived L-arginine intake in regulation of blood pressure and prevention of CHD. Moreover intake of L-arginine from animal sources could be a dietary risk factor for development of HTN and cardiovascular risk factor (Bahadoran *et al.*, 2016).

c) Glycine

Glycine is a non-essential amino acid that can be obtained either via the diet, or synthesized endogenously from serine, threonine, choline, or glyoxylate in the liver and kidney. It is a predominant constituent of collagen and is utilized in the synthesis of several biologically important compounds, including glutathione, creatinine, purines, and glucose. Glycine exerts anti-inflammatory and anti-oxidative effects and has been inversely associated with several traditional cardiovascular risk factors, including obesity, hypertension and diabetes mellitus. Plasma glycine was associated with decreased risk of acute myocardial infarction (AMI) in patients with suspected stable angina pectoris. Glycine helps in regulating lipid metabolism and cholesterol transport in patients with atherosclerosis (Ding *et al.*, 2016).

2.8.5 Fat

Fat is composed of fatty acids, which is made up of carbon, hydrogen and oxygen. Crude fat not only true fat but also other minor constituents such as waxes, phospholipids, sterols, fat soluble vitamins, pigments and anti-oxidants (Grodner *et al.*, 1996). It is reported that the total fat intake should be $\leq 30\%$ of total Kcal intake but less than 20% are tolerated without side effect (Srilakshmi, 2002). According to (Grodner *et al.*, 1996), out of total fat intake, saturated fat should be 8-10%, polyunsaturated fat should be $\leq 10\%$, and monounsaturated fat should be $\leq 15\%$ of total Kcal. Any fat passing through the stomach is digested and absorbed in the small intestine and is sent to the liver for the processing and shipping throughout the body.

The very low density lipoprotein (VLDL) travels through the blood vessels to unload fat throughout the body. The empty VLDL becomes low density lipoprotein (Grover, 2006). Some of the LDL pieces get stuck to the blood vessel walls, narrowing the same. High LDL decreases the endothelium derived relaxing factor and the blood vessel becomes narrow and cannot dilate. High density lipoprotein (HDL) rescues the LDL pieces and brings them back to the liver where the LDL pieces are either recycled into the new VLDL or broken down (Grodner *et al.*, 1996).

2.8.6 Monounsaturated fatty acids

MUFAs are fatty acids that have only one double bond present making it an unsaturated fatty acid. Sources of MUFAs include olive oil, canola oil, peanut oil and tree nuts. Recommendations from the Dietary Guidelines for Healthy Americans 2010 states that SFA should be replaced with MUFA and PUFA in order to decrease CVD risk as well as lower blood LDL and triglycerides (Mahan and Escott-Stump, 2008). With replacement of SFA with MUFA, evidence has been shown that MUFA intake has an inverse association with death from CVD (Lee *et al.*, 2004). Similarly (de Lorgeril *et al.*, 1995) reported that subjects who consumed a mediterranean type diet high in MUFAs had a significant reduction in the risk of death from CVD cause or non-fatal acute MI, as well as cardiac mortality and total mortality. Diets high in MUFAs and total fat do not show a beneficial effect with CVD. When total fat and MUFA consumption is high, HDL cholesterol has not been shown to change thus hindering the cholesterol lowering effects of HDL (Coulston *et al.*, 2001).

2.8.7 Polyunsaturated fatty acids

Just like MUFAs, PUFAs need to have a double bond in the fatty acid chain but PUFAs must contain more than one double bond. There are two forms of PUFAs: Omega-6 polyunsaturated fatty acid (linoleic acid) and Omega-3 polyunsaturated fatty acid (linolenic acid), both of which are essential fatty acids. Omega-6 PUFAs are found mainly in flaxseed, canola oil, hemp oil, pumpkin seeds, sunflower seeds, and meats. Omega-3 PUFAs are traditionally found in cold water fish and nut oils. Both Omega-6 and Omega-3 have been shown to have beneficial effects with regards to CVD. In the Nurse Health Study, consumption of fish was associated with a decreased risk of cardiovascular events and death from CVD (Hu and Willett, 2002). Omega-3 PUFA consumption was also linked to an inverse effect on blood pressure. Thirty-one controlled trials showed that intakes of Omega-3 higher than three grams of fish oil per day were needed in order to observe a significant reduction in blood pressure (3.0 mmHg systolic and 1.5 mmHg diastolic) in HTN individuals (Morris *et al.*, 1993).

Protection from CVD through fish consumption has been linked to Docosahexanoic acid (DHA) and Eicosapentanoic acid (EPA). DHA and EPA are made in the body with the presence of Omega-3 and are needed for developmental growth and may be a preventative in the areas of heart disease, rheumatoid arthritis and hypertension. The mechanism behind PUFA protective qualities are not known with confidence but may be related to an antithrombotic effect, retarding the growth of atherosclerotic plaque, an anti-inflammatory effect and being mildly hypotensive (Harris, 1997).

2.8.8 Dietary fiber

Dietary fiber has been defined as the plant polysaccharide and lignin which are resistant to hydrolysis by the digestive enzyme in human beings and consists of a heterogeneous mixture of complex polysaccharide and non-polysaccharide polymers. The main components of dietary fiber are cellulose, hemicellulose, galactans and fructosans, pectic substances, gums, mucilages and lignin (Grodner *et al.*, 1996).

Fiber has been shown to decrease CVD. Dietary fiber is found in fruits, vegetables, whole grains and legumes. Research has shown that dietary fiber is associated with a reduced risk of fatal and non-fatal MI (Rimm *et al.*, 1996). Soluble fiber including oat bran, psyllium, guar gum and pectin has been shown to reduce CVD risk through its action on lipids, lipoprotein and glucose metabolism. Likewise, fiber has also demonstrated an effect on lowering glucose and insulin levels in non-diabetics and increasing insulin sensitivity in those with diabetes. (Coulston *et al.*, 2001). Fiber has also been shown to decrease cholesterol by binding to bile acid, which lowers serum cholesterol, as it depletes the bile acid pool. When bound, bacteria in the colon ferment the fiber and produce acetate, propionate and butyrate, which inhibit cholesterol synthesis (Mahan and Escott-Stump, 2008). Comparing soluble fiber to insoluble fiber, soluble fiber may have a greater effect on blood pressure than insoluble fiber. For the benefit of fiber recommendations are 25 to 30 grams per day with six to ten grams being soluble fiber (Lazarou and Kouta, 2008).

2.8.9 Minerals

It is stated that minerals are necessary for constituents of bones and teeth, body cells of soft tissues such as muscles, liver etc, as soluble salts which give to the body fluids and cell contents, their composition and stability which are both essential for life. Some minerals are required in small quantities for specific function including for the activity of various enzymes. For the heart patient, normal allowances are recommended (Srilakshmi, 2002).

i) Some important minerals for the heart patients

a) Potassium

Potassium is the major cation of the intercellular fluid. The movement of the potassium out of the cell and sodium within the cell change the electrical potential in the nerves and muscles to allow them to function effectively. Potassium restriction is not necessary for these patients as the symptoms of oliguria and anuria are not present. The suggested dietary target for the potassium is 4700 mg/day (man and women) to take advantage of potassium's ability to blunt the effect on the blood pressure particularly in the hypertensive individuals (Swaminathan, 2000). Dietary advice for the blood pressure management includes a diet rich in fruit and vegetables which provide potassium (Grodner *et al.*, 1996). Potassium is found in numerous fruits and vegetables including: bananas, cantaloupe, avocados, potatoes, tomatoes and spinach.

In a study, administration of 60–120 mmol/day of potassium supplements decreased systolic and diastolic pressure at 4.4 and 2.5 mmHg in individuals with high blood pressure. Individuals without high blood pressure decreased by 1.8 and 1.0 mmHg. Consistent results were found in further studies providing support to use potassium as a reduction and prevention of high blood pressure and thus a decrease in CVD (Appel, 2000).

b) Calcium

Calcium is the fifth most abundant element in our body. It is a metal necessary for maintaining the nervous, muscular and skeletal systems and for cell wall and blood vessel health. Calcium's role in bone and muscle strength is well known, but calcium is also important in blood clotting, nerve function and electrical conduction in the heart. Calcium can be used to control heart arrhythmias associated with low potassium levels. Growing evidence shows that calcium is the main ingredient in the plaques that clog arteries, although this does not seem related to taking calcium supplements (Swaminathan, 2000).

c) Magnesium

Magnesium, the major intercellular divalent cation is a co-factor in reactions utilizing adenosine triphosphate (ATP) and is essential for deoxyribonucleic acid (DNA) replication and ribonucleic acid (RNA) and protein synthesis. Magnesium deficiency is associated with an increase in the rate of ventricular ectopic beats, both in the presence of left ventricular dysfunction and normal cardiac function. Hypomagnesemia may potentiate the contractile response of smooth muscle to oxidizing agents, thereby accelerating atherosclerosis. Magnesium supplementation is associated

with a fall in the rate of ventricular arrhythmias in patients with chronic heart failure, atrial fibrillation, and digoxin toxicity. It may also be useful in the management of atrial fibrillation in patients with Wolff-Parkinson-White syndrome (Wittle and Clark, 2004).

d) Selenium

Selenium is absorbed principally in the duodenum. Its main function is as an antioxidant in glutathione peroxidase, an enzyme and major intracellular antioxidant. Selenoprotein-p is postulated to serve as a major extracellular antioxidant. Selenium is a powerful antioxidant in its own right and also supports other antioxidant processes. Pure selenium deficiency is rare, but symptoms may occur in cases of additional oxidative stress due to deficiency of other antioxidant systems such as vitamin E. Selenium may preserve the ability of myocardial cells to produce ubiquinone, another powerful antioxidant, and also reduce its breakdown. Low selenium levels may predispose an individual to ischemic heart disease and peripheral vascular disease (Wittle and Clark, 2004).

e) Zinc

Zinc is absorbed mainly through the duodenum, and although absorption is unaffected by age, it is reduced by low protein diets. Elderly patients may show a 5% incidence of frank biochemical deficiency, with 20% displaying symptoms suggestive of moderate deficiency such as loss of taste acuity. Zinc is a powerful site-specific antioxidant. Deficiency leads to elevated oxidative stress and cholesterol levels. A combination of zinc deficiency and ethanol can lead to contractile dysfunction in pre-ischemic conditions in the rat (Wittle and Clark, 2004).

f) Sodium

The amount of sodium in the diet is a problem only for the people who have a high blood pressure and who are the salt sensitive. This means their blood pressure goes up when they consume too much sodium. When there is more sodium salt, the body will retain the more fluid. Extra fluid causes an increase in the blood volume. The greater the blood volume, the circulatory system has to work hard to keep the blood flowing. This leads to increase in blood pressure (Grover, 2006). In the diet sodium is restricted when there is hypertension. Usually a restriction of sodium (1.5gm-3gm/day) is satisfactory in patients with coronary heart disease (Khanna *et al.*, 2005). In many primitive societies where salt intake is habitually low (<4gm of salt/day), blood pressure does not rise with age, and hypertension is rare or absent. Sodium is therefore, is one of the most important considerations in treatment of hypertension (Grodner *et al.*, 1996). It is

suggested that the people consuming high potassium salts and low sodium diets have all been described as the population with low blood pressure (Khanna *et al.*, 2005).

2.8.10 Vitamin

Normal allowance are allowed for the heart patient. Vitamins are necessary for the growth and maintainance of good health in human beings (Swaminathan, 2000). Vitamins C, E, and beta-carotene which are all antioxidants thought to be protective because they can inactivate free radicals which would otherwise damage the cells. Three servings of vegetables and two servings of fruits daily are recommended to fulfill these antioxidants requirements (Srilakshmi, 2002). Similarly the water soluble vitamins including folate are the most important supplements for the stressed patients.

i) Some important vitamins

a) Vitamin C

It is an antioxidant found in the blood and cytosol of cells, which reduces the damage by the free radicals during oxidant process. Vit.C may work synergistically in preventing cellular oxidation. Vitamin C (ascorbic acid) can be synthesized from glucose or galactose in a wide variety of plants and most animal species. The ability to participate in redox reactions is the basis for most of the functions of this antioxidant vitamin. Cigarette smokers have lower plasma and leukocyte levels of Vitamin C; overt deficiency of vitamin C. Higher levels of intake of Vitamin C correlate with a reduced risk of death from stroke as closely as diastolic blood pressure in otherwise normal elderly subjects. Low Vitamin C levels increase the risk of stroke, particularly in hypertensive overweight men. Vitamin C supplementation can reduce oxidative stress-mediated postprandial endothelial dysfunction. Oral vitamin C improves endothelial-dependent vasodilation of the brachial artery. In addition to these acute effects, vitamin C also reduces apoptosis in cardiomyocytes in rats with experimental heart failure, suggesting a potential long-term benefit in CHF patients (Wittle and Clark, 2004).

b) Vitamin E

Vitamin E (tocopherol) is a powerful antioxidant and is ubiquitous in cell membranes, protecting them from free radical damage. High vitamin E intake is associated with a lower incidence of coronary heart disease in middle-aged subjects. Men with high vitamin E intakes have a 40% reduced risk and women a 34% reduction. In healthy volunteers, diabetics, and cardiac transplant patients, vitamin E can lead to a reduction of platelet aggregation. This is a direct effect of

vitamin E on platelet activity through the inhibition of platelet protein kinase C. Alpha-tocopherol can also control the proliferation of smooth muscle cells through a similar mechanism. High-dose vitamin E reduces the oxidation of LDL. Oxidized LDL may enhance the generation of foam cells in the arterial walls, proliferation of smooth muscle cells, and platelet adhesion and aggregation, and trigger thrombosis (Wittle and Clark, 2004).

c) Carotenoids and Beta carotene

B-carotene is an antioxidant and quenches singlet oxygen and reacts directly with free radicals generated during lipids peroxidation. Green leafy vegetables and yellow orange, coloured vegetable are the good source of carotenoids (Swaninathan, 2004).

d) Niacin

Niacin is a generic term for nicotinic acid and nicotinamide. Nicotinamide is a component of nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), and the pyridine nucleotides. Deficiency causes pellagra. No evidence suggests a consequence of niacin deficiency on the cardiovascular system, although niacin supplementation reduces cellular apoptosis to oxidative stress. Nicotinic acid was used as a lipid lowering agent prior to development of the co-enzyme A reductase inhibitors (Wittle and Clark, 2004).

e) Thiamine

Thiamine is a co-enzyme for several decarboxylation steps in carbohydrate metabolism. Deficiency leads to impaired tissue oxygenation through inhibition of both the citric acid cycle and hexose monophosphate shunt. High output cardiac failure is seen with acute beri beri. Moderate thiamine deficiency can occur in hospitalized elderly patients and chronic heart failure patients on loop diuretics. Thiamine deficiency reduces myocardial contractile performance. Thiamine uptake by cardiac myocytes is impaired by both digoxin and frusemide; the drugs have an additive effect if taken together. Thiamine supplementation in patients with moderate to severe chronic heart failure can increase left ventricular ejection fraction and improve symptoms. Post-transplant heart failure can also respond to high dose thiamine supplementation (Wittle and Clark, 2004).

f) Riboflavin

Riboflavin forms parts of two important coenzymes, Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD); both are oxidizing agents. Rats fed riboflavin-deficient diets

showed abnormal lipid metabolism and a reduction in the beta-oxidation of fatty acids.⁸⁰ Children with chronic heart failure due to congenital heart disease have increased risk of riboflavin deficiency. Riboflavin plays an important role in the many enzymes systems involved in the metabolism of carbohydrate, fats and proteins (Swaminathan, 2004).

2.9 Raw materials and their nutritive value

2.9.1 Legume

Leguminous plants belong to family of leguminosae. The seed of leguminous plants are known as legumes/pulses. Legumes such as French bean, lima bean, or others that contain a small amount of fat are termed pulses, and legumes that contain a higher amount of fat, such as soybean and peanuts are termed leguminous oilseeds. Legumes are reasonably priced sources of protein, generally about double that of most cereals, and have a high food value as, they are fair sources of some vitamins and minerals. Legumes have almost the same caloric value per unit weight as cereals. Legumes are a better source of calcium than cereals and contain 100–200 mg of calcium per 100 gm. Legumes when compared with cereals are a better source of iron, thiamine, riboflavin, and nicotinic acid. Legumes are a good source of dietary fiber; the crude fiber, protein, and lipid components have a hypocholesterolemic effect. Legumes also contain some antinutritional factors, such as trypsin and chymotrypsin, phytate, lectins, polyphenols, flatulence-provoking and cyanogenic compounds, lathrogens, estrogens, goitrogens, saponins, antivitamin and allergens. However heat treatment is known to destroy the antinutrients such as protease inhibitors and lectin although it also destroys the vitamins and amino acid (Chakraverty *et al.*, 2001).

i) Soybean

It is the legumes which contains the highest percentage of protein, matures great gift to man. Soybean is a good source of thiamine, niacin and fair source of riboflavin. It consists of all essential fatty acids and all the essential amino acids except methionine and cysteine. Soybean has a characteristic beany, grassy and green odor, due to the presence of volatile compounds such as alcohol, aldehyde, ketones and enzymes lipoxygenase etc. The presence of undesirable beany odor and anti-nutritional factor such as trypsin inhibitors and haemagglutinins can be inactivated by autoclaving at 14lb/sq. inch for 30 minutes (Shrestha, 1989). The lecithin granules which is an emulsifier present in the soybean reduced cholesterol to about 23-25% in eight weeks, increased red blood cell. Lecithin emulsify fats, breaking them up and suspending them

in the blood. Lecithin protects the blood fats from being sticking on the lining of the arteries. It help to raise the level of HDL in the blood (Grosvenor and Smolin, 2000).

ii) Bengal gram

Bengal gram is a helpful source of zinc, folate and protein. They are also very high in dietary fiber and hence a healthy source of carbohydrates for person with insulin sensitivity or diabetes. Chickpeas are low in fat and most of this is polyunsaturated fat. Bengal gram help to decrease LDL cholesterol and VLDL cholesterol while increase in HDL cholesterol has a protective effect on the atherosclerotic process (Swaminathan, 2000).

2.9.2 Cereals

Cereals are monocotyledonous plants that belong to the grass family. They consist of wheat, rice, corn, potato, barley, sweet potato, cassava, soybean, oat, sorghum, millet, rye, peanut, field bean, maize, pea, banana, and coconut. The cereal grains such as wheat, rice, corn, barley, oat, rye, sorghum and millet provide 50% of the food energy and 50% of the protein consumed on earth. . In general, cereal grains have been considered as the source of carbohydrates to supply food energy to the diet. They are also good sources of proteins, certain minerals and B-group of vitamins (Chakraverty *et al.*, 2001).

i) Maize

Maize is one of the few major cultivated crop species that originated in the western hemisphere. Maize (*Zea mays L.*) originated from teosinte (*Zea mays L. spp Mexicana*) in the western hemisphere about 7,000 to 10,000 years ago. The chief protein of maize are glutelin and prolamin (zein). Zein is completely lacking in the tryptophan and lysine. Maize protein contain excess of leucine and leucine interferes in the conversion of trptophan to niacin and hence aggravates the pellagrigenic action of maize. Whole maize is a good source of thiamine, pyridoxine, pantothenic acid, fair source of riboflavin but poor source of niacin (Chakraverty *et al.*, 2001).

ii) Wheat

Wheat (*Triticum aestivum*) is common wheat grown throughout the world. The chief proteins of wheat are glutelin and gliain together known as gluten. Wheat protein also contains small amounts of globulin, albumin and protease. The limiting amino acid of wheat is lysine and threonine. The protein efficiency ratio of wheat protein is about 1.6-1.8 (Swaninathan, 2004).

Wheat bran is rich in dietary fiber, minerals, antioxidants, lignans, and other phytochemicals. Furthermore, increased intakes of whole grains increase soluble fiber intakes adequately enough to lower serum cholesterol concentrations to a small but significant degree. Whole grains are also important dietary sources of water-soluble, fat-soluble, and insoluble antioxidants. In addition to dietary fibers, the whole-grain arsenal includes a wide variety of nutrients and phytochemicals that reduce the risk of CHD. The compounds that have hypo-cholesterolemic effects include polyunsaturated fatty acids, oligosaccharides, plant sterols and stanols and saponins (Anderson, 2004).

2.9.3 Vegetable

i) Cauliflower

Cauliflower is low in fat, high in dietary fiber, folate, water and vitamin C. It also contains phytochemicals which are beneficial to human health including sulforaphane. Cauliflower contains allicin, which can improve heart health and reduce the risk of strokes and selenium, a chemical that works well with Vitamin C to strengthen the immune system. Cauliflower can also help to maintain a healthy cholesterol level (Chable *et al.*, 2005).

2.9.4. Fruits

i) Banana

Potassium found in banana helps in controlling the blood pressure. Pectin is a soluble fiber found in a banana is a cholesterol lowering agent (Dewan, 1994).

ii) Apple

When LDL oxidizes or deteriorates in the blood, plaque accumulates along the walls of the coronary artery and causes atherosclerosis. Compounds in apples called phytonutrients act in much the same way that red wine and tea do to delay the breakdown of LDL or bad cholesterol (Grosvenor and Smolin, 2000). Pectin of apple has got something like a constituent of jelly, which helps blood to congeal. It helps to raise the good HDLs. The organic potassium salt found in apple reduces the body sodium and thus good for the blood pressure (Dewan, 1994).

2.9.5 Spices

i) Cinnamon

Cinnamon, which is derived from a Greek word that means sweet wood, comes from the inner bark of tropical evergreen cinnamon trees. Cinnamon is a plant with many uses as an herbal medicine and it contains mucilage, tannin, sugar, resin, and essential oil among which essential oil is the most important constituent. The compounds present in Cinnamon have shown to be beneficial for glucose uptake, insulin regulation and blood lipid profile. A study was performed on patients with type 2 diabetes consuming cinnamon. The results showed that there were significant decreases in fasting serum glucose (18–29%), triglycerides (23–30%), total cholesterol (12–26%) and low-density lipoprotein cholesterol (7–27%) for patients who consumed 1 gm, 3 gm or 5 gm of cinnamon for 40 days (Hamidpour *et al.*, 2015).

ii) Fenugreek

Fenugreek being rich in phytochemicals has traditionally been used as a food, forage and medicinal plant. Fenugreek seeds contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, saponins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects. Seed powder normalized the enhanced lipid peroxidation and increased susceptibility to oxidative stress associated with depletion of antioxidants in diabetic rats. In normal rats supplementation resulted in increased antioxidant status with reduction in peroxidation. The steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and synthesis and hence its potential role in arteriosclerosis. Clinical studies demonstrated statistically significant decline in human serum total cholesterol, triglycerides and LDL cholesterol by fenugreek consumption (Mullaicharam *et al.*, 2013).

Amirthaveni and Thirumanidevi studied the effect of supplementation of fenugreek seeds (10g/day for 90 days) for noninsulin dependent diabetes mellitus patients. The study proved that blood glucose level in diabetic patients could be reduced effectively and gradually through daily intake of fenugreek seeds either in soaked or powdered form. The effect of fenugreek seeds on lipid profile showed significant reduction of total cholesterol (from 219.0 mg/dl to 164.4 mg/dl), low density lipoprotein (149.1 mg/dl to 127.9 mg/dl) and triglycerides from 188.8 mg/dl to 167.7mg/dl). The HDL level increased significantly (from 33.6 mg/dl to 47.4 mg/dl) which is considered to be desirable effect for good health (Amirthaveni and Thirumanidevi, 2004).

2.9.6 Honey

Honey is a heart tonic and medicine for all ailments of heart and blood pressure. Acetylcholine, present in the honey increase the blood flow in the heart and thus decrease the blood pressure and heart rate. Honey has antioxidant, anti-inflammatory, and antimicrobial activities. More studies are exploring other aspects of honey activity such as its effect on blood sugar, body weight, lipid profile, C-reactive protein, nitric oxide, pro-inflammatory prostaglandins, and homocysteine. Growing evidence and scientific data support the use of honey in patients with diabetes, HTN, dyslipidemia, obesity, and CVDs. Honey contains fructose, oligosaccharides, minerals, and antioxidants. In normal individuals, a daily consumption of 1.2 g/kg body weight honey during a 2-week test increased blood Vitamin C concentration by 47%, B-carotene by 3%, uric acid by 12% and glutathione reductase by 7%. Honey increased serum copper by 33%. Daily consumption of 30-75 g honey for 15 days decreased total cholesterol (8%) and LDL (11%) in normal and hyperlipidemic subject (Al-Waili *et al.*, 2013).

2.9.7 Soybean oil

Apart from the phytosterols and the other components of the bean (proteins, isoflavones) soybean oil presents several characteristics that give it a preventing effect on cardiovascular risk and coronary heart disease. With 61% of polyunsaturated fatty acids and only 15% of saturated fatty acids, without transfatty acids, soybean oil diets may decrease total and LDL cholesterol. Soybean oil is also rich in Vitamin E especially in gamma tocopherol whose antioxidant activity deserves more attention today. Finally, its large content in alpha linolenic acid may induce an anti-thrombotic and anti-arrhythmic effect, but this effect is perhaps restricted by the poor bioavailability of this fatty acid in soybean oil (Lecerf and Borgies, 2002).

Soybean fatty acids may have a protective effect for cardiovascular diseases independently of the others components of soybean oil such as phytosterols and of the other components of the bean (proteins, isoflavones). Although the high PUFA content may lead to an enhanced lipoprotein oxidation effect, which is cancelled by the high vitamin E content of the soybean oil, and to a lower HDL cholesterol, the low SFA associated with a high PUFA content of the soybean oil without trans fatty acid (in non-hydrogenated oils) may decrease LDL cholesterol in a positive way (Lecerf and Borgies, 2002).

2.9.8 Sugar

It is used for the test and fulfillment of energy requirement.

2.9.9 Walnut

Nuts are a rich source of many bioactive compounds that have antioxidant properties, including tocopherols, phenolic compounds, phytosterols, melatonin, and selenium. In addition, polyphenols in nuts seem to enhance the antioxidant effects of other nutrients in nuts and other foods. For example, polyphenols enhance the action of vitamin C, β -sitosterol, and α -tocopherol, the last of which synergizes the effects of γ -tocopherol. Walnuts are a source of β -sitosterol, α -tocopherol, and γ -tocopherol. Moreover, walnuts have the highest total polyphenol content of common nuts and peanuts as well as the greatest total antioxidant concentration compared with selected nuts and seeds. Walnut consumption confers benefits on many CVD risk factors. Acute and chronic consumption of walnuts (42.5–85 gm/day) has been shown to lower total and LDL-cholesterol concentrations, decrease blood pressure, improve endothelial function, decrease both oxidative stress and markers of inflammation and increase cholesterol efflux. Collectively, these biologic effects provide a mechanistic basis to account for the cardio-protective benefits of walnuts (Kris-Etherton, 2014).

2.10 Processing technology of Supplementary product

Traditional treatments such as soaking, cooking, germinating and fermenting have been used to improve nutritional quality of the cereals and legumes. Processing of food such as soaking, germination and fermentation leads to a reduction in phytic acid and increases of the minerals solubility in foods and also improves the bioavailability of minerals in cereals and legumes. Processing techniques reduce the levels of anti-nutritional organic factors, which includes phytates, phenols, tannins and enzyme inhibitors by releasing exogenous and endogenous enzymes such as phytase enzyme formed during processing (Tarek, 2002).

2.10.1 Soaking

Soaking or steeping is a pretreatment for decortification of grain facilitate the removal of the husk or skin. Non-corticated grains are soaked in water for a short time lead themselves to easy husk removal. Soaking process increases hydration coefficient, seed weight, total protein, ash, fat, fiber, while non-protein nitrogen, total carbohydrates, starch, stachyose, raffinose, reducing sugars, and minerals of cereals and legumes. Soaking the seeds in water and processing effectively removed the anti-nutrients. All anti-nutritional factors such as phytic acid, tannin, trypsin inhibitor and hemagglutinin activity were decreased during soaking in 0.5% sodium bicarbonate (El-Adawy *et al.*, 2000).

2.10.2 Germination

Germination or sprouting of legumes and cereals increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytates and protease inhibitors. Germination was more effective in reducing phytic acid than heat treatment, and therefore it improves the nutritional quality of cereals and legumes. Germination also slightly increases the total essential amino acids in cereals and legumes. Dehusking, germination, cooking, and roasting have been shown to produce beneficial effects on nutritional quality of legumes (Kadam and Salunkhe, 1985).

The desirable nutritional changes that occur during sprouting are mainly due to the breakdown of complex compounds into a simpler form, transformation into essential constituents and breakdown of nutritionally undesirable constituents. The metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Complex biochemical changes occur during hydration and subsequent sprouting. The reserve chemical constituents, such as protein, starch and lipids, are broken down by enzymes into simple compounds that are used to make new compounds. Sprouting causes increased activities of hydrolytic enzymes, improvements in the contents of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins, and a decrease in dry matter, starch and anti-nutrients. The increased contents of protein, fat, fiber and total ash are only apparent and attributable to the disappearance of starch. However, improvements in amino acid composition, B-group vitamins, sugars, protein and starch digestibility, and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process (Chavan and Kadam, 1989).

2.10.3 Drying

Drying produce a friable, readily milled stable product that may be stored for long periods, and from which roots may easily be removed. In drying green malt, the removal of moisture at low temperature allows the maximal survival of enzyme and the least development of aroma and color. Diastatic enzyme survives if the green malt is dried in a rapid air-flow at 40°C to not less than 10% moisture (Hough *et al.*, 1982).

2.10.4 Roasting

Roasting is a cooking method that uses dry heat where hot air envelops the food, cooking it evenly on all sides with temperatures of at least 150 °C (~300 °F) from an open flame, oven, or another heat source. Roasting can enhance flavor through caramelization and Maillard browning

on the surface of the food. Dry roasting is a process by which heat is applied to dry foodstuffs without the use of oil or water as a carrier. Unlike other dry heat methods, dry roasting is used with foods such as nuts and seeds. Dry roasted foods are stirred as they are roasted to ensure even heating (Gahlawat and Sehgal, 1994).

Roasting reduces the moisture content, thereby concentrating the food value. Roasting also enhance acceptability by imparting a nutty flavor to the food. Most of the anti-nutritional factors or toxic effects of legumes (trypsin inhibitor, hemagglutinin, goitrogenic agents, cyanogenic glucosides, alkaloids, etc.) are partially or fully eliminated by roasting. Similarly, on roasting, in vitro protein and starch digestibility of weaning foods increased by 15-21% and 16-19%, respectively. Roasting also improved in vitro iron availability by 12-19% (Gahlawat and Sehgal, 1994).

2.10.5 Milling and sieving

The outer bran in coarse grains is fibrous, bitter, astringent, or colored. Milling of the coarse grains is therefore desirable to confer adequate consumer acceptability to them. It is obvious that over milling or very high refining must be avoided, since it removes the aleuronic layers and germ rich in protein, vitamins, and minerals (Viraktamath et al., 1971).

2.10.6 Blending

It is the homogenous mixing of the entire ingredient. It is the process of combining two or more ingredients together so that they lose their individual characteristics and become smooth and uniform. The main objective of blending is to combine or mix so that the constitute parts are indistinguishable from one another resulting into the lipid based paste product (Amagloh *et al.*, 2012).

2.11 Anti-nutritional factors in cereals and legumes

2.11.1 Wheat and Maize

Phytate are the principal storage form of phosphorus and are particularly abundant in cereals and legumes. These chelate divalentcations such as calcium, magnesium, zinc and iron, thereby also reducing their bioavailability. Anti-nutrient phytic acid reduces the bioavailability of minerals.. Phytic acid as powerful chelating agent reduces the bioavailability of divalent cations by the formation of insoluble complexes. The high polyphenole content in plant food grains may also adversely affect the mineral availability. Phytic acid is widely distributed in commonly

consumed foods. It is found in high concentrations in the seeds of grains, pulses and oleaginous products, and in lesser amounts in tubers and garden produce. In cereals, approximately 1-2% weight of the seed is phytic acid, and it can even reach 3-6%. Referring to its location, 90% is found in maize germ, while in wheat and rice it is distributed in larger proportions in the external covers in the pericarp and in the aleurone layer (Nadeem *et al.*, 2010).

Germination has been an effective treatment to remove anti-nutritional factors in cereals e.g. phytate. Bran contains the high amount of phytic acid. The removal of undesirable components of wheat is essential to improve the nutritional quality. Fermentation and germination may improve the quality of cereals due to the removal of some anti-nutritional factors (Nadeem *et al.*, 2010).

2.11.2 Soybean

Soybeans contain significant amounts of bioactive compounds with toxic and/or anti-nutritional properties that can alter the body metabolism of consumers and exert a negative impact on the nutritional quality of the seed protein. Proteinase inhibitors in soybeans are responsible for inhibition of growth in rats, chicks and mice and effect generally accompanied by a depression in the digestibility of the protein in the diet. Soybean agglutinin shows specificity towards N-acetylgalactosamine. In mammals, about 60% of the lectin survives intestinal transit, where it becomes bound to the intestinal epithelium, causing disruption of the brush border membranes, atrophy of the microvilli and reduced viability of the epithelial cells (Becker-Ritt *et al.*, 2004).

Soyabean has a characteristics beany, grassy and green order, due to the presence of volatile compounds such as alcohol, aldehyde, ketones and enzyme lipoxygenase etc. The presence of undesirable beany odour and anti-nutritional factor such as trypsin inhibitors, haematoglutinins can be inactivated by autoclaving at 14lb/sq inch for 30 minutes (Swaninathan, 2004).

2.11.3 Bengal gram

Bengal gram constitutes an important part of the diet of a large section of the population in the developing world, as a good source of proteins, carbohydrates, minerals and B-vitamins. But in the raw state, they contain certain toxic substances which include trypsin inhibitors, phytohemagglutinins, lathyragens, compounds causing favism, cyanogenetic and goiterogenic factors, saponins and alkaloids. It is reported that these substances are generally eliminated by soaking and subsequent discarding of the liquid and/or by heat treatment at relatively elevated temperatures (50-60°C for 10-12hr). The presence of saponins, glycosides, tannins, alkaloids,

conjugates of protein with phytin or hemi-cellulose and substances inhibiting the action of digestive enzyme trypsin in different food legumes adversely affect their digestibility as these substances are indigestible or are antagonistic to digestion (Gupta, 1987).

Part III

Materials and methods

3.1 Materials

3.1.1 Soyabean and Bengal gram

Soyabean and Bengalgram were collected from Dharan Bazar. This variety of soyabean used was locally known as Nepali Bhatmas (*Glycine max*) and Bengal gram is Rato chana (*Cicer arietinum*).

3.1.2 Wheat and Maize

Locally available wheat and maize were collected from Dharan Bazar. This variety of wheat used is locally known as Rato ganhu (*Triticum aestivum*) and maize is murali makai (*Zea mays*).

3.1.3 Banana and Apple

Both were collected from the Dharan Bazar available in shop in Bhanu chowkh. The scientific name of apple is *Malus pumila* and banana is *Musa acuminata* respectively.

3.1.4 Cauliflower

Cauliflower was collected from the Dharan Sabji Mandi. Its scientific name is *Brassica oleracea* and locally known as Fulkopi.

3.1.5 Cinnamon and Fenugreek seeds

Cinnamon and fenugreek seeds were collected from the Dharan Bazar. It is locally known as Dalchini (*Cinnamomum verum*) and Methi (*Trigonella foenum – graecum*) respectively.

3.1.6 Sugar

White sugar available in Dharan Bazar was used.

3.1.7 Honey

Dabour honey available in Dharan was used as a heart tonic for heart patient. Its scientific name is *Apis mellifera* and locally known as Maha.

3.1.8 Walnut

Walnut available in the pouches at Bhatbhateni super market was used. Its scientific name is *Juglans regia* and locally known as Okhar.

3.1.9 Soybean oil

Refined soyabean oil manufactured by Swastik oil industries Pvt Ltd, Hattimuda, Morang Nepal was used as a source of fat in the diet.

3.1.10 Equipments

- Electronic balance
- Kjeldahl digestion and distillation set
- Soxhlet assembly
- Buchner filter assembly
- Suction pump
- Muffle furnace
- Spectrophotometer

3.1.11 Chemicals

- Petroleum ether
- Acetone
- Phenolphthalein indicator
- Methyl orange indicator
- Bromocresol green
- Boric acid
- Digestion mixture (catalyst mixture)
- Methyl red indicator
- Potassium Permanganate
- Potassium thiocyanide
- Saturated Potassium Persulfate
- Reagent grade Ferrous Ammonium Sulfate.6 H₂O
- Analytical grade Potassium Chloride
- Concentrated Hydrochloric acid, Concentrated Sulphuric acid
- Concentrated Ammonia, Acetic acid
- Silver nitrate, TBHQ (Tertiary Butylated Hydroxy quinine)

- DPPH (2, 2-Diphenyl-1-picril hydrazyl radical)

3.1.12 Glassware

- Petri dish
- Silica crucible
- Conical flask
- Glass rod
- Burette
- Pipette
- Test tubes
- Volumetric flask
- Digestion flask
- Photometer
- Dessicator
- Hotair oven

3.2 Methods

3.2.1 Formulation

3.2.1.1 Basis of Formulation

The preparation of supplementary product was done on the basis of RDA of cardiovascular disease patients which is shown in table 3.1 below.

Table 3.1 RDA for Cardiovascular disease Patients

Components	Daily requirement
Total energy (Kcal)	1800-2000
Carbohydrate (gm/day)	275 ($\geq 55\%$ of total Kcal)
Protein (gm/day)	75 (15% of total Kcal)
Fat (gm/day)	66.66 ($< 30\%$ of total Kcal)
Crude fiber (gm/day)	16 (0.5-0.8% of total Kcal)
Saturated Fat	8-10% of total Kcal
Polyunsaturated Fat	$\leq 10\%$ of total Kcal
Monounsaturated Fat	$\leq 15\%$ of total Kcal
Cholesterol	Not more than 200 mg/day
Thiamine (mg/day)	1.25
Riboflavin (mg/day)	1.50
Niacin (mg/day)	20
Vit C (mg/day)	60
Ca (mg/day)	800
Iron (mg/day)	10
Potassium (mg/day)	3500
Iodine (microgram/day)	150
Sodium (mg/day)	less than 2300
Vit E (mg/day)	10

Source: (Grodner *et al.*, 1996), (Khanna *et al.*, 2005), (Srilakshmi, 2002), (Grosvenor and Smolin, 2000), (Swaninathan, 2004).

3.2.1.2 Calculation of amounts of ingredients

For formulation, the amounts of all ingredients were calculated and weighed. Since during formulation, the cereals were taken as a source of energy, legumes were taken as a source of protein, fruits were taken as a source of vitamins and minerals, vegetable was as a source of crude fiber, minerals and vitamins, sugar and honey were as a source of energy and for the taste of the product. The vegetable oil were mainly for the maintenance of level of fat and walnut for maintaining essential fatty acids. Cinnamon and fenugreek were used a source of spice in the product. Finally from these calculation so far, three formulae mixes were established.

3.2.2 Processing of raw materials

3.2.2.1 Cleaning and Washing

The raw materials like cereals and legumes contain foreign matters, immature grains and discolored grain etc. These were removed by aspiration using Nanglo. It is made of bamboo sheet. fruits, vegetables and spices were washed in a tap water.

3.2.2.2 Wheat

Cleaned wheat was soaked for 24 hr. The surplus water was then drained, spread in tray about 2cm depth and covered with the thin muslin cloth. Water was sprinkled for every 24hr. The germinated wheat grain was dried at 50-60°C for about 10-12 hr to m.c. 4-6%. The dried malted wheat was then cleaned and ground then sieved and packed into a low density polythene (LDPE) bag.

3.2.2.3 Maize

The cleaned and weighed maize was roasted to about 4-5 min and then ground,sieved and packed in LDPE bag.

3.2.2.4 Soyabean

Soyabean was soaked overnight in sufficient amount of water. As a result it becomes easy to removethe hull from soybean by means of hand rubbing and hulls washed away. The dehulled soybean was then pressure cooked at 121 °C, 15lb/sq.in. for 20 min. The cooked soybean was dried at 50-60°C for about 10-12 hr to m.c. 4-5% and then ground, sieved and packed in a LDPE bag.

3.2.2.5 Bengal gram

Bengal gram was soaked overnight in water for about 48hr, drained and allowed to germinate. The germinated bengalgram was dried at 50-60°C for 10-12 hr and then cleaned,ground, sieved and packed in LDPE bag.

3.2.2.6 Cauliflower

Cleaned cauliflower was cut into small pieces, blanched in boiling water for 4-5 minute and then dried at 60-70°C till the 5-6% moisture content. The dried product was ground,sieved and packed in LDPE bag.

3.2.2.7 Apple

The inner core of the apples were removed and cut into small pieces of 1-2 mm, blanched into boiling water for about 3 minutes, dried at 50°C for 24 hr and to 65°C for about 5-6hr. The dried product was ground, seived and packed in LDPE bag.

3.2.2.8 Banana

Ripe and peeled banana was cut into small pieces about 0.8 to 1mm, and deeped into 1.25% solution of potassium metabiosulphate for 3 minute drained, spread on the tray. The pieces were dried first at 50°C for 24hr and the 65°C for 5-6hr. The dried product was ground, sieved and packed in LDPE bag.

3.2.2.9 Sugar

Sugar was ground in a grinder and then sieved and packed in LDPE bag.

3.2.2.10 Honey

It was used as such and mixed in the final product.

3.2.2.11 Soyabean oil

It was heated at 55°C in frying pan and mixed in the final product.

3.2.2.12 Walnut

It was dried in a dryer at about 55°C for 10-12 hr and then 65 °C for 3-4 hr and then ground, sieved and packed in LDPE bag.

3.2.2.13 Cinnamon

It was roasted for about 4-5 min and then ground,sieved and packed in LDPE bag.

3.2.2.14 Fenugreek

Fenugreek seeds were first cleaned and freed from broken seeds, dust and other foreign materials and then soaked in tap water for 12 hrs at 37°C. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried at 55-60°C. The soaked seeds were germinated in sterile petri dishes lined with wet filter paper for 36 hrs at 37°C with frequent watering. The sprouts were rinsed in distilled water and dried at 55-60°C. Fenugreek seeds were roasted in an open pan at 130°C for 7 min. It was continuously stirred with ladle for proper and uniform roasting until it became slight brown and left a peculiar aroma. Raw and processed fenugreek seeds were ground in grinder and passed through standard test sieve to get uniform sized flour. Flours were collected and stored in air tight food grade containers separately at ambient temperature for further use.

3.2.3 Product Preparation

3.2.3.1 Grinding and milling

All the dried cereals and legumes were ground by using corn mill available in pilot plant of Central Campus of Technology (CCT), Dharan but the other dried ingredients such as fruits and vegetables, spices were ground by using grinder available in CCT laboratory.

3.2.3.2 Sieving of the ground powder product

All the ground flour was sieved by using 200 μ sieve available in laboratory.

3.2.3.3 Mixing

The calculated amount of ingredients were weighed according to the formulation and mixed together homogenously.

3.2.3.4 Packing

After the completion of the homogenization, the products were immediately packed in LDPE bag.

3.2.3.5 Amounts of ingredients in formulae

The calculated amounts of ingredients for three different formulae (A,B and C) are shown in table 3.2 below. Amounts of these ingredients were calculated on dry basis. For the maintainance of desirable level of fat in the product, soyabean oil and walnuts were used.

Table 3.2 Amounts of ingredients used in supplementary product

Components/Ingredient(gm)	Formulae A	Formulae B	Formuale C
Wheat	100	100	100
Maize	100	100	100
Bengal gram	50	50	50
Soyabean	50	50	50
Cauliflower	30	30	30
Apple	25	25	25
Banana	30	30	30
Cinnamon	20	20	20
Fenugreek	28	10	56.7
Walnut	28	28	28
Sugar	30	30	30
Soybean oil	15	15	15
Honey	30	50	-
Salt	1.5	1.5	1.5
Total	539.5	537.5	536.2

The flow chart diagram of different ingredients used for the preparation of the diet is shown in figure 3.1.



Fig 3.1 Outline for the Preparation of Supplementary Product for Cardiovascular disease patients

3.2.4 Evaluation of prepared diets

(i) Sensory Evaluation

Sensory evaluation was performed by panelist using Hedonic Rating test using a 9 points scale ranging from like extremely to dislike extremely. The evaluation was carried out by 10 panelists including teachers, students and staff of Central Campus of Technology, Dharan. Three different products were mixed and homogenized and the provided to the panelists and asked to identify the acceptability of these three products.

(ii) Physicochemical analysis of products

Each prepared product were analyzed for the following parameters.

a) Moisture content

Moisture content was determined by hot air oven method following the procedure of Rangana, 2000.

b) Crude protein

Crude protein of all product was determined by kjeldhal method following the procedure described by Rangana, 2000. Percentage of crude protein was calculated as $\text{Crude protein} = \% \text{ nitrogen} \times \text{Factor (F)}$. Factor used were 6.25 for all products.

c) Crude fat content

Fat content was determined by using solvent extraction method using the petroleum ether as a solvent, according to Rangana, 2000.

d) Total ash

Ash content was determined by furnace method according to Rangana, 2000.

e) Crude fiber

Crude fiber in all products was determined by following the procedure of Rangana, 2000.

f) Total carbohydrate

Total carbohydrate was determined by difference method i.e. total carbohydrate=100- (sum of the content of moisture, protein, fat, ash, and crude fiber), following the procedure of Rangana, 2000.

g) Vitamin C

Vitamin C content in the product was determined according to Ranagana, 2000. The ascorbic acid content in fruits and vegetables can be estimated by macerating the sample with stabilising agents such as 20% metaphosphoric acid. 2, 6 -dichlorophenol indophenol is reduced to a colourless form by ascorbic acid. The reaction is specific for ascorbic acid at pH 1 to 3.5. The dye is blue in alkaline solution and pink in acid.

h) Potassium and sodium

Flame photometer was used to determine the potassium and sodium content of the product according to Ranagana, 2000. Potassium solution was atomized into an oxyhydrogen or oxyacetylene flame. The flame excited atoms of potassium caused emitted radiations at specific wavelength. The amount of radiation emitted is measured on a spectrophotometer. Five samples (total 15 samples) were used for estimation of potassium content by taking readings in triplicate. Dilute an aliquot of ash solution containing less than 150 ppm potassium. Add sufficient amount of HCl so that the concentration of acid was same as that in the standard solution. Atomized the diluted extract in a calibrated flame photometer with the wavelength dial set at 768 nm and the transmittance set at 100% for the top standard solution of potassium. Check the instrument periodically with the top standard solution. From the standard curve note the concentration.

i) Calcium

The calcium content of the product was determined according to Titrimetric Method (NIN, 2003). Calcium is precipitated as oxalate when titrated with standard potassium permanganate solution. 2 ml of sample was taken into a 15ml centrifuge tube with 2ml of distilled water and 1ml of 4% ammonium oxalate solution and mixed them thoroughly and left the mixture as such overnight. The contents were mixed again and centrifuged for 5min at 1500 rpm. The supernatant liquid was poured off and the centrifuge tube was drained by inverting the tube for 5min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube was wiped with a piece of filter paper. The precipitate was stirred and the sides of the tubes were washed with 3ml of dilute ammonia. It was centrifuge again and drained as before. The

precipitate was washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate was then dissolved in 2ml of 1 N H₂SO₄. The tube was heated in water bath for 1 min and titrated against 0.01N KMnO₄ solution to a definite pink color persisting for at least 1 min.

(iii) Microbiological Analysis

It is done to access bacterial, fungal, and yeast load under laboratory condition. For analysis, 10 gm of each sample is aseptically weighted and diluted to (1:10), i.e., 10gm in 90 ml sterilized distilled water and mixed well. Pour plate method and spread plate method can be used for yeast and fungus (AOAC, 2005).

a) Total plate count(TPC)

TPC was carried out using the methods of AOAC (2005).

b) Yeast and Mold

Yeast and Mold was determined according to AOAC (2005).

3.3 Cost Calculation

The cost of the best product from these three different formulations were calculated including a profit of 10%.

3.4 Data analysis

Data on analysis of sensory analysis were tabulated for comparison and were graphically represented using Microsoft excel-2010. Data were statistically processed by Gene stat version 12.1.0.3338 for analysis of variance (ANOVA). Means of the data were separated whether they are significant or not by using LSD (least square difference) method at 5% level of significance.

Part IV

Results and Discussion

4.1 Formulation of the product

In the present work, three different formulae decoded as A, B and C were developed only changing in the amount of honey and fenugreek, all other ingredients remains constant. Product A, B and C has same ingredient in which product A was formulated with 28g fenugreek and 30g honey, product B with 10g fenugreek and 50g honey and product C with 56.7g fenugreek and 0% honey. Amount of these ingredients were calculated on dry basis. For maintenance of energy, minerals, vitamins and desirable level of fat in a product, the amount of cereals and legumes was 56%, fruits, vegetables, spices, honey and sugar as 38% and vegetables oil (soybean oil) and walnut were used as about 8%.

From the calculation of nutrient composition from food composition table by DFTQC, three products were developed. Product A and B are same however product C is formulated from non-germinated fenugreek seeds and product A and B are formulated with addition of honey and germinated fenugreek seeds. All the processed food ingredients were mixed in the accurate amounts and proportion to meet out the nutrient requirement of patients. On the basis of RDA of cardiovascular disease patients, three products was prepared. Out of three products, the product B was found as the best product on the basis of sensory evaluation and then the proximate analysis and microbiological quality of the best product was carried out.

4.2 Sensory quality of the product

The prepared three products were subjected to sensory evaluation. The samples were provided to 10 panelists comprising of teachers and students of CCT. The panelists evaluated for various parameters of the product namely color, flavor, taste, texture and overall acceptability. The panelists were requested to provide scores in the score sheets as per their perception. Data were analyzed statistically and best product was found out. Average sensory score of different products can be studied from figure 4.1.

The ANOVA at 95% level of confidence ($p < 0.05$) showed that the product A, B and C were significantly different from each other in sensory attributes.

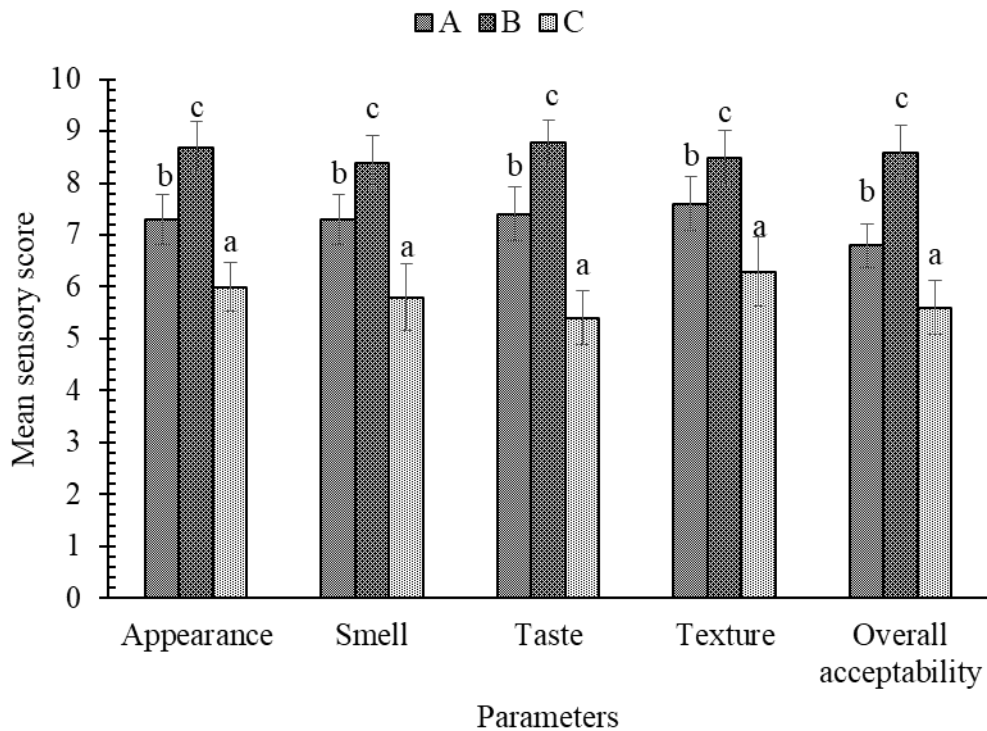


Fig 4.1 Average sensory score for three different products

4.2.1 Appearance/ Color

The average sensory score for appearance was 7.345, 8.695 and 5.995 for Product A, B and C respectively. The analysis of variance showed that there was significant differences ($p < 0.05$) between these three product.

Effect on the colour of cookies was observed as the level of the supplementation of germinated fenugreek seed powder was increased in the wheat flour that may be subjected to the colour of fenugreek seed. Similar effect on colour was obtained by (Hallen *et al.*, 2004) who observed that the level of raw cowpea incorporation increased (5, 10, 15 and 20 per cent); the darkness in the crumb and crust of the bread was increased.

4.2.2 Smell

The average sensory score for smell was 7.364, 8.386 and 5.736 for product A, B and C respectively. The analysis of variance showed that these three product were significantly different ($p < 0.05$) from each other.

4.2.3 Taste

The average sensory score for taste was 7.326, 8.803 and 5.403 respectively. In case of taste, product A, B and C were significantly different from each other.

Sensory scores revealed that the flavour of the cookies varied significantly among different treatments. The results indicated that the cookies prepared from control with 5% germinated fenugreek seed were scored high (8.00) for flavour which was significantly decreased for 15% germinated fenugreek seed (6.98). This might be due to unacceptable bitter flavour imparted by fenugreek seed flour (Agrawal, 2017).

4.2.4 Texture

The average sensory score for texture was 6.920, 8.586 and 5.586 for A, B and C respectively. The analysis of variance shows that these products were significantly different from each other.

With respect to the texture, judges accepted cookies prepared from all the treatments of the flour containing 5%, 10% and 15% germinated fenugreek seed powder (Agrawal, 2017).

4.2.5 Overall acceptability

The average sensory score for mouth was 7.636, 8.514 and 6.314 for A, B and C respectively. The analysis of variance shows that these products were significantly different from each other.

Germinated Fenugreek Seed Powder could be incorporated up to 10 per cent level in the formulation of cookies without affecting their sensory quality to produce acceptable and high nutritional value. Thus, further FC2 (cookies with 10 per cent GFSP) was further selected for storage study (Agrawal, 2017).

4.3 Chemical Analysis of the Product

The Chemical analysis of the superior product (B) found from sensory analysis was carried out. The result is tabulated in table 4.1.

Table 4.1 Nutritional composition of Supplementary Product (B)

Parameters	Guidelines for Nutritional composition	Amount in Product B
Energy (kcal)	2000	2057.85
Protein (gm)	75	78.23 (1.12)
Carbohydrate (gm)	275	326.47 (1.19)
Fat (gm)	66.66	48.792 (2.12)
Crude fiber (gm)	16	16.635 (1.17)
Ash (%)	-	2.32 (0.7)
Moisture (%)	-	8.9 (1.2)
Sodium (mg)	< 2300mg	750 (2.3)
Potassium (mg)	3500	4300 (1.2)
Calcium (mg)	800	1500 (2.1)
Vitamin C (mg)	60	139.5 (1.5)

Note- The values are the means of triplicate samples and the values in the parenthesis are standard deviation.

Source:(Grodner *et al.*, 1996), (Khanna *et al.*, 2005), (Srilakshmi, 2002), (Grosvenor and Smolin, 2000), (Swaninathan, 2004).

4.3.1 Energy density

The total weight of the developed supplementary product (537.5 gm) provides 2057.8 kcal or 384.68 kcal/100 gm. The product was designed in such a way that after reconstitution with water to a total volume of about 2000ml, it provides energy density of 1 kcal per ml per day. The major ingredients in supplementary feed which were contributing majorly to the total energy content were cereals (701 Kcal), legumes (371.45 Kcal), nuts and oil (562.28 Kcal), sugar and honey (248.9 Kcal) and spices (85.7 Kcal). Other ingredients like vegetables and fruits also contributed however, to lesser extent in the energy density of the supplementary product.

The nutritional composition of the supplementary and enteral feed formulated under various research studies were referred during the present investigation. The major findings of these studies indicated that the calorie density of the feed has tremendous implication in terms of health management of the patient. Most of the standard enteral formula provides 1 Kcal/ml and the major contributors for energy density are carbohydrate (55%) followed by fat (30%) and protein (15%) (Verma, 2006). Similarly it has been stated that a standard formula has concentration of 1 kcal per ml which falls in the accordance with the present research (Nilesh *et al.*, 2011).

4.3.2 Protein

The data presented in the table 4.1, portray that the supplementary product contains 14.63 gm protein per 100 gm of the feed weight. The total formula feed provided 78.23 gm of protein per 537.5 gm of formula powder, which accounts for the 15.21% per cent of the total calorie content of the developed supplementary feed. The sources of protein were wheat and maize soybean and bengal gram walnuts and spices. According to Sharma (1999), criteria for selection of standard formulae feed is more depending upon the physiological stage of the patient. The guideline for the RDA of cardiovascular patient stated that protein content of the supplementary product must be 15% of 2000 Kcal (75 gm). Results of the present study was also found to be comparable with the amount of protein required by CVDs patient. This justifies the adequacy of the protein delivered by the developed standard formula feed.

4.3.3 Carbohydrate

The table 4.1 shows that the total carbohydrate content of the developed supplementary feed was calculated to be 64.02 gm per 100g of the feed. The total quantity of the formula i.e. 537.5 gm, thus supplied 326.47 gm of carbohydrate. The total carbohydrate content of the developed

supplementary feed was accounted to provide 63.52 percent of the total calorie content (2057.8 kcal/537.5 gm). Wheat, maize, soybean, bengal gram, apple and banana and sugar contributed major part of carbohydrate followed by walnuts, fenugreek and cinnamon.

Various studies have shown the results, which were found to be in accordance with the present findings and prove the sufficiency of carbohydrate content in the developed standard enteral feed. Verma (2006) and Lord (1996) have documented different forms and concentrations of carbohydrate amongst various formulas. The forms may be starch, glucose polymers, disaccharides and monosaccharides in their polymeric form with 50 to 60% of total calories. Parallel to these observations, the compositional data of a standard enteral formula feed reported by a study exhibits that a major source of energy in the feed should come from carbohydrates (45 to 65%), as it known to be the essential fuel for the cells of body and brain (Nilesh *et al.*, 2011).

4.3.4 Fat

According to Gunter (2001), the fat source in formulas may include nuts and oil seeds and some vegetable oils. These contain long chain triglycerides which keep the osmolarity of the formula down and contribute essential fatty acids to the feed. The supplementary product developed under the present study contained 9.077 gm fat from the 100g of the feed, eventually 48.79 gm fat from the total amount of the feed (537.5 gm). This accounted for 21.34% of the total energy content from fat. The major part of fat was supplied by oil and walnuts (55.124 gm) followed by cereals and legumes.

According to a recent study, it has been stated that fat content of enteral formula feeds should provide 15 to 30 per cent of the total calories (Nilesh *et al.*, 2011). Similarly, Sharma (1999) has also reported that fat should provide 1 to 47% of total calories depending upon the type and severity of the disease. The RDA of the cardiovascular disease patient stated that fat % should be between 20-30% of total feed. This justifies the adequacy of the fat delivered by the developed supplementary feed for cardiovascular disease patient.

4.3.5 Crude fiber

Fibers are not digested in the GI tract but are fermented by colonic bacteria. They improve intestinal flora, reduce constipation and improve blood lipid levels. Most of the formulas contain fiber in a range of 5 to 14g per Litre of the feed (Lord, 1996). As shown in the table 4.1, the crude fiber content of the developed supplementary feed accounted to be 3.11 gm per 100 gm of the formula feed and 16.63 gm from the total amount of the feed (537.5 gm) or total

reconstituted volume of the feed (2000ml). The sources of fiber in the developed supplementary feed were cereals, legumes, fruits and vegetables and fenugreek.

Various earlier studies have reported the fiber content of standard enteral formula feed. Nilesh et.al have reported the fiber content of a standard feed to range from 4.24g to 7.0g /L, which was found to be similar of present study (8.315g/Litre or 16.63g/2L) to meet the daily requirements and to maintain on the safe level. The standard feed prepared by (Sharma and Joshi, 2014) has 12 g of fiber which is in the safe range of fiber intake of enteral feeding as the supplementary feed that was produced which has 16.63 gm The RDA of crude fiber for the CVDs patient is recognized as 16 gm per 2000 kcal and the value of crude fiber (16.63 gm) which is similar to the present studies.

4.3.6 Vitamin C

Berner (1989) has stated that various enteral feeding solutions which are designed to provide 1500 to 2000 kcal, are intended to suffice the recommended dietary allowances (RDAs) for vitamins and minerals in the patient. Young (2003) and Olree (1998) have also supported the above statement and reported that most nutritionally complete feeds in terms of macronutrients, contains adequate amount of micronutrients as well, in volume or daily dose of feed ranging from 1.5 to 2 litres. The composition of standard formula feed presented in table 4.1 depicts the Vitamin C content as 26 mg per 100 gm of the feed and 139.5mg per 537.5 gm.of the supplementary feed. The sources of Vitamin C in the supplementary feed majorly were fruits and vegetables. The requirement of Vitamin C for cardiovascular disease patients is 60 mg/day which can be sufficiently fulfilled by this product.

4.3.7 Minerals

The estimated mineral composition of the presently developed supplementary feed is demonstrated in table 4.1. The data exhibits the presence of 750 mg of sodium, 4300 mg of potassium and 1500 mg of calcium from the total amount (537.5 gm) of the developed supplementary feed. The Recommended Dietary Allowances (RDA) of these nutrients for a CVDs patient is < 2300 mg sodium per day, 3500 mg potassium per day and 800 mg calcium per day. These allowances could completely be provided by the administration of developed supplementary feed in the present study.

4.4 Microbiological quality of product

Highly acceptable products were subjected to microbial analysis during storage period of 2 months as per method described by American public health association (1984). The Nutrient agar media (NA) was used for total plate count, while potato dextrose agar (PDA) for yeast and mold. Serial dilutions were made for each sample and 1 ml of the appropriate dilution was poured, 10^{-2} and 10^{-1} serial dilution were used for pour plate in triplicate on selective media. Culture media was incubated at 37°C for 24 hrs for enumeration of the total microbial load in incubator. Developed colonies were expressed as colony forming units per gram (cfu/g) of sample. Total plate count (TPC) and mold count of the product as received by the microbiological assay are shown in table 4.2.

Table 4.2 Microbiological assay of the product

Parameters	Cfu/gm
Total plate count	1565
Yeast and mold	46

The total plate count of prepared therapeutic food was found to be 1565 cfu/g which was within the acceptable limit as specified on a Joint Statement by WHO, the WFP, the United Nations System Standing Committee on Nutrition and the UNICEF, the maximum acceptable limit of microorganisms is 10000 cfu/g (UNICEF, 2007).

Yeasts and molds are ubiquitous in the environment and can contaminate food through inadequately sanitized equipment or airborne contaminants. Yeast and mold counts frequently predominate when conditions for bacterial growth are less favorable, such as lower water activity, low pH, high salt, or high sugar content. The yeast and mold count was found to be a 46 cfu/g which was within the acceptable limit of 50 cfu/g in the product (UNICEF, 2007).

4.5 Cost of the product

The average cost of the prepared diets were calculated adding a profit of 10%. It was found that the products of formulae B can be produced in large scale at cost about Rs 15 per 100 gm respectively. These cost of the products however are substantially lower than that of Horlicks

Cardia+, which cost Rs 260/100gm in the retail shop of Nepal. Further these prepared products are nutritionally superior to horlicks and other food drinks like viva, alprovit, Bourn vita.

Horlicks Cardia+ is a malted food prepared from the ingredients – cereal products (48.2%), wheat dextrin (18%), milk solids (47%), corn solids hydrolyzed, calcium carbonate, edible vegetable oil and vitamins. Added nutrients per 100gm of horlicks manufactured and sold by Cloudbtail, India include 3g fat, 43gm carbohydrate, 15 gm protein, 25 gm dietary fiber, 617% Calcium, 56% Vit C providing 304 kcal. Based on the information available in the label of horlicks bottle and proximate composition of this products, it can be stated that the horlicks does not contain of the pharmacological important nutrients such as omega-3, omega-6 fatty acids and very low amount of fat and no sodium, potassium and sugar. From the composition of horlicks, it can be concluded that there is no balance between energy, fat, protein, carbohydrate and fatty acid composition which must be required for heart patient. This showed that the prepared diet products are superior to the heart patients than that of horlicks.

Part V

Conclusion and Recommendations

5.1 Conclusions

From the above result and discussion, it is concluded that;

a) From the sensory analysis of the product, the product B containing 10g fenugreek and 50g honey was found to be superior than the product C (58.7g fenugreek) and product A (28g fenugreek and 30g honey).

b) Diet prepared according to formula will meet our objective i.e. each diet provide energy with the recommended amounts. The percentage of energy provided by the fat, protein and carbohydrate are within the recommendation. Therefore this diet is suitable for the patient suffering from heart disease.

c) The total plate count, and yeast and mold count in weaning food was 1565Cfu/g and 46 Cfu/gm respectively. The product was safe from microbiological point of view.

d) From the analysis of product B, it was concluded that it provides 14.63 gm protein, 9.077 gm fat, 60.73 gm carbohydrate, 139.5 mg sodium, 279.06 mg calcium, 26 mg Vit.C, 800 mg potassium, 3.11 gm crude fiber and 384.68 Kcal energy per 100 gm of the product respectively. The moisture and ash content of the product were found to be 8.9%, and 2.32% respectively.

e) The total cost of the prepared therapeutic diet was calculated and found to be NRs. 15 per 100 gm. Prepared product was cost effective and is helpful to people with low economic status.

5.2 Recommendations

The following recommendation is made for the further study.

1. Evaluation of nutritional quality (e.g. Digestibility, NPU etc.), functional, toxicological and anti-nutritional quality of the product can be done.
2. Study of the amino acid profile and fatty acids composition of the prepared diet products can be done.
3. Study on the shelf life of the products using the selective packaging materials can be done.

Part VI

Summary

Nepal is being a developing country and due to its poor socio-economic condition and public awareness, chronic disease such as cardiovascular disease is now becoming the major cause of morbidity and mortality. It is believed that, along with the genetic factors and age, diet and lifestyle factors are also considered important risk factors for this disease. Many different factors are associated with the cardiovascular disease, out of them diet is one of the most important factor for causing the heart disease by providing cholesterol to the body. The dietary management is necessary for maintenance of good nutrition and for the maximum rest of the heart.

One of the major nutritional problems faced by heart patients is the management of the diet so patients suffering from the heart disease could not get the appropriate nutrients. Therefore for fulfilment of such deficiency, combination of cereals, legumes, fruits and vegetables, spices, honey and vegetable oil changed into a single form as a powder diet. It is believed that, this diet is sufficient to fulfill daily nutrient requirement for the heart patient. Three different formula were made from composite flour varying the addition and substitution of honey and fenugreek. On sensory analysis, the product B was found to be superior and was further analyzed. Protein, fat, carbohydrate, crude fiber, total ash of the product was found to be 14.63 gm, 9.077 gm, 60.73 gm, 3.11 gm and 2.32% respectively. The diet supplies 384.68 Kcal per 100gm. The energy contributed by the protein, fat and carbohydrate of product were found to be 15.21%, 21.34% and 63.52% respectively. Calcium, Potassium and Sodium content was found to be 279.06 mg, 800 mg and 139.53mg per 100gm of the product respectively.

Since color, flavour and taste have a combined effect on the overall acceptability of the diet samples. The average sensory score for appearance was 7.345, 8.695 and 5.995 for Product A, B and C respectively. The analysis of variance showed that there was significant differences ($p < 0.05$) between these three product. The average sensory score for smell was 7.364, 8.386 and 5.786 for product A, B and respectively. The analysis of variance showed that these three product were significantly different ($p < 0.05$) from each other. The average sensory score for taste was 7.326, 8.803 and 5.403 respectively. In case of taste, product A, B and C were significantly different from each other. The average sensory score for texture was 6.920, 8.586 and 5.586 for A, B and C respectively. The analysis of variance shows that these products were significantly different from each other. The average sensory score for overall acceptability was

7.636, 8.514 and 6.314 for A, B and C respectively. The analysis of variance shows that these products were significantly different from each other.

The microbiological analysis of the fresh product was carried out where total plate count was 1565 CfU/g and yeast and mold count was 46 CfU/g respectively. Experimentally the product B was found to be superior in terms of sensory evaluation and microbiological point of view.

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Appendices

Appendix-A

Table A.1 Cost of the product

Particular	Cost(Rs/kg or L)	Weight(gm)	Cost(Rs)
Wheat	30	100	3
Maize	35	100	3.5
Soybean	90	50	4.5
Bengal gram	120	50	6
Cauliflower	35	30	1.05
Apple	130	25	3.25
Banana	40	30	1.42
Cinnamon	80	20	1.6
Fenugreek	200	10	2
Walnut	700	28	19.6
Honey	280	50	14
Sugar	65	30	1.95
Soybean oil	120	15	1.8
Processing and labor cost	-	-	6.3
Profit 10%	-	-	6.3
Total cost	1925	-	77
Cost/100g	-	-	15

Appendix B

1. Sensory evaluation card

Sensory analysis of therapeutic diet for cardiovascular disease patients

Name of the panelist:

Date:

Name of the product: Therapeutic diet for cardiovascular disease patients

Dear panelist, you are provided with 3 sample of diets designed mainly for the cardiovascular disease patients. Please, score the provided samples of the products using the following scale.

	Appearance	Taste	Smell	Texture	Overall acceptability
A					
B					
C					

Hedonic Scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Comments (if any)

.....
.....

Signature

.....

Appendix C

Sensory evaluation of the product

Table C.1 Two way ANOVA for Color

Source of Variation	d.f.	s.s.	m.s.	Vr	Fpr.
Sample	2	36.4667	18.2333	67.44	<0.01
Panelist	9	1.3333	0.1481	0.55	0.801
Residual	18	4.86667	0.2704		
Total	29	42.667			

Since $p < 0.05$, there is significant difference between the samples so LSD testing is required.

Table C.2 LSD for Color

Sample	Mean
C	6 (a)
A	7.3 (b)
B	8.7 (c)

Table C..3 Two way ANOVA for Overall acceptance

Source of Variation	d.f.	s.s.	m.s.	Vr	Fpr.
Sample	2	24.4667	12.2333	45.25	<0.01
Panelist	9	4.1333	0.4593	1.7	0.162
Residual	18	4.8667	0.2704		
Total	29	33.4607			

Since $p < 0.05$, there is significant difference between the sample, so LSD testing is required.

Table C.4 LSD for overall acceptance

Sample	Mean
C	6.3 a
A	7.6 b
B	8.5 c

Table C.5 Two way ANOVA for Smell

Source of Variation	d.f.	s.s.	m.s.	Vr	Fpr.
Sample	2	34.067	17.033	42.19	<0.01
Panelist	9	0.8333	0.0926	0.23	0.985
Residual	18	7.2667	0.4037		
Total	29	41.1667			

Since $p < 0.05$, there is significant difference between the sample, so LSD testing is required.

Table C.6 LSD for Smell

Sample	Mean
C	5.8 a
A	7.3 b
B	8.4 c

Table C.7 Two way ANOVA for Taste

Source of Variation	d.f.	s.s.	m.s.	Vr	Fpr.
Sample	2	58.4	29.2	146	<0.01
Panelist	9	2.8	0.3111	1.56	0.203
Residual	18	3.6	0.2		
Total	29	64.8			

Since $p < 0.05$, there is significant difference between the sample, so LSD testing is required.

Table C.8 LSD for Taste

Sample	Mean
C	5.4 a
A	7.4 b
B	8.8 c

Table C.9 Two way ANOVA for Texture

Source of Variation	d.f.	s.s.	m.s.	Vr	Fpr.
Sample	2	45.6	22.8	71.58	<0.01
Panelist	9	0.6667	0.0741	0.23	0.985
Residual	18	5.733	0.3185		
Total	29	52			

Since $p < 0.05$, there is significant difference between the sample, so LSD testing is required

Table C.10 LSD for Texture

Sample	Mean
C	5.6 a
A	6.8 b
B	8.6 c

Appendix D

Product A

Table D.1 Diet plan of product A on the basis of Food Composition Table, 2012

Food items	Weight(gm)	Energy(Kcal)	CHO(gm)	Fat(gm)	Protein(gm)
Wheat	100	341	69.4	2.55	12.1
Maize	100	360	72.1	3.9	9.2
Bengal gram	50	174.95	29.5	1.75	10.3
Soybean	50	198.5	15.65	7.5	16.65
Cauliflower	30	12.3	1.98	0.72	0.225
Apple	25	16.38	3.72	0.138	0.05
Banana	30	49.01	11.49	0.126	0.5
Cinnamon	20	51.8	2.92	0.38	1.22
Fenugreek	28	93.23	12.345	1.61	7.33
Walnut	28	427.28	6.83	40.124	9.68
Sugar	30	1194	29.82	-	0.03
Soybean oil	15	135	-	15	-
Honey	30	98.7	23.85	-	0.09
Total	536	2072.55	279.6	73.798	67.375

Food items	Weight (gm)	Ca(mg)	P(mg)	Fe(mg)	Carotene(μ g)	Vit.C(mg)	Thiamine(mg)	Riboflavin(mg)	Niacin(mg)
Wheat	100	48	355	4.9	29	-	0.49	0.17	4.3
Maize	100	20	256	2.4	305	-	0.38	0.11	2
Bengal gram	50	29.6	137	2.65	8	-	-	-	-
Soybean	50	106	255	4.75	5	-	32	-	1.4
Cauliflower	30	3.45	22	0.25	-	25.88	-	0.11	-
Apple	25	2.5	3.5	0.16	-	0.25	-	-	-
Banana	30	5.1	11	0.111	23.4	2.1	0.015	0.024	0.15
Cinnamon	20	83	13.8	4.5	-	-	-	-	-
Fenugreek	28	44.8	104	1.82	26.88	-	0.0952	0.0812	0.308
Walnut	28	28	106	0.57	-	-	-	-	-
Sugar	30	-	-	-	-	-	-	-	-
Soybean oil	15	-	-	-	-	-	-	-	-
Honey	30	1.5	4.8	0.27	-	-	-	-	-
Total	536	372	127	22.3	397.2	28.2	32.9	0.49	8.1

Product B

Table D.2 Diet plan of product B on the basis of Food Composition Table, 2012

Food items	Weight(gm)	Energy(Kcal)	CHO(gm)	Fat(gm)	Protein(gm)
Wheat	100	341	69.4	2.55	12.1
Maize	100	360	72.1	3.9	9.2
Bengal gram	50	174.95	29.5	1.75	10.3
Soybean	50	196.5	15.65	7.5	16.65
Cauliflower	30	12.3	1.98	0.72	0.225
Apple	25	16.38	3.72	0.138	0.05
Banana	30	49.01	11.49	0.126	0.5
Cinnamon	20	51.8	2.92	0.38	1.22
Fenugreek	10	33.29	4.4	0.578	2.61
Walnut	28	427.28	6.83	40.124	9.68
Sugar	30	119.4	29.82	-	0.03
Soybean oil	15	135		15	-
Honey	50	159.5	39.75	-	0.15
Total	536	2076.41	287.63	72.766	78.715

Food items	Weight(gm)	Ca(mg)	P(mg)	Fe(mg)	Carotene(μ g)	Vit.C(mg)	Thiamine(mg)	Riboflavin(mg)	Niacin(mg)
Wheat	100	48	355	4.9	29	-	0.49	0.17	4.3
Maize	100	20	256	2.4	305	-	0.38	0.11	2
Bengal gram	50	29.6	137	2.65	8	-	-	-	-
Soybean	50	106	254	4.75	5	-	32	-	1.4
Cauliflower	30	3.45	22.3	0.255	-	25.88	-	0.11	-
Apple	25	2.5	3.5	0.165	-	0.25	-	-	-
Banana	30	5.1	10.8	0.111	23.4	2.1	0.015	0.024	0.15
Cinnamon	20	83	13.8	4.5	-	-	-	-	-
Fenugreek	10	16	37	0.65	9.6	-	0.032	0.029	0.11
Walnut	28	28	106	0.573	-	-	-	-	-
Sugar	30	-	-	-	-	-	-	-	-
Soybean oil	15	-	-	-	-	-	-	-	-
Honey	50	0.25	8	0.45	-	-	-	-	-
Total	536	342	120	21.4	380	28.23	32.980	0.4952	8.158

Product C

Table D.3 Diet plan of product C on the basis of Food Composition Table, 2012

Food items	Weight(gm)	Energy(Kcal)	CHO(gm)	Fat(gm)	Protein (gm)
Wheat	100	341	69.4	2.55	12.1
Maize	100	360	72.1	3.9	9.2
Bengal gram	50	174.95	29.5	1.75	10.3
Soybean	50	196.5	15.65	7.5	16.65
Cauliflower	30	12.3	1.98	0.72	0.225
Apple	25	16.38	3.72	0.138	0.65
Banana	30	49.01	11.49	0.126	0.5
Cinnamon	20	51.8	2.92	0.38	1.22
Fenugreek	56.7	188.81	25	3.28	14.79
Walnut	28	427.28	6.83	40.124	9.68
Sugar	30	119.4	29.82	0	0.03
Soybean oil	15	135		15	-
Total	534.7	2072.43	278.41	75.46	75.345

Food items	Weight(gm)	Ca(mg)	P(mg)	Fe(mg)	Carotene (µg)	Vit.C(mg)	Thiamine (mg)	Riboflavin(mg)	Niacin(mg)
Wheat	100	48	355	4.9	29	-	0.49	0.17	4.3
Maize	100	20	256	2.4	305	-	0.38	0.11	2
Bengal gram	50	29.6	137	2.65	8	-	-	-	-
Soybean	50	106	254	4.75	5	-	32	-	1.4
Cauliflower	30	3.45	22.3	0.25	-	25.88	-	0.11	-
Apple	25	2.5	3.5	0.16	-	0.25	-	-	-
Banana	30	5.1	10.8	0.11	23.4	2.1	0.015	0.024	0.15
Cinnamon	20	83	13.8	4.5	-	-	-	-	-
Fenugreek	56.7	90.7	209	3.68	54.432	-	0.192	0.164	0.623
Walnut	28	28	106	0.57	-	-	-	-	-
Sugar	30	-	-	-	-	-	-	-	-
Soybean oil	15	-	-	-	-	-	-	-	-
Total	534.7	417	1369	23.9	411.83	28.23	33	0.578	8.473

Color plates



P.1 Determination of total protein



P.2 Samples prepared